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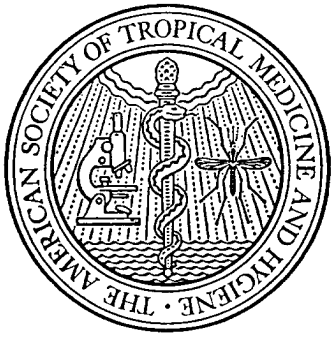
PROGRAM AND ABSTRACTS OF THE 44TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

The Hyatt Regency
San Antonio, Texas
November 17–21, 1995

Supplement to
THE AMERICAN JOURNAL OF
TROPICAL MEDICINE AND HYGIENE



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ADDITIONAL COPIES WILL BE $10.00

The abstracts in this issue represent preliminary communications and are *not* citable as publications.

AD_____

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TITLE: Annual Meetings of the American Society of Tropical Medicine and Hygiene

PRINCIPAL INVESTIGATOR: Peter F. Weller, Ph.D.

CONTRACTING ORGANIZATION: Beth Israel Hospital
American Society of Tropical Medicine and Hygiene
Boston, Massachusetts 02215-5491

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13. ABSTRACT *(Maximum 200*

There were two main objectives of the Army funding to the American Society of Tropical Medicine and Hygiene (ASTMH). The first was to support and enhance the Annual Meeting of the ASTMH, as a means for promoting research, collaboration between scientists, and exchange of information on the etiology, pathogenesis, diagnosis, prevention and control of tropical diseases. The second objective was to increase the opportunities for scientists from developing countries, students and young investigators to participate in the Annual Meeting. Through this funding, the ASTMH was able to sponsor 15 scientists, students and young investigators from developing countries to participate and present at the last three Annual Meetings. Both objectives were met and the funding was critical to the promotion of various advances in prevention and treatment as well as research for tropical diseases that are prevalent in developing countries.

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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature

1/21/98
Date

AMERICAN SOCIETY OF

TROPICAL MEDICINE AND HYGIENE

44th ANNUAL MEETING

NOVEMBER 17 - NOVEMBER 21, 1995

HYATT REGENCY

SAN ANTONIO, TEXAS

AMERICAN SOCIETY OF

TROPICAL MEDICINE AND HYGIENE

CONTENTS

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REGISTRATION INFORMATION

Los Rios Foyer, 2nd Floor, Hyatt Regency

Friday	November 17	7:00 AM - 7:00 PM
Saturday	November 18	7:00 AM - 5:00 PM
Sunday	November 19	7:00 AM - 5:00 PM
Monday	November 20	7:00 AM - 5:00 PM
Tuesday	November 21	7:00 AM - 1:00 PM

SPONSORS OF THE ANNUAL MEETING

Burroughs Wellcome Fund
National Institute of Allergy and Infectious Diseases
Rockefeller Foundation
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- recognition in Society publications and at the annual meeting
- discounts on annual meeting exhibit space fees, journal advertising rates and list rentals

Affiliate membership is available at the Patron, Donor and Contributor levels. Contact ASTMH headquarters for details or to request an application.

EXHIBITS

Be sure to visit the exhibits in the
Regency Foyer

Saturday	November 18	10:00 AM - 5:00 PM
Sunday	November 19	10:00 AM - 5:00 PM
Monday	November 20	10:00 AM - 3:00 PM

RECEPTION FOR STUDENTS AND FELLOWS

Students and fellows attending the Annual Meeting are invited to a pizza reception hosted by the Society's Officers and Council on Monday evening from 5:00 - 6:00 PM in the Pecan Room.

LATE BREAKERS IN MOLECULAR BIOLOGY

This Session is specifically designed for brief presentation of important, new data obtained after the closing date for abstract submission. Presentations are restricted to 5 minutes plus 5 minutes discussion time. Submit abstracts of 200 words or less to:

Dr. B. Kim Lee Sim
EntreMed Inc.
9610 Medical Center Drive
Suite 200
Rockville MD 20850
Fax: (301) 217-9594
Phone: (301) 217-9858

Dr. Anthony James
Dept. Molecular Biology & Biochemistry
University of California, Irvine
Irvine CA 92717
FAX (714) 824-2814
Phone: (714) 824-5930
e-mail: aajames@uoi.edu

prior to the Meeting or at the Meeting, but no later than 5:00 PM, Saturday, November 18, 1995. The list of presenters will be posted Sunday and the session will be held Monday morning 9:00 - 11:30 AM, Pecos Room. Check the bulletin board at the Meeting for further information.

AUDIOVISUAL FACILITIES

Slide preview and submission facilities are provided in the **Guadalupe Room** (second floor) beginning 1:00 PM on Friday, November 17. Speakers scheduled for AM sessions should preview slides and place them in carousels on the afternoon before their scientific presentation. Speakers scheduled for afternoon sessions should prepare slides on the morning of their presentation.

MESSAGES AND EMERGENCY CALLS

A message board will be available near the Registration Desk, Hyatt Regency Hotel. Emergency calls should be directed to (210) 222-1234, the main switchboard of the hotel. Faxes can be sent to (210) 227-4925.

EMPLOYMENT OPPORTUNITIES

Bulletin boards for posting employment opportunities will be available in the Registration area.

POSTER PRESENTATIONS

Poster Sessions on Monday, November 20 and Tuesday, November 21 will be located in the **Regency Ballrooms E/C/W** on the second floor of the Hyatt. A continental breakfast will be served for the Monday session and sandwiches for the Tuesday session. Posters may be set up beginning at 8:00 PM on the night before the session or in the morning before the session begins. Numbers on poster boards correspond to abstract numbers in the Program Booklet. Authors should be in attendance for the entire period at each poster session. Posters should be taken down during the lunch hour or afternoon following the session.

Poster Boards accommodate 2 posters and are 4 x 8 ft in size. Pins and numbers will be available in the presentation hall.

CONTINUING MEDICAL EDUCATION

INFORMATION ON CME FOR PRE-MEETING WORKSHOP WILL BE GIVEN SEPARATELY TO PARTICIPANTS.

The American Society of Tropical Medicine & Hygiene is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians.

The American Society of Tropical Medicine & Hygiene designates this continuing medical education activity for up to 30 credit hours in Category 1 of the Physician's Recognition Award of the American Medical Association.

Attendees desiring CME credit are advised to preregister for this activity (see materials mailed in the Registration Package) or to register at the ASTMH Registration Desk whereupon they will receive instructions and required forms. Documentation fees will be collected. Certificates based on recorded attendance at medical education activities will be mailed shortly after the meeting.

forms. Documentation fees will be collected. Certificates based on recorded attendance at medical education activities will be mailed shortly after the meeting.

NOTICES

Badges must be worn to attend all functions.

Smoking will be permitted only where specifically authorized. This rule is in compliance with the Resolution on Smoking adopted at the ASTMH Annual Business Meeting on November 5, 1976. The cooperation and thoughtfulness of smokers is requested to minimize embarrassment and discomfort for all persons.

The time and/or location of all activities are subject to change. Change notices will be posted in the Registration area.

Suggestions for changes in the Annual Meetings may be directed to members of the Scientific Program Committee.

ASTMH 1996 ANNUAL MEETING

The 1996 Annual Meeting of the American Society of Tropical Medicine and Hygiene will be held December 1-5 at the Hyatt Regency Hotel, Baltimore, Maryland.

The Deadline for Abstract submission will be 1 June 1996.

The executive offices for ASTMH are located at 60 Revere Drive, Suite 500, Northbrook, Illinois 60062 (phone 708/480-9592; FAX 708/480-9282). Requests for abstract discs and questions about the 1996 meeting should be referred to the executive offices. Watch for announcements and order forms, which will be mailed with the Journal and in the Call for Abstracts booklet in the winter of 1996. Note: As of January 20, 1996 the area code for the ASTMH office will be (847).

ASTMH TRAVEL GRANT AWARDEES

Sponsored by

U.S. Army Medical Research and Development Command
Rockefeller Foundation
National Institute of Allergy and Infectious Diseases

Graduate Student Awardees

C. Leptak	University of California, San Francisco
S. Sidjanski	New York University School of Medicine, New York, N.Y.
A. V. Gundlapalli	University of Connecticut Health Center, Farmington,
L. Gonzalez-Ceron	Center for Malaria Research, Tapachula, Mexico
V. J. Jennings	Emory University, Atlanta, Georgia
D. M. Isaza	Instituto de Colombiano de Medicina Tropical, Medellin, Colombia

Standard Travel Awardees

S. C. Levis	Inst. Nacional de Enfermedades Virales Humanas, Pergamino, Argentina
I. S. Metcheva	Medical College of Virginia, Richmond
G. Aviles	Ministry of Health, Cordoba Province, Argentina
D. D. Duc	Institute of Clinical Research in Tropical Medicine, Hanoi, Vietnam
B. Raengsakulrach	Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand
K. El-Shewy	University of Alberta, Edmonton, Alberta, Canada
D. A. Oliveira	Centers for Disease Control and Prevention, Atlanta, Georgia
J. E. Gigstad	University of Massachusetts, Worcester, Massachusetts
I. Ghosh	Johns Hopkins University, Baltimore, Maryland
C. N. Wamae	Kenya Medical Research Institute, Nairobi, Kenya
W. G. Dos Santos	ICB, UFMG, Belo Horizonte, MG, Brazil
D. G. Bausch	Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana
R. Haque	International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh
A. V. Ofulla	Kenya Medical Research Institute, Nairobi

SUMMARY OF

SCIENTIFIC PROGRAM

THURSDAY NOVEMBER 16, MORNING/AFTERNOON

9:00 - 5:30	Meeting of the WHO working group on research, development and introduction of dengue and Japanese encephalitis vaccine and diagnostic tests (open to all meeting attendees).	Rio Grande West

FRIDAY NOVEMBER 17, MORNING/AFTERNOON

7:00 - 7:00	Registration	Los Rios Foyer
8:00 - 5:00	Pre-Meeting Workshop: Migration Medicine (Separate Registration Fee)	Regency Ballroom - Center
8:00 - 5:00	ASTMH Council Meeting	Frio
8:00 - 11:30	SIRACA Subcommittee of ACAV	Chula Vista
8:00 - 10:00	ACAV Executive Council	Blanco
8:30 - 10:00	ACME Council	Llano
12:00 - 5:00	Young Investigator Award Competition	Chula Vista/Blanco/Llano

FRIDAY NOVEMBER 17, EVENING

6:00 - 9:00	Opening Reception	Regency Ballroom East/Center

SATURDAY NOVEMBER 18, MORNING

6:30 - 7:45	Scientific Program Committee Breakfast	Pecos
8:00 - 12:00	Plenary Session #1	Regency Ballrooms Center/West
10:00 - 5:00	Exhibits	Foyer
11:30 - 12:00	Commemorative Fund Lecture	Regency Ballrooms Center/West

SATURDAY NOVEMBER 18, AFTERNOON

1:30 - 5:30	Symposium: DNA Vaccines	Regency Ballroom Center
1:30 - 5:30	Scientific Session A: Malaria Chemotherapy I	Regency Ballroom East-2
1:30 - 5:30	Scientific Session B: Malaria Cytokine & Immunology	Regency Ballroom West
1:30 - 4:30	Scientific Session C: Bunyaviruses	Live Oak
1:30 - 5:15	Scientific Session D: Filariasis - Clinical & Epidemiology	Chula Vista
1:30 - 5:00	Scientific Session E: Amebiasis	Regency Ballroom East-1
2:00 - 5:45	Scientific Session F: Clinical Tropical Medicine I	Regency Ballroom East-3
1:30 - 5:30	Symposium: Global Surveillance of Emerging Diseases	Blanco/Llano
1:30 - 5:30	ACME Symposium: Transmission of Arthropod-Borne Pathogens	Nueces/Frio
1:30 - 5:30	Symposium: Toxoplasmosis - Clinical and Basic Science Update	Pecos

SATURDAY NOVEMBER 18, EVENING

5:00 - 6:00	Pizza reception for students and fellows hosted by ASTMH Officers/Council	Pecan
6:00 - 9:15	Scientific Session G: Alphaviruses	Rio Grande E
6:00 - 9:15	Scientific Session H: Schistosomiasis: Molecular Biology	Rio Grande C
6:00 - 9:45	Scientific Session I: Malaria Vaccines	Rio Grande W

SUNDAY NOVEMBER 19, MORNING

7:00 - 8:00	American Journal of Tropical Medicine and Hygiene Editorial Board Breakfast	Llano
7:00 - 8:00	Past Presidents Breakfast	Blanco
8:00 - 9:00	NIH Workshop on Grants and Grantsmanship	Nueces/Frio
8:30 - 11:30	Symposium: Design of Antimalarial Drugs	Regency Ballroom Center
8:00 - 11:30	Symposium: Host Defense Against Mucosal Pathogens	Regency Ballroom West
8:30 - 12:00	Scientific Session J: Filariasis - Immunology	Pecan
8:00 - 12:15	Scientific Session K: Kinetoplasts - Molecular Biology & Immunology	Rio Grande E
8:00 - 12:15	Scientific Session L: Malaria Vaccines & Immunology	Rio Grande C
8:00 - 12:15	Scientific Session M: Flaviviruses	Rio Grande W
8:00 - 12:00	Scientific Session N: Entomology-Field Ecology & Population Genetics	Blanco/Llano
9:00 - 12:15	Scientific Session O: Schistosomiasis - Immunopathology	Nueces/Frio
10:00 - 5:00	Exhibits	Foyer

SUNDAY NOVEMBER 19, AFTERNOON

1:30 - 5:30	ASTMH Plenary Session #2	Regency Ballroom Center/West
5:30 - 6:45	ICTDR Directors' Meeting	Frio

SUNDAY NOVEMBER 19, EVENING

6:00 - 10:00	New Developments in Hepatitis A Disease Prevention	Rio Grande
8:00 - 10:00	Discussion Group: Epidemiology of Malaria	Pecan

MONDAY NOVEMBER 20, MORNING

7:30 - 9:00	Poster Session # 1	Regency Ballroom East/Center/West (Coffee and donuts provided)
9:00 - 11:30	Late Breakers in Molecular Biology	Pecos
9:00 - 11:45	Scientific Session P: Filariasis - Molecular Biology & Chemotherapy	Pecan
9:00 - 12:00	Scientific Session Q: Entomology - Physiology of Vector Competence	Rio Grande E
9:00 - 12:00	Scientific Session R: Malaria - Chemotherapy II	Rio Grande C
9:00 - 11:45	Scientific Session S: Hepatitis	Nueces/Frio
9:00 - 11:30	Symposium: *Plasmodium falciparum* Antigens for Vaccine Design	Rio Grande W
9:00 - 12:00	Symposium: Bacterial Symbionts & Arthropod Genetic Manipulations	Chula Vista
9:00 - 12:00	Symposium: Allen Cheever Symposium on Schistosomiasis	Blanco/Llano
10:00 - 3:00	Exhibits	Foyer
11:30-1:30	London School Reunion Lunch	Chula Vista Boardroom

MONDAY NOVEMBER 20, AFTERNOON

1:30 - 2:15	Soper Lecture	Regency Ballroom East/Center
2:30 - 6:00	American Committee on Clinical Tropical Medicine and Travelers Health	Rio Grande C
2:30 - 6:00	Symposium: The Spf 66 Malaria Vaccine	Rio Grande W
2:30 - 6:00	Symposium: Burroughs Wellcome Molecular Parasitology	Chula/Vista
2:30 - 6:30	Symposium: American Committee on Arthropod-Borne Viruses	Regency Ballroom Center
2:30 - 6:30	Scientific Session T: Malaria - Biology & Molecular Biology	Rio Grande E
2:30 - 6:15	Scientific Session U: Kinetoplast - Epidemiology & Chemotherapy	Blanco/Llano
2:30 - 6:15	Scientific Session V: Schistosomiasis - Diagnosis, Chemotherapy &Epidemiology	Nueces/Frio
2:30 - 5:00	Scientific Session W: Giardia & Trichomonas	Pecan
2:30 - 6:15	Scientific Session X: Opportunistic Infections	Regency Ballroom East- 1&2
2:30 - 5:45	Scientific Session Y: Tick-borne Diseases	Regency Ballroom East- 3

MONDAY NOVEMBER 20 - EVENING

7:00 - 10:00	ASTMH Banquet	Institute of Texas Cultures/University of Texas

TUESDAY NOVEMBER 21 - MORNING/AFTERNOON

8:00 - 11:00	ASTMH Council Meeting	Pecan
8:00 - 11:00	Scientific Session Z: Hantavirus & Arenavirus	Rio Grande E
7:30 - 11:15	Scientific Session AA: Malaria - Epidemiology & Diagnosis	Rio Grande C
8:00 - 10:45	Scientific Session BB: Clinical Tropical Medicine II	Chula/Vista
8:00 - 11:30	Symposium: Cytokines in Infection	Rio Grande W
7:30 - 11:00	Symposium: Lipid Mediation of Host-Pathogen Interaction	Blanco/Llano
8:00 - 11:00	Symposium: Cryptosporidiosis	Nueces/Frio
11:00 - 12:30	Poster Session #2	Regency Ballroom East/Center/West (Sandwiches provided)

DETAILED

SCIENTIFIC PROGRAM

PRE-MEETING WORKSHOP
MIGRATION MEDICINE: MANAGING CURRENT AND FUTURE PROBLEMS*

Friday, November 17, 1995 Regency Ballroom Center
8:00 AM - 5:00 PM
Chairs: V. Lawrence and B. Gushulak

8:00 REGISTRATION, CONTINENTAL BREAKFAST, AND INTRODUCTION.

MORNING: OVERVIEW: INTERNATIONAL AND UNITED STATES.

8:30 MIGRATION HEALTH - INTERNATIONAL PROBLEMS AND PERSPECTIVES. Siem H. International Organization for Migration, Geneva.

9:15 MEDICAL ASSESSMENT AND SCREENING OF IMMIGRANTS AND REFUGEES DESTINED TO THE U.S. Tipple M. CDC, Atlanta, Ga.

10:00 Coffee Break.

10:20 EPIDEMIOLOGY OF TUBERCULOSIS IN US IMMIGRANTS. Castro K. CDC, Atlanta, GA.

11:05 GROUP DISCUSSION: INCREASING IMPACT OF MIGRATION-RELATED HEALTH PROBLEMS.

12:00 Lunch Break.

AFTERNOON: FOCUS: CLINICAL CARE AND LA FRONTERA (MEXICO/TEXAS BORDER)

1:30 CLINICAL CARE OF IMMIGRANTS - THE MINNESOTA EXPERIENCE. Walker P. Ramsey Medical Center, St. Paul, MN.

2:15 PUBLIC HEALTH AND TOXICOLOGY IN UNOFFICIAL BORDER COMMUNITIES. Garza V. Environmental Health Program, Mexico/US Border.

3:00 Coffee Break.

3:15 TUBERCULOSIS CONTROL IN MIGRANTS. Gonzales F. Binational Tuberculosis Project, El Paso, TX.

4:00 GROUP DISCUSSION: CLINICAL PROBLEMS, PRESENT AND FUTURE.

4:45 EVALUATION AND CLOSING

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*Registration for Pre-Meeting Workshop is required and is in addition to Annual Meeting Registration.

YOUNG INVESTIGATOR AWARD COMPETITION

Friday, November 17, 1994 Chula Vista/Blanco/Llano
12:00 N - 5:00 PM
Chairs: R. Blanton and M. Wilson

(Program to be posted at Registration)

PLENARY SESSION #1

Saturday, November 18, 1995 Regency Ballrooms Center/West
8:00 AM - 12:00
Chair: W. Petri, Jr.

8:00 RECOGNITION OF ASTMH TRAVEL GRANT AWARDEES. LeDuc JW.

8:10 PRESENTATION OF YOUNG INVESTIGATOR AWARD. Blanton R.

8:20 IMMUNITY AND DISEASE IN *TRYPANOSOMA CRUZI* INFECTION. Tarleton R. University of Georgia, Athens, GA (*Introduced by MG Peck, Burroughs Wellcome Fund*).

8:50 THE SPECIALIZED PARASITOPHOROUS VACUOLE SURROUNDING *TOXOPLASMA GONDII*. Joiner K. Yale University, New Haven, CT (*Introduced by MG Peck, Burroughs Wellcome Fund*).

9:20 CREATION OF TRANSGENIC PLANTS FOR ORAL IMMUNIZATION AGAINST DIARRHEAL DISEASES. Richter L. Texas A&M University, Houston, TX.

9:50 Coffee Break.

10:30 EBOLA VIRUS: THE PERSPECTIVE FROM CDC. Peters CJ. CDC, Atlanta, GA.

11:00 EBOLA VIRUS: REFLECTIONS FROM HONOLULU AND KIKWIT. Garrett L. New York, NY.

11:30 CONGRESSIONAL UPDATE: The Honorable Henry Bonilla, Member of Congress (Invited).

SYMPOSIUM: DNA VACCINES AGAINST TROPICAL DISEASES

Saturday, November 18, 1995 Regency Ballroom Center
1:30 - 5:30 PM
Chair: S. Hoffman

1:30 INTRODUCTION. Hoffman S. Naval Medical Research Institute, Rockville, MD.

1:40 THE DEVELOPMENT OF IMPROVED VECTORS AND SAFETY ISSUES FOR DNA-BASED VACCINES. Norman JA. VICAL, Inc., San Diego, CA.

2:15 SM23 NAKED DNA VACCINE *SCHISTOSOMA MANSONI*. Harn D. Harvard School of Public Health, Boston, MA.

2:50 PROTECTION AGAINST TUBERCULOSIS BY VACCINATION WITH DNA ENCODING SINGLE MYCOBACTERIAL ANTIGENS. Lowrie DB. National Institute for Medical Research, London, England.

3:25 Coffee Break

3:45 DNA VACCINES AGAINST LEISHMANIASIS. Liew FY. University of Glasgow, Scotland.

4:20 DNA VACCINES AGAINST MALARIA. Hoffman S. Naval Medical Research Institute, Rockville, MD.

4:55 EXPRESSION LIBRARY IMMUNZATION AS A GENERAL, SYSTEMATIC PROTOCOL TO DEVELOP VACCINES TO PATHOGEN. Johnston SA. University of Texas, Dallas, TX.

SCIENTIFIC SESSION A: MALARIA CHEMOTHERAPY I

Saturday, November 18, 1995 Regency Ballroom East 2
1:30 - 5:30 PM
Chairs: G. Heppner and J.K. Baird

1:30 1 WORLD-WIDE CONTROLLED CLINICAL TRIALS WITH ATOVAQUONE AND PROGUANIL FOR TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA. Canfield CJ*, Boudreau EF, Altstatt LB, Dausel LL, and Hutchinson DB. Pharmaceutical Systems Inc., Gaithersburg, MD; and Glaxo Wellcome, UK.

1:45 2 TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA WITH ATOVAQUONE PLUS PROGUANIL IN BRAZILIAN ADULTS. Cerutti C*, Alencar F, Piovesan-Alves F, Milhous WK, and Pang LW. U.S. Army Medical Research Unit-Brazil, Rio de Janeiro, Brazil; and University of Sao Paulo, Sao Paulo, Brazil.

2:00 3 HYDROXY-ANTHRAQUINONES AS ANTIMALARIAL AGENTS. Riscoe MK*, Winter RW, Cornell KA, Ignatushchenko M, and Hinrichs DJ. Medical Research Service, Department of Veterans Affairs Medical Center, Portland, OR; Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR; and Interlab Inc., Lake Oswego, OR.

2:15 4 THERAPEUTIC CONCENTRATIONS OF CHLOROQUINE INDUCE CHANGES IN THE CELLULAR ROUTAGE OF CATHEPSIN D-LIKE PROTEIN IN *PLASMODIUM FALCIPARUM*. Jambou R *, Bailly E, Rabe T, Benedetti EL, and Jaureguiberry G. INSERM U13, Hopital Claude Bernard, Paris France; Institut Jacques Monod du CNRS, Université Paris VII, Paris France; Institut Pasteur de Madagascar, Antananarivo, Madagascar.

2:30 5 SURVEYS OF RESISTANCE TO CHLOROQUINE BY *PLASMODIUM VIVAX* IN INDONESIA AND THE PHILIPPINES. Baird JK*, Masbar S, Fryauff DJ, Basri H, Tjitra E, Nalim MF, and Canete-Miguel E. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; National Institute of Health Research, Jakarta, Indonesia; and ALYKA, Puerto Princesa City, Palawan, Philippines.

2:45 6 TREATMENT OF UNCOMPLICATED *IN VITRO* CHLOROQUINE RESISTANT FALCIPARUM MALARIA IN IRIAN JAYA. Tjitra E*, Pribadi W, Budiono W, Arbani PR, Naibaho P, Supriyanto S, Romzan A, and Dewi RM. National Institute of Health Research and Development, Jakarta, Indonesia; Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; Freeport Hospital, Tembagapura, Timika, Irian Jaya, Indonesia; and Directorate General of Communicable Diseases Control and Environmental Health, Ministry of Health, Jakarta, Indonesia

3:00 Coffee Break

3:30 7 MEFLOQUINE IN THE TREATMENT OF *PLAMODIUM FALCIPARUM* MALARIA IN PREGNANCY. Nosten F*, McGready R, Thein Cho T, Chongsupadjaisiddhi T, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

3:45 8 INTRARECTAL QUININE ADMINISTERED THROUGH A NEW GALENIC FORMULATION TO *PLASMODIUM FALCIPARUM* MALARIA CHILDREN IN NIGER. Barennes H*, Mahaman Sani A, Pussard E, Clavier F, Kahiatani F, Granic G, Ravinet L, and Verdier F. Coopération franaise; Hôpital de Niamey, Niger; INSERM U13/IMEA, Hôp.C. Bernard, Paris, France; and Sanofi-Winthrop, Gentilly, France.

4:00 9 SURVEILLANCE OF DRUG RESISTANT MALARIA IN BRAZIL. Zalis MG*, Cruz CM, Alcencar FE, Cerutti CJ, Pang LB, Wirth DF, and Milhous WK. Instituto de Biofisica Carlos Chagas Filo, Federal University of Rio de Janeiro, Brazil; US Army Medical Research Unit-Brazil, American Consulate, Rio de Janeiro, Brazil; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

4:15 10 PRIMAQUINE-RESISTANT STRAIN OF *PLASMODIUM VIVAX* MALARIA FROM BRAZIL. Nayar JK*, Collins W, Baker RH, and Knight JW. Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences, University of Florida, Vero Beach, FL; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

4:30 11 ANTIMALARIAL DRUG METABOLISM IN A RAT DISEASED LIVER MODEL. Leo KU*, Grace JM, Peggins JO, Aguilar AJ, and Brewer TG. Division of Experimental Therapeutics, WRAIR, Washington, DC; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

4:45 12 DOES IRON THERAPY ENHANCE HEMATOLOGIC RECOVERY IN CHILDREN TREATED FOR MALARIA WITH SULFADOXINE-PYRIMETHAMINE? Kazembe PN*, Nwanyanwu OC, Ziba C, Gamadzi D, and Redd SC. Ministry of Health, Lilongwe, Malawi; and Centers for Disease Control and Prevention, Atlanta, GA.

5:00 13 THE EFFICACY OF SULFADOXINE-PYRIMETHAMINE (SP) IN THE PREVENTION OF PLACENTAL MALARIA IN WESTERN KENYA. Parise ME*, Schultz LJ, Ayisi JG, Nahlen BL, Oloo AJ, and Steketee RW. Malaria Section, Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

5:15 14 PROGRESSION OF PYRIMETHAMINE-RESISTANT *PLASMODIUM FALCIPARUM* IN MALIAN VILLAGES WHERE USE OF PYRIMETHAMINE-SULFADOXINE IS CONTROLLED VS. UNRESTRICTED. Plowe CV*, Djimde A, Wellems TE, and Doumbo O. Division of Geographic Medicine, University of Maryland School of Medicine, Baltimore, MD; Malaria Genetics Section, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and Malaria Research & Training Center, National School of Medicine & Pharmacy, Bamako, Mali.

SCIENTIFIC SESSION B: MALARIA CYTOKINES AND IMMUNOLOGY

Saturday, November 18, 1995 Regency Ballroom West
1:30 - 5:30 PM
Chairs: V. Udhayakumar and E. Nardin

1:30 15 LYMPHOCYTE SUBPOPULATIONS THAT FUNCTION IN THE PATHOGENESIS OF MURINE CEREBRAL MALARIA. Yanez D, Manning DD, van der Heyde HC, and Weidanz WP*. Department of Medical Microbiology & Immunology, University of Wisconsin, Madison, WI.

1:45 16 EFFECT OF NITRIC OXIDE ON NEURONAL NMDA CHANNELS HAS IMPLICATIONS FOR HUMAN CEREBRAL MALARIA. Rockett KA, Kwiatkowski DK, Bate CA, Premkumar LS, Gage PW, Awburn MM, and Clark IA*. Australian National University, Canberra, ACT, Australia; and Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

2:00 17 DECREASED NITRATE EXCRETION IN TANZANIAN CHILDREAN WITH UNCOMPLICATED AND CEREBRAL MALARIA. Anstey NM*, Hassanali MY, Mwaikambo ED, Manyenga D, Mlalasi J, McDonald MI, and Granger DL. Duke-Muhimbili Clinical Research Lab, Muhimbili Medical Centre, Dar es Salaam, Tanzania; Department Paediatrics, Muhimbili Medical Centre, Dar es Salaam, Tanzania; and Division of Infectious Diseases & International Health, Duke University Medical Center, Durham, NC.

2:15 18 CYTOKINE PRODUCTION BY ENDOTHELIAL CELL AFTER STIMULATION WITH *PLASMODIUM FALCIPARUM* BLOOD STAGE ANTIGENS. Xiao L*, Yang C, Saekhou AM, Udhayakumar V, and Lal AA. Division of Parasitic Disease, NCIC, CDC, PHS, U. S. Department of Health and Human Services, Atlanta, GA.

2:30 19 EXPRESSION-CLONING AND IMMUNOLOGIC ANALYSIS OF THE CD36-BINDING MALARIA PROTEIN, SEQUESTRIN: ADHERENCE CHARACTERISTICS AND MECHANISMS OF REVERSAL. Duffy PE*, and Ockenhouse CF. US Army Medical Research Unit- Kenya/Kenya Medical Research Institute, Kisumu, Kenya; Immunology, Walter Reed Army Institute of Research, Washington, DC.

2:45 20 CYTOKINE SECRETION IN PLACENTAS OBTAINED FROM MALARIA-INFECTED WOMEN. Fried M*, and Duffy PE. US Army Medical Research Unit- Kenya/Kenya Medical Research Institute, Kisumu, Kenya.

3:00 Coffee Break

3:30 21 CYTOKINE PROFILES IN RESPONSE TO MALARIA ACQUIRED ON THE PACIFIC COAST OF COLOMBIA. Duque S, Montenegro-James S*, Praba A, Hutchinson L, Herrera MA, Herrera S, and James MA. Department of Tropical Medicine, Tulane School of Public Health and Tropical

Medicine, New Orleans, LA; Ochsner Medical Foundation, New Orleans, LA; and Instituto Nacional de Salud, Bogota, Colombia; Universidad del Valle, Cali, Colombia.

3:45 22 CYTOKINE EXPRESSION IN AOTUS MONKEYS AFTER IMMUNIZATION WITH SYNTHETIC AND RECOMBINANT *PLASMODIUM VIVAX* AND *P. FALCIPARUM* ANTIGENS. Montenegro-James S, Duque S, Herrera MA, Praba A, Hutchinson L, Herrera S, and James MA*. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA; Ochsner Medical Foundation, New Orleans, LA; and Instituto Nacional de Salud, Bogota, Colombia; Universidad del Valle, Cali, Colombia.

4:00 23 IMMUNOREGULATORY ROLE OF *PLASMODIUM FALCIPARUM* SCHIZONT-INDUCED IL-12 PRODUCTION. Pichyangkul S *, Saengkrai P, Yongvanitchit K, and Heppner DG. Department of Immunology and Parasitology, U.S. Army Medical Component, AFRIMS, Bangkok, Thailand.

4:15 24 ALTERED COURSE OF *PLASMODIUM YOELII* INFECTION IN CBA/J MICE WITH PRE-EXISTING *SCHISTOSOMA MANSONI* INFECTIONS. Bosshardt SC*, Louis-Wileman V, Freeman GL, Colley DG, and Lal AA. Vanderbilt University School of Medicine, Nashville, TN; and Immunology Branch, DPD/NCID/CDC, Atlanta, GA.

4:30 25 MUCOSAL IMMUNIZATION WITH RBC INFECTED WITH LETHAL *PLASMODIUM CHABAUDI ADAMI* PROTECTS AGAINST CHALLENGE WITH THE SAME STRAIN. Dimayuga FO*, Dimayuga ER, and Wei Y. Department of Biological Sciences, Ohio University, Athens, OH; and Edison Biotechnology Institute, Ohio University, Athens, OH.

4:45 26 REQUIREMENTS FOR THE EXPRESSION OF PRIMARY AND REINFECTION IMMUNITY TO MURINE MALARIA *PLASMODIUM YOELII*. Yadava A*, Denkers E, Ahlers J, Gorden J, Berzofsky JA, Miller LH, and Kumar S. Laboratory of Parasitic Diseases, National Institute of Allergy & Infectious Diseases & Metabolism Branch, National Cancer Institute, NIH Bethesda, MD.

5:00 27 INDUCTION OF CTL RESPONSES AGAINST TWO DISTINCT EPITOPES ON THE *PLASMODIUM YOELII* CIRCUMSPOROZOITE PROTEIN BY IMMUNIZATION WITH PEPTIDES. Franke ED*, Sacci J, Corradin G, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Institute of Biochemistry, University of Lausanne, Epalinges sur Lausanne, Switzerland.

5:15 28 CYTOTOXIC T CELL RESPONSES TO CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM FALCIPARUM* IN NATURALLY IMMUNE KENYA ADULTS REACTIVITY OF DIFFERENT POLYMORPHIC SEQUENCES. Udhayakumar V*, Ongecha JM, Shi YP, Orago AS, Duffy PE, Hawley WA, Nahlen B, Hoffman SL, Weiss W, and Lal AA. Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; CDC-KEMRI, VBCRC, Kissian, Kenya; Kenyatta University, Kenya; NMRI, Bethesda, MD; and USAMRU, KEMRI, Kenya.

SCIENTIFIC SESSION C: BUNYAVIRUSES

Saturday, November 18, 1995 Live Oak
1:30 - 4:30 PM
Chairs: B. Beaty and G. Ludwig

1:30 29 PROBLEMS ASSOCIATED WITH IMMUNIZATION OF EUROPEAN BREED CATTLE WITH LIVE, ATTENUATED SMITHBURN ANTI-RIFT VALLEY FEVER VACCINE. Botros BA*, Moussa A, Taylor K, Soliman A, Salib A, Abdel Fattah M, and Arthur RR. US Naval Medical Research Unit No. 3, Cairo, Egypt; and General Organization for Veterinary Services, Ministry of Agriculture, Cairo, Egypt.

1:45 30 SURVEILLANCE OF RIFT VALLEY FEVER IN EGYPT USING SENTINEL ANIMALS. Arthur R*, Taylor K, Soliman A, Caliamaio C, Ibrahim H, Berry J, Moussa A, and Botros B. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt; and General Organization for Veterinary Services, Ministry of Agriculture, Cairo, Egypt.

2:00 31 ENHANCED TRANSMISSION OF RIFT VALLEY FEVER VIRUS BY *ANOPHELES STEPHENSI* MOSQUITOES CO-INFECTED WITH MALARIA. Vaughan JA*, and Turell MJ. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

2:15 32 A SPATIAL ANALYSIS OF THE DISTRIBUTION OF LACROSSE ENCEPHALITIS AND ITS ENVIRONMENTAL DETERMINANTS IN THE UPPER MIDWEST. Kitron U*, Kazmierczak JJ, Haramis LD, Aufmuth J, and Michael J. College of Veterinary Medicine, University of Illinois, Urbana, IL; Communicable Disease Section, Wisconsin Division of Health, Madison, WI; and Vector Control Program, Illinois Department of Public Health, Springfield, IL.

2:30 33 ARE NORTH AMERICAN BUNYAMWERA SEROGROUP VIRUSES THE ETIOLOGIC AGENTS OF CONGENITAL DEFECTS OF THE HUMAN CENTRAL NERVOUS SYSTEM? Calisher CH*, and Sever JL. Colorado State University, Fort Collins, CO; and NINCDS, NIH, Bethesda, MD.

2:45 34 EPIDEMIOLOGY OF OROPOUCHE FEVER IN THE AMAZAON REGION OF PERU. Hayes C*, Phillips I, Wi SJ, Callahan J, Griebenow W, Hyams K, and Watts D. Naval Medical Research Institute Detachment, Lima, Peru; and Naval Medical Research Institute, Bethesda MD.

3:00 Coffee Break

3:30 35 MOSQUITO SALIVA POTENTIATES LACROSSE VIRUS VIRULENCE. Edwards JF*, Higgs S, and Beaty BJ. AIDL, Colorado State University, Ft. Collins, CO.

3:45 36 QUALITATIVE ANALYSIS OF LAC S SEGMENT TRANSCRIPTION IN *AEDES TRISERIATUS* MIDGUTS BY TRANSCRIPT SPECIFIC RT-PCR. Wasieloski LP*, Blair CD, and Beaty BJ. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Arthropod Borne and Infectious Diseases Laboratory, Department of Microbiology, Colorado State University, Ft Collins CO.

4:00 37 AMPLIFICATION OF LA CROSSE VIRUSES FROM AUTOPSIED CNS TISSUES AND CHARACTERIZATION OF THE VIRUSES BY SSCP ANALYSIS. Beaty BJ*, Chandler LJ, Dobie D, Vanlandingham DL, Sweeney WP, and Black WC. Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

4:15 38 DETECTING BUNYAVIRUSES OF THE CALIFORNIA-BUNYAMWERA COMPLEX BY PCR. Kuno G*, Mitchell CJ, Chang GJ, and Smith GC. Division Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO.

SCIENTIFIC SESSION D: FILARIASIS - CLINICAL / EPIDEMIOLOGICAL / DIAGNOSTIC

Saturday, November 18, 1995 Chula Vista
1:30 - 5:15 PM
Chairs: J. Kazura and W.C. Nicolas

1:30 39 RISK FACTORS FOR ACUTE MORBIDITY IN BANCROFTIAN FILARIASIS. Kazura J*, Bockarie M, Alexander N, Dagoro H, Bockarie F, Hyun P, Perry R, and Alpers M. Case Western Reserve University, Cleveland, OH; and Papua New Guinea Institute of Medical Research, Maprik, Madang, and Goroka.

1:45 40 FIVE HUMAN FILARIASIS IN EQUATORIAL AFRICA: FIRST DESCRIPTIONS OF MULTIFILARIAL ENDEMIC AREAS IN GABON. Richard-Lenoble D*, Kombila M, Chandenier J, Eyang Obame E, Thérizol Ferly M, and Duong TH. Department of Parasitology and Tropical Medicine University School of Medicine Libreville Gabon and Tours France.

2:00 41 EFFECT OF REPEATED IVERMECTIN TREATMENTS ON OCULAR ONCHOCERCIASIS: EVALUATION AFTER SIX TO EIGHT DOSINGS. Chippaux JP*, Boussinesq M, Lafleur C, Fobi G, Auduge A, Banos MT, Ngosso A, Ernould JC, and Prod'hon J. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon; Hopital Central, Yaounde, Cameroon; Hopital General, Douala Cameroon; Ministère franais de la Cooperation, Paris, France; and Ophtalmo sans frontieres, France.

2:15 42 ONCHOCERCIASIS IN ECUADOR: EVOLUTION OF CHORIRETINOPATH AFTER AMOCARZINE TREAMTENT. Cooper PJ*, Proano R, Beltran C, Anselmi M, and Guderian RH. Department of Pathology, Cambridge University, Cambridge, United Kingdom; Onchocerciasis Control Progamme, Hospital Vozandes, Quito, Ecuador.

2:30 43 EFFECT OF REPEATED IVERMECTIN TREATMENTS ON THE INCIDENCE OF ONCHOCERCIASIS: A 7-8 YEARS FOLLOW-UP IN NORTHERN CAMEROON. Boussinesq M, Prod'hon J, and Chippaux JP*. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon.

2:45 44 IVERMECTIN BASED COMMUNITY CONTROL PROGRAM FOR LYMPHATIC FILARIASIS. Streit TG*, LeConte FA, Addiss DG, Lammie PJ, and Eberhard ML. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; and Hopital Ste. Croix, Leogane, Haiti.

3:00 Coffee Break

3:30 *KEYNOTE ADDRESS:* GLOBAL STRATEGY FOR LYMPHATIC FILARIASIS CONTROL - AND THE DATA SUPPORTING IT. Ottesen EA.

4:00 45 IMMUNODIAGNOSIS FOR SURVEILLANCE OF ONCHOCERCIASIS : EVALUATION OF AN ENZYME-IMMUNOASSAY USING A "COCKTAIL" OF RECOMBINANT ANTIGENS ("TRI-COCKTAIL"). Weiss N*, Toe L, Bradley J, Oettli A, Back C, and Boatin B. Swiss Tropical Institute, Basel, Switzerland; Onchocerciasis Control Programme, Ouagadougou, Burkina Faso; and Imperial College, London, U.K.

4:15 46 EARLY IMMUNODIAGNOSIS OF *ONCHOCERCA VOLVULUS* INFECTIONS IN TRANSMIGRANTS TO AN ENDEMIC AREA IN CAMEROON. Schneider D*, Chippaux JP, Boussinesq M, Vogt I, and Weiss N. ORSTOM, Centre Pasteur, Yaoundé, Cameroon; and Swiss Tropical Institute, Basel, Switzerland.

4:30 47 SERODIAGNOSIS OF EARLY ONCHOCERCIASIS IN CHILDREN: IMPLICATIONS FOR MONITORING TRANSMISSION. Ogunrinade AF*, Chandrashekar R, and Weil GJ. University of Ibadan, Nigeria; Washington University School of Medicine, St. Louis, MO.

4:45 48 TRANSMISSION OF *ONCHOCERCA VOLVULUS* STRAINS BY *SIMULIUM DAMNOSUM S.L.* SIBLING SPECIES IN WEST AFRICA. Toe L, Tang J, Back C, and Unnasch TR*. Onchocerciasis Control Programme, Bouake, Cote d'Ivoire; and Division of Geographic Medicine, University of Alabama at Birmingham, Brimingham, AL.

5:00 49 MONITORING BY PCR OF *WUCHERERIA BANCROFTI* INFECTIONS IN WILD POPULATIONS OF *AEDES POLYNESIENSIS* IN FRENCH POLYNESIA. Nicolas L*, Luquiaud P, Lardeux F, and Mercer DR. Institut Territorial de Recherches Medicales Louis Malarde, Papeete, Tahiti, French Polynesia.

SCIENTIFIC SESSION E: AMEBIASIS

Saturday, November 18, 1995 Ballroom East - 1
1:30 - 5:00 PM
Chairs: B. Mann and M. Goettke

1:30 *KEYNOTE ADDRESS:* THE CYTOLYTIC ARMAMENT IN GRANULES OF ENTAMOEBA HISTOLYTICA. Leippe M. Department of Molecular Biology, Bernhard Nocht Institute of Tropical Medicine, Hamburg, F.R. Germany.

2:00 50 THE CYTOLYTIC ARMAMENT IN GRANULES OF *ENTAMOEBA HISTOLYTICA*. Jacobs T, Berninghausen O, Andrä J, and Leippe M*. Department of Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, F.R. Germany

2:15 51 REPETITIVE ELEMENTS FROM THE *ENTAMOEBA HISTOLYTICA* RIBOSOMAL DNA EPISOME CONFER STABILITY TO TRANSFECTED DNA. Dhar SK*, Vines RR, Mann BJ, Bhattacharya S, Bhattacharya A, and Petri, Jr. WA. University of Virginia, Charlottesville, VA, USA; and School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India.

2:30 52 PHYSICAL MAPPING AND THE STUDY OF EXPRESSION OF GENE FAMILIES ENCODING N-ACETYLGALACTOSAMINE ADHERENCE LECTIN OF *ENTAMOEBA HISTOLYTICA*. Mann BJ*, Ramakrishnan G, Ragland BD, Purdy JA, and Petri, Jr. WA. University of Virginia, Charlottesville, VA, USA.

2:45 53 INDUCTION OF IL-8 RELEASE FROM HUMAN CELLS BY *E. HISTOLYTICA* TROPHOZOITES IS MEDIATED BY PREFORMED IL-1A AND THE GALACTOSE-INHIBITABLE LECTIN. Eckmann L,

Kagnoff MF, and Reed SL*. Departments of Medicine and Pathology, University of California, San Diego School of Medicine, San Diego, CA.

3:00 Coffee Break

3:30 54 MOLECULAR TRANSFER FROM *ENTAMOEBA HISTOLYTICA* TO HUMAN ENTEROCYTES IN COCULTURE. Leroy A*, De Bruyne G, Bailey G, Mareel M, and Nelis H. Laboratory of Pharmaceutical Microbiology, University of Ghent, Ghent, Belgium; Laboratory of Experimental Cancerology, University Hospital, Ghent, Belgium; and Morehouse School of Medicine, Atlanta, GA.

3:45 55 LYMPHOCYTE CYTOKINE RELEASE PATTERNS IN GERBILS WITH AMOEBIC LIVER ABSCESS. Campbell JD*, and Chadee K. Institute of Parasitology, Macdonald Campus of McGill University, Montreal, Quebec, Canada.

4:00 56 HUMAN COLONIC MUCIN HETEROGENEITY FOR DIFFERENTIAL INHIBITION OF *ENTAMOEBA HISTOLYTICA* ADHERENCE TO TARGET CELLS. Goettke MU*, and Chadee K. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

4:15 57 DEVELOPMENT OF A SCID-HU-INT MODEL FOR INVASIVE INTESTINAL AMEBIASIS. Seydel K*, and Stanley, Jr. SL. Department of Medicine Washington University School of Medicine, St. Louis, MO.

4:30 58 RAPID DIAGNOSIS OF AMEBIASIS BY ANTIGEN DETECTION. Haque R*, Hahn P, Neville LM, Shamsuzzaman SM, and Petri, Jr. WA. International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh; TechLab, Blacksburg, VA; Dhaka Medical College, Dhaka, Bangladesh; and University of Virginia, Charlottesville, VA.

4:45 59 FIRST RECOVERY OF THE LAB-MADE *ENTAMOEBA HISTOLYTICA* ZYMODEME XX IN HUMANS. Gatti S*, Cevini C, Bruno A, Novati S, Marchi L, AND Scaglia M. Lab. Clinical Parasitology, Institute of Infectectios Diseases, University-IRCCS S, Matteo, Pavia, Italy; and Sospiro Hospital, Sospiro, Cremona, Italy.

SCIENTIFIC SESSION F: CLINICAL TROPICAL MEDICINE I

Saturday, November 18, 1995 Ballroom East - 3
2:00 - 5:45 PM
Chairs: J. Bryan and B.L. Herwaldt

2:00 61 HEPATITIS E VIRUS EXCRETION AND SEROLOGIC RESPONSE FROM AN EPIDEMIC OF HEPATITIS IN PAKISTAN. Zhang HY, Iqbal M, Bryan JP*, Tsarev S, Longer CF, Rafiqui AR, Caudill JD, Duncan JF, Ahmed A, Miele TA, Malik IA, and Purcell RA. Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC; Pakistan United States Laboratory for Seroepidemiology, Rawalpindi, Pakistan; Department of Preventive Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD; and Hepatitis Viruses Section, National Institutes of Allergy and Infectious Diseases, Bethesda, MD.

2:15 62 GNATHOSTOMIASIS CONTRACTED IN EAST AFRICA. Wolfe MS*. Traveler's Medical Service of Washington, Clinical Professor of Medicine, George Washington University Medical School, Washington, DC.

2:30 63 EPIDEMIC VISCERAL LEISHMANIASIS IN SOUTHERN SUDAN: PATIENT CHARACTERISTICS AND TREATMENT OUTCOMES. Seaman J, Mercer AJ, Sondorp HE, and Herwaldt BL*. Medecins Sans Frontieres-Holland, Amsterdam; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

2:45 64 *OESOPHAGOSTOMUM BIFURCUM* IN MAN: TRANSMISSION IN NORTHERN TOGO AND GHANA. Polderman AM*, Eberhard ML, Pit D, Blotkamp J, and Baeta S. Department of Parasitology, University of Leiden, The Netherlands; Division of Parasitic Diseases, Centres for Disease Control & Prevention, Atlanta, GA; Regional Hospital of Dapaong, Ministry of Health, Togo & Department of Parasitology, Leiden, The Netherlands; Department of Parasitology, University of Leiden, The Netherlands; and Department of Gynaecology, University of Lome, Togo.

3:00 Coffee Break

3:30 65 CHARACTERIZATION OF THE INFLAMMATORY RESPONSE IN THE HUMAN BRAIN INFECTED BY THE CYSTICERCUS OF THE FLATWORM *TAENIA SOLIUM*. Restrepo BI*, Llaguno P, Sandoval MA, Enciso JA, and Teale JM. Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, TX; and Division Investigaciones en Enfermedades Infecciosas y Parasitarias, Hospital Pediatria CMN-IMSS Mexico D.F.

3:45 66 LYMPHOCYTE RESPONSE TO TETANUS IMMUNIZATION DURING EXTENDED USE OF CHLOROQUINE OR PRIMAQUINE CHEMOPROPHYLAXIS. Fryauff DJ*, Church P, Mouzin E, Widjaja H, Sutamihardja MA, Ratiwayanto S, Hadiputranto H, Saraswati A, Subianto B, Tjitra E, Wignall FS, and Hoffman SL. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; Provincial Health Service, Irian Jaya, Indonesia; National Institute of Health Research, Jakarta, Indonesia; Provincial Health Service, Irian Jaya, Indonesia; and U.S. Naval Medical Research Institute, Bethesda, MD.

4:00 67 HUMAN COENUROSIS IN NORTH AMERICA: REPORT OF TWO CASES AND REVIEW. Ing MB*, Schantz PM, and Turner JA. Division of Infectious Diseases, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA; and Epidemiology Branch, Division of Parasitic Diseases, NCID, Centers for Disease Control, Atlanta, GA.

4:15 68 MYIASIS: AN EMERGING OR ENDEMIC PROBLEM IN KUWAIT? Hira PR*, Farooq R, Al-Ali F, Hajj B, Al-Muzairai IA, and Hall MJ. Department of Microbiology, Faculty of Medicine, Kuwait University, Safat,Kuwait; Laboratory Departments, Adan and Farwaniya Hospitals, Kuwait City, Kuwait; Ophthalmology Department, Ibn Sina and Mubarak Hospitals, Kuwait City, Kuwait; and Natural History Museum, London, England.

4:30 69 STRATEGIC CONTROL OF CYSTICERCOSIS THROUGH SEROLOGIC MONITORING OF SENTINELS AND TREATMENT OF INFECTED PIGS WITH ALBENDAZOLE AND OXFENDAZOLE. Gonzales AE, Garcia HH, Gilman RH, Gavidia C, Pilcher JB, and Tsang VC*. Universidad Nacional Mayor de San Marcos, Lima, Peru; Universidad Peruana Cayetano Heredia, Lima, Peru; Division of Parasitic Diseases, NCID, Centers for Disease Control & Prevention, Atlanta, GA; and School of International Health, The Johns Hopkins University, Baltimore, MD.

4:45 70 ROLE OF FINE NEEDLE ASPIRATION BIOPSY IN DIFFERENTIAL DIAGNOSIS AND TREATMENT OF LIV ER ECHINOCOCCOSIS. Stefaniak J*, Lemke A, Paul M, and Pawlowski ZS. Clinic of Parasitic and Tropical Diseases and Institute of Radiology, University of Medical Sciences, Poznan, Poland.

5:00 71 GEOGRAPHIC INFORMATION SYSTEMS (GIS) DURING THE CEASE FIRE INITIATIVE IN SOUTHERN SUDAN: PROBLEMS WITH THE AVAILABLE DATA BASES. Richards F*, Roberts J, Doyle R, Ruiz-Tiben E, and Pietrantonio F. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; The Carter Center, Atlanta, GA; and Somalia/Sudan Mission, Medicins sans Frontieres-Belgium, Nairobi, Kenya.

5:15 72 GENETIC ANALYSIS OF ROUNDWORM BURDEN IN A NEPALESE POPULATION. Williams-Blangero S*, Blangero J, Upreti RP, Adhikari BN, Upadhayay RP, Jha B, Rai SK, Subedi J, and Robinson ER. Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; Tribhuvan University Institute of Medicine, Kathmandu, Nepal; and Department of Sociology/Anthropology, Miami University, Miami, OH.

5:30 73 *ASCARIS LUMBRICOIDES* ASSOCIATED WITH LOW HEIGHT FOR AGE Z-SCORES IN INFANTS AND YOUNG CHILDREN FROM A RURAL AREA IN SUB-SAHARAN AFRICA. Rockhold P*. Department of Epidemiology, UCLA, Dr. L. Ash.

SYMPOSIUM: GLOBAL SURVEILLANCE OF EMERGING DISEASES

Saturday, November 18, 1995 Blanco/Llano
1:30 - 5:30 PM
Chair: W. Bancroft

1:30 MILITARY INTEREST IN GLOBAL SURVEILLANCE. Joseph S. Assistant Secretary of Defense for Health Affairs.

2:10 THE NEED FOR GLOBAL SURVEILLANCE. LeDuc J. WHO, Geneva, Switzerland.

2:50 THE THREAT OF EMERGING DISEASES. Hughes J. CDC, Atlanta, GA.

3:30 Coffee Break.

3:50 ROLE OF DOD LABORATORIES IN GLOBAL SURVEILLANCE. Bancroft W. Military Infectious Diseases Research Program.

4:30 IMPLEMENTATION OF SURVEILLANCE AT THE REGIONAL LEVEL. Kaye B. Naval Medical Research Unit-3, Cairo, Egypt.

SYMPOSIUM: AMERICAN COMMITTEE ON MEDICAL ENTOMOLOGY THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE TRANSMISSION OF ARTHROPOD-BORNE PATHOGENS

Saturday, November 18, 1995 Nueces/Frio
1:30 - 5:30 PM
Chairs: M. Turrell and M. Wilson

1:30 THE ROLE OF ENVIRONMENTAL TEMPERATURE IN THE EPIDEMIOLOGY OF ARTHROPOD-BORNE DISEASES. Wilson M..

2:05 THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE TRANSMISSION OF MALARIA PARASITES. Beier J.

2:40 THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE TRANSMISSION OF VIRUSES BY MOSQUITOES. Turell M.

3:15 Coffee Break.

3:45 ENVIRONMENTAL TEMPERATURE AS A FACTOR IN THE EPIDEMIOLOGY OF FILARIASIS. Lok J.

4:20 TEMPERATURE AND THE TRANSMISSION OF DEER TICK-BORNE ZOONOSES. Telford S.

4:55 THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE TRANSMISSION OF PLAGUE. Gage K.

SYMPOSIUM: TOXOPLASMA

Saturday, November 18, 1995 Pecos
1:30 - 5:30 PM
Chair: J. Schwartzman

1:30 INTRODUCTION. Schwartzman J. Dartmouth College, Hanover, NH.

1:45 TOXOPLASMOSIS IN AIDS. Remington J. Palo Alto Research Foundation, Palo Alto, CA.

2:25 ADVANCES IN THE UNDERSTANDING OF CONGENITAL TOXOPLASMOSIS. McLeod R. Michael Reese Hospital, Chicago, IL.

3:05 Coffee Break.

3:30 IMMUNITY TO *TOXOPLASMA GONDII.* Kasper L. Dartmouth College, Hanover, NH.

4:10 INVASION AND INTRACELLULAR SURVIVAL OF *TOXOPLASMA GONDII.* Sibley D. Washington University, St. Louis, MO.

4:50 ADVANCES IN MOLECULAR GENETICS OF *TOXOPLASMA GONDII.* Roos D. University of Pennsylvania.

SCIENTIFIC SESSION G: ALPHAVIRUSES

Saturday, November 18, 1995 Rio Grande East
6:00 - 9:15 PM
Chairs: J. Smith and S. Weaver

6:00 74 COMPARISON OF THE CAPACITY OF ROBINS AND STARLINGS AS RESERVOIRS OF EEE VIRUS. Komar N*, Turell MJ, Pollack RJ, Monath TP, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

6:15 75 MULTIPLE EMERGENCES OF EPIDEMIC/EPIZOOTIC VENEZUELAN EQUINE ENCEPHALITIS VIRUSES FROM ENZOOTIC PROGENITORS. Weaver SC*, Oberste MS, Smith JF, Tesh RB, Shope RE, and Rico-Hesse R. Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX; US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Yale Arbovirus Research Unit, Yale School of Medicine, New Haven, CT.

6:30 76 ATTENUATED VENEZUELAN EQUINE ENCEPHALITIS VIRUS VACCINE VECTORS EXPRESS IMMUNOGENS OF HETEROLOGOUS PATHOGENS *IN VIVO* AND INDUCE MUCOSAL IMMUNITY. Davis NL, Brown KW, Charles PC, Caley IJ, Swanstrom RI, Smith JF, and Johnston RE*. Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC; and Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

6:45 77 DEVELOPMENT OF RNA REPLICON AND HELPER SYSTEMS FROM ATTENUATED STRAINS OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS. Pushko P *, Parker M, Ludwig G, Davis N, Johnston R, and Smith J. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC.

7:00 78 VENEZUELAN EQUINE ENCEPHALITIS VIRAL INFECTION AMONG PERUVIAN MILITARY TROOPS IN THE AMAZON RIVER BASIN OF PERU. Watts DM*, Callahan J, Cropp CB, Rossi C, Oberste MS, Karabatos N, Nelson W, Roehrig JT, Lavera V, Wooster MT, Smith JF, and Gubler DL. U.S. Naval Medical Research Institute Detachment, Lima, Peru; U.S. Army Medical Research Institue of Infectious Diseases, Frederick, MD; Division of Vectroborne Infectious Diseases, CDC, Ft. Collins, CO; and Peruvian Military, Iquitos, Peru; and U.S. Naval Medical Research Institute, Bethesda, MD.

7:15 79 ARBOVIRUSES ASSOCIATED WITH HUMAN INFECTION IN THE PERUVIAN AMAZON RIVER BASIN. Wooster MT, Watts DM*, Rossi C, Oberste MS, Callahan J, Smith JF, Hayes CG. U.S. Naval Medical Research Institute Detachment, Lima, Peru; U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD; and U.S. Naval Medical Research Institute, Bethesda, MD.

7:30 Coffee Break

8:00 80 IDENTIFICATION AND SUBTYPING OF HUMAN ISOLATES OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN PERU BY SEQUENCING AND PHYLOGENETIC ANALYSIS. Oberste MS*, Weaver SC, Watts DM, and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX; U.S. and Naval Medical Research Institute Detachment, Lima, Peru.

8:15 81 SUSCEPTIBILITY OF CENTRAL AND SOUTH AMERICAN MOSQUITOES FOR EPIZOOTIC AND ENZOOTIC STRAINS OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS. Turell MJ*, Coleman RE, Dohm DJ, and Barth J. Applied Research Division, U. S. Army Medical Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

8:30 82 PROTECTION OF MICE AGAINST AEROSOL CHALLENGE WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS BY ALPHAVIRUS SPECIFIC MONOCLONAL ANTIBODIES. Pratt WD, and Ludwig GV*. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

8:45 83 EFFECT OF GAMMA IRRADIATION ON VENEZUELAN EQUINE ENCEPHALITIS VIRUS ENVELOPE PROTEIN EPITOPES. Lind CM, Kondig JP*, Shoemaker MO, Smith JF, and Ludwig GV. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

9:00 84 IMMUNOMAGNETIC SEPARATION AS A SIMPLIFIED PROCEDURE FOR PROCESSING VENEZUELAN EQUINE ENCEPHALITIS VIRUS SAMPLES FOR IDENTIFICATION BY RT-PCR. Knauert FK*, Parrish BA, Ibrahim SI, Johnson DE, Craw PD, and Lofts RS. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

SCIENTIFIC SESSION H: SCHISTOSOMIASIS - MOLECULAR BIOLOGY

Saturday, November 18, 1995 Rio Grande C
6:00 - 9:15 PM
Chairs: D. Harn and T. Wynn

6:00 85 BIOCHEMICAL CHARACTERIZATION OF AN ANTIGEN LOCALIZED TO DISCOID BODIES IN THE PARASITIC TREMATODE *SCHISTOSOMA MANSONI*. Hoffmann KF*, Lewis SA, and Strand M. The Johns Hopkins University, School of Medicine, Baltimore, MD.

6:15 86 ASPARAGINYL ENDOPEPTIDASE ACTIVITY IN ADULT *SCHISTOSOMA MANSONI*. Dalton JP, and Brindley PJ*. School of Biological Sciences, Dublin City University, Dublin, Ireland; and Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

6:30 87 PUTATIVE MOLECULAR IDENTIFICATION OF ANDROGEN RECEPTOR GENE(S) IN *SCHISTOSOMA MANSONI*. Fantappie MF*, Sluder AE, Colley DG, and Secor WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; and Department of Zoology, University of Georgia, Athens, GA.

6:45 88 CLONING AND CHARACTERIZATION OF A CALPONIN HOMOLOGUE FROM *SCHISTOSOMA MANSONI*. Karim AM*, Shalaby KA, and LoVerde PT. Department of Biochemistry, Ain Shams University, Cairo, Egypt; and Department of Microbiology, State University of New York, Buffalo, NY.

7:00 89 EXPRESSION AND CHARACTERIZATION OF GLUTATHIONE PEROXIDASE ACTIVITY IN *SCHISTOSOMA MANSONI*. Mei H, Thakur AN, and LoVerde PT*. Department of Microbiology, State University of New York, Buffalo, NY.

7:15 90 TREMATODES DIFFER IN THEIR SUSCEPTIBILITY TO FREE RADICAL KILLING *IN VITRO*: COMPARISON OF LARVAE OF *SCHISTOSOMA MANSONI* AND *FASCIOLA HEPATICA*. Piedrafita DM*, Brindley PJ, Dalton JP, Spithill TW, Sandeman M, Wood P, and Parsons JC. Victorian Institute of Animal Science, Victoria, Australia; Queensland Institute of Medical Research, Queensland, Australia; Dublin City University, Dublin, Ireland; La Trobe University, Victoria, Australia; and CSIRO Division of Animal Health, Victoria, Australia.

7:30 Coffee Break

8:00 91 EXPRESSION OF LEWIS X BY SCHISTOSOMES AND AUTOIMMUNITY TO THIS DETERMINANT IN SCHISTOSOMIASIS. Nyame AK*, Pilcher JB, Tsang VC, and Cummings RD. Oklahoma University Health Sciences Center, Department Biochemistry & Molecular Biology, Oklahoma City, OK; and Centers for Disease Control and Prevention, Division of Parasitic Disisease Immunology Branch, Atlanta, GA.

8:15 92 IL-12 BOOSTS CELL-MEDIATED AND HUMORAL IMMUNE RESPONSES IN MICE VACCINATED WITH ATTENUATED CERCARIAE OF *SCHISTOSOMA MANSONI*. Wynn TA*, Reynolds A, Fouad S, James S, Hieny S, Jankovic D, Strand M, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, MD.

8:30 93 IMMUNITY IN HUMAN URINARY SCHISTOSOMIASIS: ASSOCIATION OF CELLULAR RESPONSES WITH AGE AND INTENSITY OF INFECTION. King CL*, Malhotra I, Koech D, Wamachi A, Kioko J, Mungi P, and Ouma JH. Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH; and Kenyan Institute of Medical Research and Division Vector Borne Diseases, Nairobi, Kenya.

8:45 94 INTERLEUKIN-10 REGULATES *IN VIVO* T-CELL REACTIVITY IN HUMAN URINARY SCHISTOSOMIASIS. Malhotra IJ*, Medhat A, Nafeh M, Shata A, Helmy A, Khoudary J, and King CL. Division of Tropical Medicine, Case Western Reserve University; Departments of Medicine and Microbiology, Assiut Egypt.

SCIENTIFIC SESSION I: MALARIA: VACCINES

Saturday, November 18, 1995 Rio Grande West
6:00 - 9:45 PM
Chairs: G. Hui and R Gramzinski

6:00 95 MODELLING OF MULTIVALENT DNA VACCINES WHICH PROTECT AGAINST MALARIA IN THE *PLASMODIUM YOELII* SYSTEM. Doolan DL*, Hedstrom RC, Charoenvit Y, Rogers WO, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD.

6:15 96 PROTECTIVE IMMUNITY AFTER IMMUNIZATION WITH A PyCSP DNA VACCINE: OPTIMIZATION OF DOSAGE REGIMEN AND CHARACTERIZATION OF GENETIC RESTRICTION. Sedegah M*, Hedstrom RC, Margalith M, Hobart P, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Vical Incorporated, San Diego, CA.

6:30 97 DNA VACCINES AGAINST MALARIA BASED ON THE SSP2 ANTIGEN. Hedstrom RC*, Sedegah M, Wang H, Kaur M, Hobart P, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Vical Incorporated, San Diego, CA.

6:45 98 PROTECTIVE EFFICACY AGAINST SPOROZOITE CHALLENGE WITH A COMBINATION SPOROZOITE AND ERYTHROCYTIC STAGE MALARIA VACCINE. Charoenvit Y*, Wang R, Daly TM, Long CA, Corradin GP, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Department of Microbiology and Immunology, Hahnemann University, Philadelphia, PA; and Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland.

7:00 99 OPTIMIZATION OF IMMUNE RESPONSES TO A *PLASMODIUM* DNA VACCINE IN *AOTUS* MONKEYS. Gramzinski RA*, Maris DC, Obaldia N, Rossan R, Sedegah M, Wang B, Hobart P, Margalith M, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and ProMed/Gorgas Memorial Laboratory, Panama City, Panama; and Vical Incorporated, San Diego, CA.

7:15 100 MALARIA VACCINE TRIALS AGAINST PREERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM* IN CHIMPANZEES: 1) EFFECT ON PARASITEMIA ELICITED BY FOUR VACCINE CANDIDATES. Millet P*, Luty A, Dubreuil G, LeRoy E, Tartar A, Eling WM, Georges AJ, and Druilhe P. Centre International de Recherche Médicale de Franceville (CIRMF), Franceville, Gabon; Institut Pasteur, Roux, Paris, France; and University Hospital Nijmegen, Department of Parasitology, Nijmegen, The Netherlands.

7:30 Coffee Break

8:00 101 COMPARTMENTALIZED T LYMPHOCYTE RESPONSES IN *MACACA MULATTA* TO THE MULTIANTIGEN MALARIA VACCINE CANDIDATE NYVAC-PF7. Stewart A*, Tongtawe P, Ngampochjana M, Lanar D, Tine JA, Krzych U, Ballou WR, and Heppner DG. Department of Immunology and Parasitology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences,

Bangkok, Thailand; Virogenetics Corporation, Troy, NY; and Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

8:15 102 VACCINATION WITH LIVE NYVAC-PF7 CAN PRIME THE IMMUNE SYSTEM TO DEVELOP AN AMNESTIC RESPONSE TO A BOOST WITH ADJUVENTED PROTEIN. Lanar DE*, Tine JA, Wellde BT, Kaslow DC, Sadoff JC, Paoletti E, and Ballou WR. Department Immunology, WRAIR, Washington DC; Virogenetics Corp, Troy, NY; and NIAID, NIH, Bethesda, MD.

8:30 103 INDUCTION OF MULTI-SPECIES IMMUNITY TO RODENT MALARIA USING MULTPLE EPITOPE TREES. Reed RC*, Lousis-Wileman V, Fang HS, Jue D, Wohlhueter R, Hunter RL, Lal AA. Department of Pathology, Emory University; and Biotechnology Core Facility Branch and Immunology Branch, Division of Parasitic Diseases, CDC, Atlanta, GA.

8:45 104 IMMUNOGENICITY OF DI-EPITOPE TREES, SYNTHETIC PEPTIDE VACCINES CONTAINING REPEATS OF CIRCUMSPOROZOITE PROTEIN OF TWO DIFFERENT PLASMODIAL SPECIES. Lal A*, Saekhou AM, Fang S, Jue D, Wohlhueter R, and Udhayakumar V. Division of Parasitic Diseases, Scientific Resourse Program, Biotechnology Core Facility, NCID, CDC, Atlanta, GA.

9:00 105 $CD4^+$ T CELL DEPENDENT STERILE PROTECTION AGAINST MALARIA AFTER VACCINATION WITH PySSP2 PEPTIDE. Wang R*, Charoenvit Y, Corradin GP, De La Vega P, Franke ED, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland.

9:15 106 DIFFERENTIAL GENETIC REGULATION OF PROTECTIVE IMMUNE RESPONSES INDUCED BY RECOMBINANT GST-$MSPI_{19}$ OF *PLASMODIUM YOELII* IN CONGENIC MICE. Tian JH*, Miller LH, Berzofsky JA, and Kumar S. Laboratory of Parasitic Diseases, National Institute of Allergy & infectious Diseases; and Molecular Vaccine Section, NIH, National Cancer Institute, Bethsda, MD.

9:30 107 VACCINATION WITH P30P2-$MSP1_{19}$ BUT NOT $MSP1_{19}$ OR EVE-$MSP1_{19}$ ELICITS PROTECTIVE IMMUNITY IN *AOTUS NANCYMAI* CHALLENGED WITH FVO *PLASMODIUM FALCIPARUM*. Kumar S*, Wellde B, Miller LH, Ballou R, Hall T, and Kaslow DC. Malaria Vaccine Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD; and Division of Immunology, WRAIR, Washington, DC.

NIH WORKSHOP ON GRANTS AND GRANTSMANSHIP

Sunday, November 19, 1995 Nueces/Frio
8:00 - 9:00 AM
Chair: S. James

This workshop will describe NIH procedures for grant review and funding, provide hints for writing successful applications, and allow time for specific questions from the audience. NIH staff will review the current status of funding for tropical disease research.

SYMPOSIUM: DESIGN OF ANTIMALARIAL DRUGS

Sunday, November 19, 1995 Regency Ballroom Center
8:30 - 11:30 AM
Chair: L. Miller

8:30 HEME POLYMERIZATION AND CHLOROQUINE. Ridley R.

9:10 SHORTENING THE DIAMINOALKANE SIDE CHAIN CIRCUMVENTS CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM*. Krogstad DJ.

9:45 THE *PLASMODIUM* DIGESTIVE VACUOLE: METABOLIC HEADQUARTERS AND CHOICE DRUG TARGET. Goldberg D.

10:15 Coffee Break.

10:30 EXPORT OF GOLGI AND MEMBRANE TRAFFICKING FROM THE PARASITE TO THE RED CELL. Haldar K.

11:00 COMBINATORIAL CHEMISTRY: THE FUTURE OF DRUG DISCOVERY. Howard R.

SYMPOSIUM:
HOST DEFENCE AGAINST MUCOSAL PATHOGENS
Supported by OraVax Inc.

Sunday, November 19, 1995 Regency Ballroom West
8:00 - 11:30 AM
Chair: K. Chadee and T. Monath

8:00 REGULATION OF THE MUCOSAL IMMUNE RESPONSE. Ernst P. University of Texas, Galveston, TX.

8:40 MUCINS AS A FIRST LINE OF HOST DEFENCE AGAINST PATHOGENS. Chadee K. McGill University, Montreal, Canada.

9:20 IMMUNOMODULATION OF MUCOSAL EPITHELIAL CELL FUNCTIONS. Perdue M. McMaster University, Hamilton, Canada.

10:00 Coffee Break.

10:20 NEUROMODULATION OF MUCOSAL INFLAMMATORY RESPONSES. Befus D. University of Alberta, Edmonton, Canada.

11:00 STRATEGIES FOR MUCOSAL IMMUNIZATION AGAINST VIRUSES AND BACTERIA. McGhee T. University of Alabama, Birmingham, AL.

SCIENTIFIC SESSION J: FILARIASIS

Sunday, November 19, 1995 Pecan
8:30 - 12:00 AM
Chairs: T.V. Rajan and Amy Klion

8:30 108 *BRUGIA MALAYI* MICROFILARIAE UTILIZE A B-7-LIKE PATHWAY TO PROVIDE COSTIMULATION FOR HUMAN T LYMPHOCYTES. Weller PF*, Liu LX, and Kim J. Beth Israel Hospital, Harvard Medical School, Boston, MA.

8:45 109 DIFFERENTIAL REGULATION OF ANTIGEN-SPECIFIC IGG4 AND IGE BY THE RECOMBINANT FILARIAL PROTEINS OV27 AND OVD5B. Garraud O, Perler FB, Bradley JE, and Nutman TB*. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; New England Biolabs Inc., Beverly, MA; and Imperial College of Science Technology and Medicine, London, England.

9:00 110 LARVAL AND ADULT FILARIAL ANTIGENS STIMULATE DIFFERENT CYTOKINE RESPONSES IN "ENDEMIC NORMAL" AND MICROFILAREMIC INDIVIDUALS FROM A HAITIAN POPULATION. Dimock KA*, Lammie PJ, and Eberhard ML. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

9:15 111 CHANGES IN Vß RECEPTOR USAGE ARE LINKED TO *IN UTERO* EXPOSURE TO *WUCHERERIA BANCROFTI* INFECTION. Steel C*, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and Division of Control of Tropical Diseases (CTD), World Health Organization, Geneva, Switzerland.

9:30 112 *IN UTERO* EXPOSURE TO *ONCHOCERCA VOLVULUS* PREDISPOSES TO HIGHER LEVELS OF INFECTION AND ALTERS SUBSEQUENT CELLULAR IMMUNE RESPONSES. Elson LH*, Days A, Calvopina M, Parredes W, Araujo E, Guderian RH, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD; Investigaciones Clinicas, Hospital Vozandes, Quito, Ecuador.

8:45 113 T-CELL MIGRATION THROUGH ENDOTHELIAL CELL MONOLAYERS IS INCREASED IN INDIVIDUALS WITH SYMPTOMATIC BANCROFTIAN FILARIASIS. Plier DA*, Maia e Silva MC, Maciel A, de Almeida AB, and Freedman DO. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil.

10:00 Coffee Break

10:30 114 ROLE OF NITRIC OXIDE (NO) IN HOST DEFENCE AGAINST THE HUMAN FILARIAL PARASITE, *BRUGIA MALAYI*. Rajan TV*, Keefer L, Yates J, Schultz LD, and Porte P. Department of Pathology, University of Connecticut Health Center, CT; National Cancer Institute, Frederick, MD; Department of Biology, Oakland University, Rochester, MI; and Jackson Laboratory, Bar Harbor, ME.

10:45 115 CHARACTERIZATION OF MTA/SAH NUCLEOSIDASE AND MTR KINASE FROM ENTERIC BACTERIA: POTENTIAL TARGETS FOR CHEMOTHERAPEUTIC INTERVENTION. Cornell KA*, and Riscoe MK. Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR; and Medical Research Service, Veterans' Affairs Medical Center, Portland, OR.

11:00 116 CYTOKINE GENE EXPRESSION IN BLOOD, LYMPH NODES, AND SPLEEN OF JIRDS DURING A PRIMARY INFECTION OF *BRUGIA PAHANGI*. Mai Z*, Horohov DW, and Klei TR. Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton, Rouge, LA.

11:15 117 AN ORAL *SALMONELLA* VACCINE IN MICE INDUCED A TH1-LIKE RESPONSE TO *ONCHOCERCA VOLVULUS* GST BUT NOT TO THE OV103 SURFACE-ASSOCIATED PROTEIN. Catmull J*, Wilson ME, Kirchhoff LV, and Donelson JE. Howard Hughes Medical Institute, Departments of Biochemistry, Microbiology and Internal Medicine, University of Iowa; and Department of Veterans Affairs Medical Center, Iowa City, IA.

11:30 118 THE ROLE OF IL-4 IN DEVELOPMENT OF T HELPER CELL RESPONSES ASSOCIATED WITH PULMONARY EOSINOPHILIA AND RESISTANCE TO MICROFILARIAE IN MICE. Pearlman E*, Hazlett FE, Lizotte MR, Williams SA, and Kazura JW. Case Western Reserve University, Cleveland OH; and Smith College, Northampton, MA.

11:45 119 IgE RESPONSIVENESS TO A RECOMBINANT FILARIAL ANTIGEN (WB1.2) CORRELATES WITH TPE PATIENTS WITH HLA-DQB1*0301 IN THEIR DQB1 GENOTYPES. Zimmerman PA*, Wang J, Kubofcik J, Phadke PM, Raghavan NK, Mollis SN, Kumaraswami V, Vijayan V, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Tuberculosis Research Center, Madras, India.

SCIENTIFIC SESSION K: KINETOPLASTS - MOLECULAR BIOLOGY AND IMMUNOLOGY

Sunday, November 19, 1995 Rio Grande E
8:00 - 12:15 AM
Chairs: P. Melby and M.F. Lima

8:00 120 BOTH IL-4 AND IFN-γ mRNA ARE PRESENT IN THE INITIAL LYMPH NODE RESPONSES IN HUMAN CUTANEOUS LEISHMANIASIS. Barral A*, Bonfim G, Nascimento C, Barral MN, and Carvalho EM. Servico de Imunologia, Univ. Federal da Bahia, Bahia, Brazil.

8:15 121 ANALYSIS OF *IN SITU* CYTOKINE EXPRESSION IN LESIONS OF ZOONOTIC CUTANEOUS LEISHMANIASIS. Louzir H*, Ben Salah A, Marrakchi H, Zaatour A, Ftaiti A, Dellagi K, and Melby PC. Pasteur Institute of Tunis, Tunis, Tunisia; and The University of Texas Health Science Center, San Antonio, TX.

8:30 122 CYTOKINE EXPRESSION IN LEISHMANIASIS PATIENTS TREATED WITH SODIUM STIBOGLUCONATE (PENTOSTAM). Endy TP*, King AD, Macarthy PO, Magill AJ, Aronson NE, and Oster CN. Infectious Disease Service, Walter Reed Army Medical Center, Washington DC; Department of Immunology, Walter Reed Army Institute of Research, Washington DC; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington DC.

8:45 123 IL-12 UP REGULATES CYTOTOXICITY IN HUMAN LEISHMANIASIS. Barral MN*, Brodskyn A, Barral A, and Carvalho EM. Servico de Imunologia, Univ. Federal da Bahia, Bahia, Brazil.

9:00 124 PARASITE DRIVEN REGULATION OF ENDOGENOUS PARASITE ANTIGEN PRESENTATION BY MACROPHAGES INFECTED WITH *LEISHMANIA AMAZONENSIS*. Kima PE*, Ruddle NR, and McMahon-Pratt D. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT.

9:15 125 NEUTRALIZATION OF *TRYPANOSOMA CRUZI* INFECTION BY FAB' FRAGMENTS OF A MONOCLONAL ANTIBODY TO A MEMBER OF THE FAMILY 2 OF TRANS-SIALIDASES. Villalta F*, Burns, Jr. JM, Chaudhuri G, Smith C, Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

9:30 126 CALCIUM RELEASE FROM ACIDOCALCISOMES OF *TRYPANOSOMA BRUCEI*. Vercesi AE*, Catisti R, and Docampo R. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; and Departamento de Bioquimica, Universidade Estadual de Campinas, Campinas, Brazil.

9:45 127 SIGNAL TRANSDUCTION PATHWAYS IN *TRYPANOSOMA CRUZI* INDUCED BY EPIDERMAL GROWTH FACTOR. Lima MF*, Moura P, Ager EP, and Williams KL. Department of Microbiology, Meharry Medical College, Nashville, TN.

10:00 Coffee Break

10:30 128 CLONING OF FeSOD FROM *LEISHMANIA CHAGASI* AND *TRYPANOSOMA CRUZI*: ROLE IN PROTECTION AGAINST OXIDATIVE STRESS. Ismail SO*, Bhatia A, Paramchuk W, Omara-Opyene LA, Gedamu L. Dept. of Biological Science, University of Calgary, Canada.

10:45 129 STABLE INTEGRATIVE TRANSFORMATION AND AUTONOMOUSLY REPLICATING PLASMIDS IN *TRYPANOSOMA CRUZI*. Dos Santos WG*, Buck GA. Department of Biochemistry and Immunology, ICB, UFMG, Belo Horizonte, MG, Brazil; and Medical College of Virginia, Virginia Commonwealth University, Richmond,VA.

11:00 130 GENE DISRUPTION OF THE ELONGATION FACTOR 1 α LOCUS IN *TRYPANOSOMA BRUCEI* BY HOMOLOGOUS RECOMBINATION: IMPLICATIONS FOR THERAPY DESIGN. Ridgley EL*, Kaur KJ, and Ruben L. Department of Biological Sciences, Southern Methodist University, Dallas, TX; and Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX.

11:15 131 SEQUENCE SPECIFIC RNA ENDONUCLEASE ACTIVITY OF *LEISHMANIA* RNA VIRUS CAPSIDS. MacBeth K*, and Patterson JL. Division of Infectious Diseases and Department of Microbiology & Molecular Genetics, Harvard Medical School.

11:30 132 CHARACTERIZATION OF A MULTI-DRUG RESISTANCE GENE (*lemdr1*) IN *LEISHMANIA ENRIETTII*. Chow LM*, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:45 133 EXPRESSION OF A *LEISHMANIA CHAGASI* ANTIGEN IN LISTERIA MONOCYTOGENES FOR USE IN VACCINE DEVELOPMENT. Wilson ME*, Clark MA, Portnoy DA, and Jones S. Department of Internal Medicine and Microbiology, University of Iowa and VA Medical Center, Iowa City, IA; and Department of Microbiology, University of Pennsylvania, Philadelphia, PA.

12:00 134 MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION IN *ESCHERICHIA COLI* OF THREE DISTINCT CYSTEINE PROTEASE cDNAS FROM *LEISHMANIA DONOVANI CHAGASI*. Omara-Opyene AL*, Ismail SO, and Gedamu L. Department of Biological Science, University of Calgary, Canada.

SCIENTIFIC SESSION L: MALARIA VACCINES AND IMMUNOLOGY

Sunday, November 19, 1995 Rio Grande C
8:00 - 12:15 AM
Chairs: Y.P. Shi and D.C. Kaslow

8:00 135 IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* $(T1B)_4$ MAP VACCINE IN *AOTUS* MONKEYS. Moreno CA*, Rodriguez RJ, Oliveira GA, Calvo-Calle JM, Nussenzweig RS, and Nardin EH. Instituto de Inmunologia, Hospital San Juan De Dios, Santa Fe de Bogota, Colombia, South America; and Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

8:15 136 GENETIC RESTRICTION OF PRIMATE AND MURINE IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM* MAP VACCINE. Calvo-Calle JM*, Hurley CK, Clavijo P, and Nardin EH. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY; and Department of Microbiology and Immunology, Georgetown University, Washington, DC.

8:30 137 PROTECTIVE EFFICACY OF A LIPOSOME ENCAPSULATED R32NS1 *PLASMODIUM FALCIPARUM* MALARIA VACCINE IN A HUMAN CHALLENGE MODEL. Magill AJ*, Fries BT, Gordon DM, Wellde BT, Owens R, Krzych U, Schnieder IP, Wirtz RA, Kester K, Ockenhouse CF, Stoute JA, and Ballou WR. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC; Department of Entomology, Walter Reed Army Institute of Research, Washington DC; and Center for Immunization Research, Johns Hopkins University, Baltimore MD.

8:45 138 HUMAN CTL RESPONSES TO THE *PLASMODIUM FALCIPARUM* SPOROZOITE SURFACE PROTEIN 2: IDENTIFICATION OF EPITOPES RESTRICTED BY SIX HLA CLASS I ALLELES. Wizel B*, Houghten R, Ballou WR, Paoletti E, Setti A, Coligan JE, Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Department of Molecular Microbiology & Immunol, The Johns Hopkins University, Baltimore, MD; Torrey Pines Institute for Molecular Studies, San Diego, CA; Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; Virogenetics Corporation, Troy, NY; Cytel Corporation, San Diego, CA; Laboratory of Molecular Structure, National Institute of Allergy and Infectious Diseases, Rockville, MD; Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Malaria Program, Naval Medical Research Institute, Bethesda, MD.

9:00 139 KILLING OF *PLASMODIUM YOELII* EXOERYTHROCYTIC STAGES, *IN VITRO*, BY NYLS3 MONOCLONAL ANTIBODY REQUIRES A HEAT INACTIVATABLE SERUM COMPONENT. Sacci Jr. JB*, Porrozzi R, de la Vega P, Charoenvit Y, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Rockville, MD; and Department de Ultraestrutura, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil.

9:15 140 INHIBITORY MONOCLONAL ANTIBODIES TARGET A CONFORMATIONAL EPITOPE WITHIN THE 83 kDa *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN (PF83/AMA1). Narum DL*, van der Wel A, Dubbeld M, and Thomas AW. Division of Parasitology, National Institute for Medical Research/MRC, Mill Hill, London, United Kingdom; and Laboratory of Parasitology, Biomedical Primate Research Centre, Rijswijk, The Netherlands.

9:30 141 IMMUNOGENIC CHARACTERISTICS OF THE *PLASMODIUM FALCIPARUM* RHOP-3 PROTEIN DEFINED BY A C-TERMINAL RECOMBINANT. Yang J *, Blanton RE, King CL, and Sam-Yellowe TY. Department of Biology, Cleveland State University, Cleveland, OH; Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH.

9:45 142 ANTIBODY RESPONSES TO THE MEROZOITE SURFACE PROTEIN, MSP1 IN NAIVE *AOTUS* MONKEYS INFECTED WITH FALCIPARUM MALARIAS. Hui GS*, Hasiro C, Nikaido C, Kaslow DC, and Collins WE. Department of Tropical Medicine, University of Hawaii, Honolulu, HI; Laboratory of Malaria Research, National Institutes of Health, Bethesda, MD; and Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

10:00 Coffee Break

10:30 143 NATURAL IMMUNE RESPONSES TO RECOMBINANT C-TERMINAL 19 KDA ANTIGEN OF *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 (MSP-1) IN IMMUNE ADULTS FROM KENYA. Shi YP*, Udhayakumar V, Sayed U, Anyona D, Roberts JM, Hightower AW, Oloo A, Hawley WA, Kaslow DC, Nahlen BL, and Lal AA. Division of Parasitic Disease, NCID, Centers for Disease Control and Prevention, Atlanta, GA.; Vector Biology andControl Research Center, Kenya Medical Research Institute, Kisumu, Kenya; and Laboratory of Malaria Research, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

10:45 144 IMMUNE RESPONSES TO CONSERVED ASEXUAL-STAGE T CELL EPITOPES IN CHILDREN AND ADULTS RESIDING IN ETOA, A VILLAGE WITH HOLOENDEMIC MALARIA IN CAMEROON. Quakyi IA*, Leke R, Befidi R, Bomba-Nkolo D, Manga L, Njeungue E, Fogako J, Eno A, Sama G, Djokam R, Achidi E, and Ngu J. Department of Biology, Georgetown University, Washington, DC; and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon.

11:00 145 INDUCTION OF TRANSMISSION BLOCKADE IN MICE INFECTED WITH *PLASMODIUM BERGHEI*. Beetsma AL*, van de Wiel T, Sauerwein RW, and Eling W. Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands.

11:15 146 STUDIES OF NON-CONVENTIONAL FORMULATIONS AND VACCINE DELIVERY SYSTEMS TO ELICIT ANTI-Pfs25 TRANSMISSION-BLOCKING ANTIBODIES IN RODENTS AND PRIMATES. Kaslow DC*, Price V, Keister DB, Gupta S, Min-Ying, Kumar S, Cleland J, Tine J, Lanar D, Wellde B, Shiloach J, and Paoletti E, and Muratova O. Malaria Vaccine Section, LPD, NIAID, NIH, Bethesda, MD; Immunex Corp., Seattle, WA; NIH Clinical Center Pharmacy, NIH, Bethesda, MD; Biotechnology Unit, NIDDK, NIH, Bethesda, MD; Virogenetics Co., NY; Pharmaceutical R & D, Genentech Inc., South San Francisco, CA; Division of Immunology, WRAIR, Washington, DC.

11:30 147 CELLULAR AND HUMORAL RECOGNITION OF CANDIDATE MALARIA TRANSMISSION-BLOCKING VACCINE ANTIGENS IN NATURALLY EXPOSED PERSONS. Ohas EA*, Kaslow DC, and Duffy PE. US Army Medical Research Unit- Kenya/Kenya Medical Research Institute,Kisumu, Kenya; and Laboratory of Malaria Research, National Institutes ofHealth, Bethesda, MD.

11:45 148 ANALYSES OF PARASITOLOGICAL AND ENTOMOLOGICAL PARAMETERS IN BANACOUMANA, MALI, A POTENTIAL SITE FOR TESTING MALARIA TRANSMISSION-BLOCKING VACCINES. Doumbo O*, Toure YT, Diallo M, Sakai D, Bagayogo M, Kouriba B, Muratova O, Keister DB, and Kaslow DC. Department of Epidemiology and Parasitic Diseases, Mali National School of Medicine and Pharmacy, Bamako, Mali; and Malaria Vaccine Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.

12:00 149 MALARIA INCIDENCE MEASUREMENT AND VACCINE TEST SITE DEVELOPMENT IN VIETNAM. Doan H*, Richie TL, Nguyen DT, Tran TU, Luc NT, Church CJ, Le XH, Vu DC, Corwin AL, Le PT, Le DC, and Hoffman SL. Institute for Malariology, Parasitology and Entomology, Hanoi, Vietnam; U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; and Naval Medical Research Institute, Bethesda MD.

SCIENTIFIC SESSION M: FLAVIVIROLOGY

Sunday, November 19, 1995 Rio Grande West
8:00 - 12:15 AM
Chairs: C. Hoke and G. Clarke

8:00 150 THE 3' NONCODING REGION OF WILD-TYPE STRAINS OF YELLOW FEVER VIRUS HAVE SIGNIFICANT DIFFERENCES: GENETIC AND BIOLOGICAL IMPLICATIONS. Wang E, Tesh RB, Shope RE, Weaver SC, and Barrett AD*. Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX.

8:15 151 EFFECTIVENESS OF LIVE-ATTENUATED HAMSTER KIDNEY CELL CULTURE DERIVED JAPANESE ENCEPHALITIS VACCINE (SA_{14}-14-2). Hennessy S*, Zhengle L, Tsai TF, Strom BL, Caoming W, Huilian L, Xiangtai W, Bilker WB, Quimao L, Karabatsos N, and Halstead SB. Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA; West China University of Medical Sciences, Sichuan Province, People's Republic of China; Centers for Disease Control and Prevention, Ft. Collins, CO; and Health Sciences Division, The Rockefeller Foundation, New York, NY.

8:30 152 INTRANASAL CHALLENGE MODEL FOR TESTING JAPANESE ENCEPHALITIS VIRUS VACCINES IN RHESUS MACAQUES. Raengsakulrach B*, Nisalak A, Myint KS, Thirawuth V, Ngampochjana M, Young GD, Ferguson LM, Innis BL, and Vaughn DW. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Connaught Laboratories Inc., Swiftwater, PA; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

8:45 153 EVALUATION OF IMMUNOGENICITY OF NYVAC-JEV AND ALVAC-JEV ATTENUATED RECOMBINANT JAPANESE ENCEPHALITIS VIRUS - POXVIRUS VACCINES IN HUMANS. Kanesa-thasan N*, Smucny JJ, Konishi E, Kurane I, Shope R, Vaughn DW, Mason PW, Paoletti E, Pincus S, Marks DH, Ennis FA, and Hoke CH. Division of Communicable Diseases & Immunology, Walter Reed Army Institute of Research, Washington, DC; Yale Arbovirus Research Unit, Department of Epidemiology & Public Health, School of Medicine, New Haven CT; Division of Infectious Diseases & Immunology, Dept of Medicine, Universtiy of Massachusetts Medical Center, Worcester MA; Deptment of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Virogenetics, Inc., Troy NY; and Connaught Laboratories, Inc., Swiftwater PA.

9:00 154 THE DENGUE AND DENGUE HEMORRHAGIC FEVER EPIDEMIC IN PUERTO RICO, 1994-1995. Rigau-Perez JG*, Vorndam AV, and Clark GG. Dengue Branch, Centers for Disease Control and Prevention, San Juan, PR.

9:15 155 A STUDY OF THE SPATIAL AND TEMPORAL DISTRIBUTION OF DENGUE CASES DURING AN OUTBREAK IN PUERTO RICO (1991-1992) USING A GIS APPROACH. Morrison AC*, Santiago M, Reiter P, Rigau-Perez JG, Clark GG. Dengue Branch, Centers for Disease Control and Prevention, San Juan, PR; and Water Resources Division, Caribbean District, U.S. Geological Survey, Guaynabo, PR.

9:30 156 A GRAPHIC METHOD FOR DETECTING REGIONAL INCREASES IN DENGUE INCIDENCE IN PUERTO RICO, 1994-95. Millard PS*, Rigau-Perez JG, Deseda CC, and Clark GG. Epidemic Intelligence Service, Centers for Disease Control and Prevention, Atlanta, GA; Dengue Branch, Centers for Disease Control and Prevention, San Juan, PR; and Epidemiology Division, Puerto Rico Department of Health, San Juan, PR.

9:45 157 NATURAL VERTICAL TRANSMISSION OF DENGUE VIRUSES IN *AEDES AEGYPTI* (L.) IN FRENCH GUIANA. Fouque F*, Reynes JM, Carinci R, and Gaborit P. Laboratoire d'Entomologie médicale, Institut Pasteur de Guyane, Cayenne Cedex, French Guiana.

10:00 Coffee Break

10:30 158 EARLY DIAGNOSTIC INDICATORS OF DENGUE. Kalayanarooj S*, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachu-eke S, Kiatpolpoj S, Innis BL, Rothman AL, Nisalak A, and Ennis FA Bangkok Children's Hospital, Bangkok, Thailand; Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Division of Infectious Diseases and Immunology, University of Massachusetts Medical Center, Worcester, MA; Kamphaeng Phet Provincial Hospital, Kamphaeng Phet, Thailand; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

10:45 159 DENGUE IN THE EARLY FEBRILE PHASE: VIREMIA AND ANTIBODY RESPONSES. Vaughn DW*, Green S, Kalayanarooj S, Innis BL, Nimmanitya S, Suntayakorn S, Rothman AL, Ennis FA, and Nisalak A. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Division of Infectious Diseases and Immunology, University of Massachusetts Medical Center, Worcester, MA; Bangkok Children's Hospital, Bangkok, Thailand; Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC; Center for Disease Control, Ministry of Public Health, Nonthaburi, Thailand; and Kamphaeng Phet Provincial Hospital, Kamphaeng Phet Thailand.

11:00 160 PLASMA CYTOKINE LEVELS AND T CELL ACTIVATION MARKERS IN CHILDREN WITH ACUTE DENGUE. Green S*, Vaughn DV, Kalayanarooj S, Suntayakorn S, Nisalak A, Nimmanitya S, Hussem K, Innis BL, Kurane I, Rothman AL, and Ennis FA. Division of Infectious Diseases and Immunology, University of Massachusetts, Worcester, MA; Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Bangkok Children's Hospital, Bangkok, Thailand; Kamphaeng Phet Provincial Hospital, Kamphaeng Phet, Thailand; Center for Disease

Control, Ministry of Public Health, Nonthburi, Thailand; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

11:15 161 CYTOKINE PRODUCTION BY DENGUE-SPECIFIC MEMORY T LYMPHOCYTES. Gigstad JE*, Kurane I, Janus J, and Ennis FA. Division of Infectious Diseases and Immunology, University of Massachusetts, Worcester, MA.

11:30 162 INFECTIOUS DENGUE TYPE 2 VIRUS RNA MADE BY *IN VITRO* SYNTHESIS FROM A FULL LENGTH cDNA CLONE. Zhao B *, Warren R, Snellings N, Hoke CH, and Putnak JR. Departments of Virus and Bacterial Diseases, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

11:45 163 NUCLEIC ACID SEQUENCE POINT MUTATIONS ASSOCIATED WITH LIVE, ATTENUATED DENGUE-2 VIRUS VACCINES PREPARED IN PRIMARY DOG KIDNEY CELLS. Henchal EA*, Pedersen JC, Innis BL, Vaughn DW, Dubois DR, Eckels KH, and Hoke CH. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

12:00 164 PATHOGEN DERIVED RESISTANCE TO DENGUE-2 VIRUS IN MOSQUITO CELLS BY EXPRESSION OF CAPSID AND PREMEMBRANE CODING REGIONS OF THE VIRAL GENOME. Olson KE*, Higgs S, Gaines PJ, Beaty BJ, and Blair CB. Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

SCIENTIFIC SESSION N: ENTOMOLOGY - FIELD ECOLOGY AND POPULATION GENETICS

Sunday, November 19, 1995 Blanco - Llano
8:00 - 12:00 AM
Chairs: G. Lanzaro and D. Fuchs

8:00 165 PUPAL SURVEY: AN EPIDEMIOLOGICALLY SIGNIFICANT SURVEILLANCE METHOD FOR *AEDES AEGYPTI*. AN EXAMPLE USING DATA FROM TRINIDAD. Focks DA*, and Chadee DD. Medical and Veterinary Entomology Research Laboratory, U. S. Department of Agriculture, Gainesville, FL; and Insect Vector Control Division, Trinidad and Tobago Ministry of Health, St. Joseph, Trinidad, WI.

8:15 166 EVALUATION OF A SIMPLE BED NET TRAP FOR SAMPLING AFRICAN MALARIA VECTORS. Ofulla AV*, Hawley WA, Beach RF, Roberts JM, and Hightower AW. Kenya Medical Research Institute, Nairobi, Kenya; Division of Parasitic Diseases, NCID, CDC, Nairobi, Kenya; and Division of Parasitic Diseases, NCID, CDC, Atlanta, GA.

8:30 167 ABUNDANCE, BITING FREQUENCY, AND AGE STRUCTURE OF *LUTZOMYIA CRUCIATA* (DIPTERA: PSYCHODIDAE) IN A MEXICAN FOCUS OF LOCALIZED CUTANEOUS LEISHMANIASIS. Rebollar-Tellez EA , Fernandez-Salas I, Van Wynsberghe NR, and Andrade-Narvaez FJ. Department of Immunology, Center of Regional Research (CIR), University of Yucatan (UADY), Merida, Yucatan, Mexico; Fac. of Biological Science, Medical Entomology Lab., University of Nuevo Leon, San Nicolas de los Garza, NL, Mexico.

8:45 168 FITNESS COSTS ASSOCIATED WITH A STRAIN OF *AEDES AEGYPTI* REFRACTORY TO *PLASMODIUM GALLINACEUM*. Yan G, Severson DW, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

9:00 169 HYBRIDIZATION AMONG SPECIES OF THE SAND FLY GROUP RELATED TO *PHLEBOTOMUS PAPATASI* PARALLELS GENETIC PROFILES AND GEOGRAPHIC DISTRIBUTION. Munstermann LE*, Ghosh KN, Guzman H, Tesh RB, and Mukhopadhyay J. Yale School of Epidemiology and Public Health, Yale University, New Haven CT; and Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX.

9:15 170 MICROSATELLITE DNA VARIATION AND THE GENETIC STRUCTURE OF *ANOPHELES GAMBIAE* POPULATIONS IN MALI, WEST AFRICA. Lanzaro GC*, Zheng L, Toure YT, Petrarca V, Kafatos FC, and Vernick KD. Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX; European Molecular Biology Laboratory, Heidelberg, Germany; Malaria Research and

Training Center, National School of Medicine and Pharmacy, Bamako, Mali; Institute of Parasitology, University of Rome, Rome, Italy; and Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD

9:30 171 DEVELOPMENT OF MICROSATELLITE MARKERS FOR *ANOPHELES MACULATUS*, A MALARIA VECTOR IN THAILAND. Rongnoparut P*, Sirichotprakorn N, Yaicharoen S, Rattanarithikul R, and Linthicum KJ. Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Department of Parasitology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.

9:45 172 THE *ANOPHELES GAMBIAE* COMPLEX IN SENEGAL: A VERY HETEROGENEOUS TRANSMISSION OF MALARIA. Fontenille D*, Faye O, LeMasson JJ, Lochouarn L, Simard F, Diatta M, Konate L, and Trape JF. ORSTOM, Dakar, Senegal.

10:00 Coffee Break

10:30 173 IDENTIFICATION OF SURFACE MOLECULES OF MOSQUITO SALIVARY GLANDS WHICH MALARIA SPOROZOITES USE AS RECEPTORS FOR INVASION. Barreau C*, Touray M, Miller LH, and Vernick KD. Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD.

10:45 174 A *IN VITRO* SYSTEM FROM *AEDES AEGYPTI* TO STUDY MIDGUT CELL PATHWAYS INVOLVED DURING HOST CELL INVASION BY VIRUS AND PARASITE. Poupel O, Vazeille M, Shahabuddin M, Tardieux IC*. Institut Pasteur, Ecologie des Systemes Vectoriels, Paris, France; and Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD.

11:00 175 MICE IMMUNIZED BY BITES OF *ANOPHELES STEPHENSI* DEVELOP ANTIBODIES THAT INHIBIT ACTIVITY OF MOSQUITO SALIVARY GLAND APYRASE. Mathews GV, Sidjanski S, and Vanderberg JP*. Department of Medical & Molecular Parasitology, New York University School of Medicine, New York, NY.

11:15 176 TARGETED MAPPING OF LOCI LINKED WITH GENES AFFECTING DISEASE TRANSMISSION BY MOSQUITOES. Severson DW*, and Kassner VA. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

11:30 177 SEX-LINKAGE OF PERMETHRIN RESISTANCE IN A FIELD STRAIN OF *AEDES AEGYPTI*. Mebrahtu YB*, Taylor MF, and Norem J. University of Arizona, Department of Entomology, Tucson, AZ.

11:45 178 DO MOSQUITOES ORIENT IN MAGNETIC FIELDS? Strickman D*, Weissman M, Novak R, and Estrada-Franco J. Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, DC; Department of Physics, University of Illinois, Urbana, IL; Illinois Natural History Survey, Champaign, IL; and Department of Entomology, University of Maryland, College Park, MD.

SCIENTIFIC SESSION O: SCHISTOSOMIASIS - IMMUNOPATHOLOGY

Sunday, November 19, 1995 Nueces / Frio
9:00 - 12:15 AM
Chairs: D. Wyler and E. Secor

9:00 179 IMMUNE RESPONSES OF MICE DEFICIENT FOR 5-LIPOXYGENASE OR 12-LIPOXYGENASE DURING INFECTION WITH *SCHISTOSOMA MANSONI*. Secor WE*, and Funk CD. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Division of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

9:15 180 T CELL HYBRIDOMAS FROM HIGH AND LOW RESPONDER MOUSE STRAINS RECOGNIZE DIFFERENT SCHISTOSOMAL EGG ANTIGENS. Hernandez HJ*, Brodeur PH, and Stadecker MJ. Department of Pathology, Tufts University School of Medicine, Boston, MA.

9:30 181 A CLONED, 38 kDa *SCHISTOSOMA MANSONI* EGG PEPTIDE INDUCES TH1 TYPE LYMPHOCYTE RESPONSES. Cai Y, Langley JG, Smith DI, and Boros DL*. Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI; and Division of Hematology/Oncology Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI.

9:45 182 EXPRESSION OF TNFα AND INDUCTION OF SCHISTOSOME EGG GRANULOMAS IS DEPENDENT ON TH PRESENCE OF ADULT PARASITES. Leptak CL*, and McKerrow JH. Department of Microbiology and Immunology, University of California, San Francisco, CA; and Department of Anatomic Pathology, Veterans Affairs Medical Center, San Francisco, CA.

10:00 Coffee Break

10:30 183 ASSOCIATION OF HYPERSPLENOMEGALY SYNDROME AND HIGH TNF-A LEVELS IN LIVER HOMOGENATES OF MICE WITH CHRONIC SCHISTOSOMIASIS. Adewusi OI*, Bosshardt SC, Colley DG, and Secor WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

10:45 184 THE IL-10/Fc FUSION PROTEIN INHIBITS EGG GRANULOMA FORMATION IN SCHISTOSOMIASIS. Flores Villanueva PO*, Zheng XX, Strom TB, and Stadecker MJ. Department of Pathology, Tufts University School of Medicine, Boston, MA; and Department of Medicine, Harvard Medical School, Boston MA.

11:00 185 IN SITU ANALYSIS OF Th CELL-REGULATORY B7 MOLECULE EXPRESSION IN *SCHISTOSOMA MANSONI* EGG GRANULOMAS. Rathore A*, Ricklan DE, Flores Villanueva PO, and Stadecker MJ. Department of Pathology, Tufts University School of Medicine, Boston, MA.

11:30 186 UPREGULATION OF B7.2 EXPRESSION ON B CELLS CORRELATES WITH THE DEVELOPMENT OF Th2 PATHWAY IN MURINE EXPERIMENTAL SCHISTOSOMIASIS. Cayabyab M*, and Harn, Jr. DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:45 187 FIBROSIN, A NOVEL FIBROGENIC CYTOKINE, IS ELEVATED IN BLOOD OF MICE INFECTED WITH *SCHISTOSOMA MANSONI*. Wyler DJ*, and Talebian P. New England Medical Center and Tufts University School of Medicine, Boston, MA.

12:00 188 CROSS-SECTIONAL ANALYSIS OF CELL POPULATIONS IN PATIENTS WITH INFECTION BY THE *SCHISTOSOMA MANSONI*. Martins-Filho OA, Cunha-Melo JR, Silveira AM, Prata A, Alves-Oliveira L, Lambertucci JR, Gazzinelli G, Correa-Oliveira R*. Laboratory of Immunology Centro de Pesquisas Rene Rachou-FIOCRUZ, Belo Horizonte, M.G. Brasil; Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte. M.G. Brasil; Faculdade de Medicina do Triangulo Mineiro, Uberaba, Minas Gerais, Brasil; Universidade do Vale do Rio Doce, Governador Valadares, M.G. Brasil; and Faculade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, M.G. Brasil.

ASTMH PLENARY SESSION #2

Sunday, November 19, 1995 Regency Ballroom Center/West
1:30 - 5:30 PM
Chair: C. Long

1:30 Presidential Address: Long C.

2:30 Research Funding Update: NIAID. James S.

2:45 Research Funding Update: CDC, USAID, DOD. Zimmerman T.

3:00 Awards Ceremony.

3:30 Coffee Break

4:00 Commemorative Fund Lecture. CONTROL AND ERADICATION OF INFECTIOUS DISEASES WITH VACCINES. de Quadros CA. Pan American Health Organization (introduced by D. Burke).

5:00 Business Meeting.

SYMPOSIUM: NEW DEVELOPMENTS IN HEPATITIS A DISEASE PREVENTION

Sunday, November 19, 1995 Rio Grande
6:00 - 10:00 PM
Chair: E. Jong

DISCUSSION: EPIDEMIOLOGY OF MALARIA

Sunday, November 19, 1995 Pecan
8:00 - 10:00 PM
Chair: L. Miller

Epidemiological data which are based on studies that incorporate a denominator are beginning to shed light on both mortality and morbidity due to malaria. Such studies which derive from the examination of communities rather than hospital-based populations should enable workers to evaluate various end points of the disease. Should we only use mortality as an outcome or can various other malariometric indices be used? This will be of value in planning interventions whether vaccines, chemotherapy or vector control.

All interested individuals, including those who have data gathered from studies designed to incorporate a denominator, whether published or not, are encouraged to bring the material and participate in an open discussion under the chairmanship of Lou Miller.

POSTER SESSION I

Monday, November 20, 1995 Regency Ballroom East/Center/West
7:30 - 9:00 AM

CLINICAL MEDICINE

189 PREDICTIVE VALUE OF ABSOLUTE LYMPHOCYTE COUNT IN IDENTIFYING INPATIENTS WITH AIDS IN AN URBAN TEACHING HOSPITAL. Fisher EJ*. Infectious Diseases Division, Medical College of Virginia, Richmond VA.

190 CURRENT CAUSES OF COMMUNITY-ACQUIRED SEPTICEMIA IN TANZANIA: ROLE OF HIV-1 AND TUBERCULOSIS. Archibald LK, den Dulk MO, Pallangyo KP, and Reller LB. Department of Medicine, Muhimbili Medical Centre, Dar es Salaam, Tanzania; and Clinical Microbiology Laboratory, Duke University Medical Center, Durham, NC.

191 MALNUTRITION AND TUBERCULOSIS IN A NATIONALLY REPRESENTATIVE COHORT OF ADULTS IN THE UNITED STATES, 1971-1987. Cegielski JP*. Center for Pulmonary Infectious Disease Control, University of Texas Health Center, Tyler, TX.

192 GENERAL PRACTICE AND TROPICAL MEDICINE IN RURAL COSTA RICA. Adams DP*. Department of Family Medicine, Ohio State University College of Medicine, Columbus, OH.

193 EPIDEMIOLOGY, PREVENTIVE SERVICES, AND ILLNESSES OF INTERNATIONAL TRAVELERS. Scoville SA*, Bryan JP, and Tribble D. Department of Preventive Medicine and Biometics, Uniformed Services University of the Health Sciences, Bethesda; and Division of Infectious Diseases, National Naval Medical Center, Bethesda, MD.

194 PREVENTIVE MEASURES AND HEALTH PRECAUTIONS EMPLOYED BY TRAVEL MEDICINE ADVISORS BEFORE AND DURING TRAVEL TO MEXICO FOR AN INTERNATIONAL CONFERENCE. Caputi RA*, Cetron MS, Keystone JS, and Kozarsky PE. Emory University School of Medicine, Atlanta, GA; Center for Disease Control and Prevention, Atlanta, GA; and University of Toronto, Toronto, Canada.

195 SATISFACTION OF TRAVELERS USING A TRAVEL MEDICINE SERVICE: A PRELIMINARY REPORT. Shepherd SM, and Shoff WH*. Travel Medicine Service, Department of Emergency Medicine,University of Pennsylvania Medical Center, Philadelphia, PA.

196 FOCAL LESIONS OF THE SPLEEN IN PATIENTS WITH FEVER OF UNKNOWN ORIGIN: SONOGRAPHIC PATTERNS AND DIAGNOSIS. Farid Z*, Kamal M, Anis E, Karam M, Mousa M, and Mateczun A. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and Abbassia Fever Hospital, Cairo, Egypt.

197 FEBRILE ILLNESS ASSOCIATED WITH HANDLING IMPORTED JERBOAS. Barnett BJ*, Rawlings JA, and Hendricks KA. Infectious Disease Epidemiology and Surveillance Division, Texas Department of Health, Austin, TX.

198 SEASONALITY OF NUTRITIONAL STATUS OF YOUNG CHILDREN IN A SHANTYTOWN NEAR LIMA PERU. Marin CM, Segura JL, Bern CL*, Freedman DF, Lescano AG, Benavente LE, Cordero LG, Clavijo L, and Gilman JB. Asociacion Benefica Proyectos en Informacion Salud Medecina y Agricultura, Lima, Peru; and Division of Nutrition, Centers for Disease Control and Prevention, Atlanta, GA.

199 FALSE CHARCOT LEYDEN AND FATTY ACIDS CRYSTALS : THE PINEAPPLE CRYSTALS. Ardoin FG*, Petithory JC, and Ash LR Contrìle de Qualité National en Parasitologie, Centre Hospitalier, Gonesse, France; and School of Public Health, U.C.L.A., Los Angeles, CA.

200 COMPARATIVE EFFICIENCY AND EFFECTIVENESS OF VARIOUS SEDIMENTATION METHODS FOR CONCENTRATING INTESTINAL PARASITES AND EGGS. Cover EC*, and Price DL. Natural Science Department, Manatee Community College, Bradenton, FL.

BACTERIOLOGY

201 SPATIAL ASPECTS OF THE DISTRIBUTION OF *MYCOBACTERIUM LEPRAE* INFECTIONS IN THE US GULF COAST. Truman RW*, and Hugh-Jones ME. Laboratory Research Branch, G.W.L. Hansen's Disease Center, Baton Rouge, LA; and WHO Collaborating Center, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

202 ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS ISOLATED DURING AN OUTBREAK OF HIV-INFECTED PATIENTS BY DNA FINGER-PRINTING. Diaz R *, Montoro E, González R, Echemendía M, and Valdivia JA. Pedro Kourí Institute, Ciudad de La Habana, Cuba..

203 SEQUENCE TYPING OF MYCOBACTERIUM AVIUM COMPLEX (MAC) AS AN APPROACH TO VIRULENCE. De Smet KA, Brown IN*, Yates MD, and Ivanyi J. Dept Medical Microbiology, St.Mary's Hospital Medical School, London, UK; PHLS Regional Centre for Tuberculosis, Dulwich Hospital, London, UK; and MRC Tuberculosis Unit, Hammersmith Hospital, London, UK.

204 GROWTH OF *MYCOBACTERIUM AVIUM*-INTRACELLULARE COMPLEX (MAC) STRAIN 101 IN MACROPHAGES INFLUENCES ITS COLONY MORPHOLOGY METABOLIC STATUS AND VIRULENCE. Kansal R*, Gomez-Flores R, and Mehta RT. Department of Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center, Houston, TX.

205 ENTEROBACTERIA PROMOTE THE GROWTH OF *MYCOBACTERIUM AVIUM* COMPLEX STRAIN 101. Gomez-Flore R*, Kansal R, Tamez-Guerra R, and Mehta RT. Department of Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center, Houston,TX; and Departamento de Microbiologia, Univer sidad Autonoma de Nuevo Leon, San Nicolás de los Garza, NL, México.

206 SURVEILLANCE FOR PNEUMONIC PLAGUE IN THE US DURING AN INTERNATIONAL EMERGENCY: A MODEL FOR CONTROL OF IMPORTED EMERGING DISEASES. Fritz CL, Dennis DT, Tipple MA, Campbell CL, McCance CR, and Gubler DJ. DVBID/CDC, Ft. Collins, CO.

207 ELECTROCHEMILUMINESCENCE DETECTION OF *YERSINIA PESTIS* ANTIGEN IN SERUM. Parker RW*, Horne MF, Ezzell JW, and Lofts RS. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

208 DEVELOPMENT OF A SIMPLE 15 MINUTE ASSAY FOR THE DETECTION OF *YERSINIA PESTIS* ANTIGEN. Moss DW*, Parker RW, Ezzell JW, Kijek TM, Abshire TG, Rossi CA, Korch GW, and Lofts RS. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

209 EVALUATION OF ELISA-BASED TESTS FOR IgM AND IgG ANTIBODIES TO *YERSINIA PESTIS* F1 ANTIGEN USING SERA FROM HUMAN VACCINEES. Lewis TE*, Roberts BA, Mangiafico JA, Hile JA, Danner DK, Pittman PR, Rossi CA, Korch GW, and Lofts RS. Applied Research Division, U.S. Army

Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

210 CHARACTERIZATION OF *YERSINIA PESTIS* STRAINS ISOLATED FROM PNEUMONIC PLAGUE PATIENTS FROM INDIA (SURAT, 1994). Chu MC*, Rana UV, Yockey BM, Wilmoth BA, Bracher JE, Berrada ZL, Carter LG, and Sehgal S. Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Ft. Collins CO; and Zoonoses Division, National Institute for Communicable Diseases, Delhi, India.

211 PASSIVE IMMUNIZATION WITH MONOCLONAL ANTIBODIES AGAINST THE F1 ANTIGEN OF *YERSINIA PESTIS* PROTECTS MICE FROM FATAL BUBONIC AND PNEUMONIC PLAGUE. Anderson Jr GW*, Worsham PL, Andrews GP, Bolt CR, Welkos SL, Friedlander AM, and Burans JP. Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Naval Medical Research Institute, Bethesda, MD.

212 COMPARISON BETWEEN IMMUNODIAGNOSTIC ASSAYS FOR BRUCELLOSIS DIAGNOSIS. El-Masry N*, Farid Z, Mohareb E, Shaheen H, Kamal M, Bassily S, Brown FM. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and Abbassia Fever Hospital, Cairo, Egypt.

213 CHARACTERIZATION OF MTA/SAH NUCLEOSIDASE AND MTR KINASE FROM ENTERIC BACTERIA: POTENTIAL TARGETS FOR CHEMOTHERAPEUTIC INTERVENTION. Cornell KA*, and Riscoe MK. Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR; and Medical Research Service, Veterans Affairs Medical Center, Portland, OR.

214 SAFETY AND IMMUNOGENICITY OF A LIVE, ORAL, ATTENUATED Bah-15 *VIBRIO CHOLERA* EL TOR OGAWA VACCINE. Coster TS*, Kenner JR, Arthur JD, Killeen KP, Spriggs DR, McClain JB, Barrera-Oro M, Mekalanos JJ, and Sadoff JC. Division of Medicine, USAMRIID, Ft Detrick, MD; Division of Comm Diseases & Immunology, WRAIR, Wash, DC; Department of Microbiology and Molec Genetics, Harvard Medical School, Boston, Mass; and Virus Research Institute, Cambridge, MA.

215 PERSISTENCE OF ANTIBODIES AND BOOSTER RESPONSE AGAINST ANTHRAX AND BOTULINUM VACCINES IN MILITARY PERSONNEL VACCINATED DURING OPERATIONS DS/DS. Pittman PR*, Sjogren MH, Makuch RS, LaChance R, Hack D, Arthur J, and Franz D. Division of Medicine, U. S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, DC.

216 PCR FIELD TRIAL FOR THE DETECTION OF *CAMPYLOBACTER* DURING EXERCISES "COBRA GOLD 94", COBRA GOLD 95" AND "BALANCE TORCH #1", THAILAND. Lebron CI*, Echeverria P, Walz SE, and Wignall SF. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Armed Forces Research Institute of Medical Science, Bangkok, Thailand.

VECTOR BIOLOGY

217 SEWERS: THE PRINCIPAL *AEDES AEGYPTI* BREEDING SITES IN CALI, COLOMBIA. Gonzalez R, and Suarez MF*. Departamento de Entomologia, Facultad de Ciencias, Universidad del Valle, Cali, Colombia; and Instituto de Inmunologia del Valle, Universidad del Valle, Cali, Colombia.

218 *AEDES AEGYPTI* PRESENCE IN CORDOBA PROVINCE, ARGENTINA (1995). Aviles B, Harrington ME, Cecchini R, Asis M, and Mios C. Ministry of Health, Cordoba Province, Argentina.

219 *AEDES ALBOPICTUS* IN SOUTH CAROLINA. Mekuria Y*. International Center for Public Health Research, School of Public Health, University of S.C., McClellanville, SC.

220 SAND FLY SPECIES COMPOSITION, DISTRIBUTION AND ECOLOGY IN A NORTHERN AREA OF MOROCCO. Hamdani A *, Essari A, and Guessous-Idrissi N. Unite d'Etudes et de Recherche sur les Leishmaniaoses, Faculte de Medicine et dePharmacie, Casablanca, Morocco.

221 FIELD BEHAVIOUR OF SANDFLIES IN WADI FERAN, SINAI-EGYPT. Shehata MG, Doha S, El Hossary S, Swalem M, Abd El Mohsen A, and El Kadi G.

222 SOLATION OF A FAMILY OF SERINE PROTEASE cDNA SEQUENCES FROM THE CAT FLEA *CTENOCEPHALIDES FELIS*. Stiegler GL*, Gaines PJ, Sampson CM, Lupien S, and Rushlow KE. Paravax, Inc., Fort Collins, CO.

223 CHROMOSOMAL ABERRATIONS IN INTRASPECIFIC HYBRIDS OF THE SAND FLY *LUTZOMYIA LONGIPALPIS*. Mukhopadhyay J*, Munstermann LE, and Ghosh KN. Yale School of Epidemiology and Public Health, Yale University, New Haven, CT.

224 DIFFERENTIATION BETWEEN *CULEX PIPIENS* COMPLEX MEMBERS USING A PCR ASSAY BASED ON DIFFERENCES IDENTIFIED BY GENOMIC SUBTRACTIVE HYBRIDIZATION. Crabtree MB*, Savage HM, and Miller BR. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Ft. Collins, CO.

225 PURIFICATION AND CLONING OF THE SALIVARY NITROPHORIN (NO-CARRYING HEMOPROTEIN)FROM THE BED BUG, *CIMEX LECTULARIUS*. Valenzuela JG*, and Ribeiro J MC. Department of Entomology, University of Arizona, Tucson, AZ.

226 TOXIC ACTIVITY (LC_{50}) OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* STRAIN LFB/FIOCRUZ 710 ON *LUTZOMYIA LONGIPALPIS* (DIPTERA:PSYCHODIDAE: PHLEBOTOMINAE). Wermelinger ED, Zanuncio JC, Rangel EF*, and Rabinovitch L. Departamento de Entomologia do Instituto Oswaldo Cruz FIOCRUZ Rio de Janeiro, Brasil; Departamento de Bacteriologia do Instituto Oswaldo Cruz FIOCRUZ Rio de Janeiro, Brasil; and Departamento de Biologia Animal, Universidade Federal de Vicosa, Vicosa, Brasil.

227 DEVELOPMENT OF A EXPLANT MODEL TO TEST FOR A MIDGUT BARRIER TO BLUETONGUE VIRUS INFECTION IN *CULICOIDES VARIIPENNIS* SUBSPECIES. Spielholz J*, Dubovi EJ, and Patrican LA. Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY; and Department of Entomology, Cornell University, Ithaca, NY.

228 AN IMMUNOLOGICAL STUDY OF RIFT VALLEY FEVER VIRUS IN A SENEGALESE MOSQUITO, *AEDES FOWLERI*. Romosor WS*, Lerdthusnee K, and Leon R. Tropical & Geographical Disease Institute, Department of Biological Sciences, Ohio University, Athens, OH.

229 GARLIC - MOSQUITO REPELLENT OR ANTIMALARIAL? Goh LM*, Lowrie RC, and Wiser MF. Section of Peds. Infectious Diseases, Tulane University Medical Center, New Orleans, LA; Parasitology Department, Tulane Regional Primate Research Center, Covington, LA; and Tropical Medicine Department, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

TICK-BORNE DISEASES

230 EFFECTS OF IVERMECTIN ON TRANSMISSION OF *BORRELIA BURGDORFERI* BY *AMBLYOMMA AMERICANUM*. Presley SM, Abbassy MM*, and Arthur RR. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and Johns Hopkins University, Baltimore, MD.

231 ACQUISITION OF LYME DISEASE SPIROCHETES BY CO-FEEDING *IXODES SCAPULARIS* NYMPHS. Patrican LA*. Department of Entomology, Cornell University, Ithaca, NY.

232 THE OUTER SURFACE PROTEIN A (OspA) LYME DISEASE VACCINE BLOCKS TRANSMISSION FROM THE VECTOR TO THE HOST. de Silva AM*, Barthold SW, Telford SR, Burnet L, and Fikrig E. Yale University School of Medicine, New Haven, CT; and Department of Tropical Public Health, Harvard University School of Public Health, Boston, MA.

233 TICK-BORNE INFECTIOUS DISEASES IN A NORTHERN CALIFORNIA COMMUNITY. Fritz CL*, Conrad PA, Flores GR, Kjemtrup AJ, Campbell GL, Schriefer ME, Gallo D, and DJ Vugia. Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO; Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA; Public Health Division, Sonoma County Department of Health Services, Santa Rosa, CA; and Division of Communicable Disease Control, California Department of Health Services, Berkeley, CA.

234 GENETIC ANALYSIS OF *RICKETTSIA TSUTSUGAMUSHI* ISOLATES OBTAINED FROM HUMANS, RODENTS, AND THEIR TROMBICULID MITE VECTORS IN AUSTRALIA. Dasch GA*, Jackson LM, and Chan CT. Viral and Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

235 ENZYME IMMUNOASSAY FOR DETECTING *RICKETTSIA TYPHI* INFECTIONS IN FIELD COLLECTED RODENTS, MAUI, HAWAII. Kirschner KF*, Sasaki DM, Cope SE, and Olson JG. U.S. Navy Environmental and Preventive Medicine Unit No. 6, Pearl Harbor, HI; Zoonoses Section, Epidemiology Branch, State of Hawaii Department of Health, Honolulu, HI; Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, GA.

236 *IXODES PACIFICUS* (ACARI: IXODIDAE): A VECTOR OF *EHRLICHIA EQUI*. Richter PJ*, Kimsey RB, Madigan JE, Barlough JE, Dumler JS, and Brooks DK. Division of Comparative Medicine, University of CA, San Diego, La Jolla, CA; Department of Entomology, University of California, Davis, CA; Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA; and Department of Pathology, University of Maryland Medical Center, Baltimore, MD.

237 ECOLOGICAL ASSOCIATION BETWEEN *IXODES PACIFICUS* (ACARI: IXODIDAE) AND THE SPATIAL AND TEMPORAL DISTRIBUTION OF EQUINE EHRLICHIOSIS IN NORTHERN CA. Vredevoe LK*, Richter PJ, Madigan JE, and Kimsey RB. Department of Entomology, University of California, Davis, CA; Division of Comparative Medicine, University of CA, San Diego, La Jolla, CA; Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA; and Department of Entomology, University of California, Davis, CA.

238 MOLECULAR CLONING AND SEQUENCING OF A 120 kDa IMMUNODOMINANT PROTEIN GENE OF *EHRLICHIA CHAFFEENSIS*. Yu XJ*, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX

239 ULTRASTRUCTURE OF HUMAN GRANULOCYTIC EHRLICHIAE. Popov VL*, Han VC, Chen SM, Bakken JS, Dumler JS, Madigan JE, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX; Duluth Clinic, Duluth, MN; Department of Pathology, The University of Maryland,Baltimore, MD; and Department of Medicine and Epidemiology, School of Veterinary Medicine, The University of California, Davis, CA.

240 EVALUATION OF AN ENZYME IMMUNOASSAY IN THAI SCRUB TYPHUS PATIENTS. Kelly DJ*, Suwanabun N, Eamsila C, Chaouriyagune C, Howard R, and Dasch GA. Department of Clinical Investigation, Walter Reed Army Medical Center Washington, DC; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Medicine, Chiangrai Prachanuchroa Hospital, Chiang Rai, Thailand; and Viral and Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

INTESTINAL PROTOZOA

241 DEMONSTRATION OF CYCLOOXYGENASE-1 ENZYME IN *ENTAMOEBA HISTOLYTICA*: PARASITE PRODUCTION OF PROSTAGLANDIN E2. Belley AC*, Keller K, and Chadee K. Institute of Parasitology of McGill University, Macdonald Campus, Ste. Anne de Bellevue, Quebec.

242 EPIDEMIOLOGICAL ASPECTS OF CYST PRODUCTION OF *ENTAMOEBA*. Zurabian R*, and Acuna-Soto R. Departmento de Microbiologia y Parasitologia. Facultad de Medicina. Universidad Nacional Autonoma de Mexico. M.

243 COMPARISON OF TWO IMMUNOASSAYS FOR THE DETECTION OF *ENTAMOEBA HISTOLYTICA/DISPAR* IN STOOL SPECIMENS. Rosenblatt JE* and Sloan LM. Division of Clinical Microbiology, The Mayo Clinic, Rochester, MN.

244 EXPERIMENTAL STUDIES ON THE KITTEN SHOWING THE DIFFERENCE OF PATHOGENECITY BETWEEN *ENTAMOEBA DISPAR* AND *ENTAMOEBA HISTOLYTICA* BY E. BRUMPT. Petithory JC*, Ardoin FG, Sargeaunt PG, and Brumpt LC. Contrìle de Qualité National en Parasitologie, Centre Hospitalier, Gonesse, France.

245 IMMUNODIAGNOSIS OF ALVEOLAR ECHINOCOCCOSIS BY ELISA USING PARTIALLY PURIFIED Em18 AND Em16 ENRICHED FRACTION. Ito A*, Ma L, Itoh M, Cho S, Kong Y, Kang S, Horii T, Pang X, Okamoto M, Yamashita T, Lightowlers MW, and Liu Y. Department of Parasitology, Gifu University School of Medicine, Japan; Institute of Infectious and Parasitic Diseases, Chongqing University of Medical Sciences, China; and Department of Medical Biology, Medical School, Nagoya City University, Japan.

246 INDUCTION OF MUCOSAL ANTI-AMEBIC IgA ANTIBODIES BY IMMUNIZATION WITH *SALMONELLA TYPHIMURIUM* EXPRESSING THE AMEBIC SREHP MOLECULE. Zhang T*, Li E, and Stanley, Jr. SL. Washington University School of Medicine, St. Louis, MO.

247 *IN VITRO* SUSCEPTIBILITY OF METRONIDAZOLE-RESISTANT *TRICHOMONAS VAGINALIS* TO FURAZOLIDONE. Narcisi EM*, and Secor WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

248 IDENTIFICATION OF DEVELOPMENTALLY REGULATED TRANSCRIPTS IN *GIARDIA LAMBLIA* BY DIFFERENTIAL DISPLAY. Que X*, Aley SB, Meng TC, Hetsko ML, and Gillin FD. Department of Pathology, Division of Infectious Diseases; University of California San Diego Medical Center, CA.

249 PATHOGENESIS OF MUCOSAL DAMAGE IN GIARDIASIS: POSSIBLE ROLE OF HOST IMMUNE RESPONSE. Sehgal R, Goyal R, Ganguly NK*, and Mahajan RC. Departments of Parasitology and Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh, India.

250 PURIFICATION OF A LECTIN FROM *GIARDIA LAMBLIA* AND CHARACTERIZATION OF INTESTINAL RECEPTOR(S) FOR THE LECTIN. Sehgal R, Sriniwas K, Ganguly NK*, and Mahajan RC. Departments of Parasitology and Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh, India.

251 GASTROINTESTINAL SYMPTOMS IN HAITIAN MIGRANTS - PARASITIC EVALUATION AND ETIOLOGY, AND RESPONSE TO ANTI-PARASITIC TREATMENT. Rusnak JM*, Reed K, Rodgers J, Pace E, Ross C, and Hayashi K. Department of Infectious Diseases, Wilford Hall Medical Center, Lackland Air Force Base, TX.; MacDill Air Force Base, FL.; Portsmouth Naval Station, VA; and World Relief Organization.

252 *IN VITRO* SUSCEPTIBILITY OF *GIARDIA LAMBLIA* TROPHOZOITES TO NEWLY SYNTHESIZED METHYL-BENZIMIDAZOLES. Cedillo RR*, Hernández CA, Tapia CA, Sánchez R, Castillo R, Muñoz 0. Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias. Hospital de Pediatríca, CMN, IMSS; Departamento de Farmacia, Facultad de Química, Universidad Nacional Autínoma de México.

253 TRICHOMONIASIS IN WOMEN FROM MISSISSIPPI; A FIVE YEAR RETROSPECTIVE REVIEW OF PAPANICOLAOU SMEARS. Acholonu AD, White JD, Lushbaugh WB*, Cason Z, and Lemos LB. Alcorn State University, Department of Biology, Lorman, MS.; University of Mississippi Medical Center, Department of Pathology, Section of Cytopathology; and University of Mississippi Medical Center, Department of Microbiology, Jackson, MS.

254 CLONING AND IMMUNOLOCALIZATION OF A CALTRACTIN-LIKE GENE FROM *GIARDIA LAMBLIA*. Meng TC*, Aley SB, Kim J, Huang B, Smith MW, Harwood J, and Gillin FD. Departments of Medicine and Pathology, University of California San Diego Medical Center, San Diego, CA; Department of Cell Biology, The Scripps Research Institute, La Jolla, CA; and Molecular Genetics Laboratories, The Salk Institute, La Jolla, CA.

FILARIASIS

255 PURIFICATION AND PARTIAL CHARACTERIZATION OF A TRANSGLUTAMINASE FROM DOG FILARIAL PARASITE, *DIROFILARIA IMMITIS*. Mehta K*, Singh RN, and Chandrashekar R. Department of Bioimmunotherapy, University of Texas MD Anderson Cancer Center, Houston, TX; and Department of Medicine, Washington University Medical Center, St. Louis, MO.

256 A SMALL HEAT SHOCK PROTEIN FROM *BRUGIA MALAYI* L4 STAGE LARVAE. Raghavan N*, and Scott AL. Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

257 INITIAL CHARACTERIZATION OF THE MITOCHONDRIAL GENOME OF *ONCHOCERCA VOLVULUS*. Keddie EM*, and Unnasch TR. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL; and Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL.

258 DISRUPTION OF EMBRYOGENESIS IN *BRUGIA MALAYI* BY IMMUNIZATION WITH OVT1. Triteeraprapab S*, and Scott AL. Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; and Department of Microbiology & Immunology, Johns Hopkins University School of Hygiene & Public Health, Baltimore; MD.

259 CONSTRUCTION AND CHARACTERIZATION OF *BRUGIA MALAYI* ADULT MALE, MICROFILARIA AND L3 cDNA LIBRARIES. Saunders LJ*, Lu W, Ling N, and Williams SA. Molecular and Cellular Biology, University of Massachusetts, Amherst, MA; Department of Biological Sciences, Smith College, Northampton, MA.

260 DIRECT AMINO ACID SEQUENCING OF *ONCHOCERCA VOLVULUS* ANTIGENS SEPARATED BY TWO DIMENSIONAL GEL ELECTROPHORESIS. Bitter J, Erttmann KD, and Gallin MY*. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany.

261 FURTHER CHARACTERIZATION OF OVGALBP, AN IGE BINDING PROTEIN OF FILARIAL ORIGIN. Klion AK*, Catmull J, Garraud O, and Donelson JE. Department of Internal Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA; Department of Biochemistry, University of Iowa, Iowa City, IA; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

262 *IN VIVO* AND IN *VITRO* CELLULAR IMMUNE RESPONSE TO RECOMBINANT FILARIAL PROTEINS OF *BRUGIA* SPP. Rao UR*, Nassare CS, Coleman SU, Horohov DW, and Klei TR. Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton, Rouge, LA.

263 ANTIBODY RESPONSES TO RECOMBINANT *BRUGIA MALAYI* ANTIGENS IN EXPERIMENTALLY INFECTED RHESUS MONKEYS. Dennis VA*, Lasater BL, Lowrie, Jr. RC, Bakeer M, Chandrashekar R, Weil GJ, and Xu K. Departments of Parasitology and Microbiology, Tulane Regional Primate Research Center, Covington, LA; and Washington University School of Medicine, St. Louis, MO.

264 THE KINETICS OF TYPE 2 CYTOKINE PRODUCTION IN BANCROFTIAN FILARIASIS DIFFERS BETWEEN CLINICAL GROUPS. de Almeida AB*, Maia e Silva MC, Maciel A, Freedman DO. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil.

265 ANTIFILARIAL IMMUNE RESPONSE IN JIRDS CONCURRENTLY INFECTED WITH *STRONGYLOIDES STERCORALIS* AND *BRUGIA PAHANGI*. Chisholm ES*, and Lammie PJ. Division of Parasitic Diseases, Center for Disease Control and Prevention, Atlanta, GA.

266 FIELD EVALUATION OF A NEW MICROSCOPIC TECHNIQUE FOR MONITORING FILARIAL INFECTIONS IN MOSQUITOES. Yates JA*, Green DF, Levesque MA, and Bockarie MJ. Department of Biological Sciences, Oakland University, Rochester, MI; and Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea.

267 MOLECULAR CHARACTERIZATION AND FIELD EVALUATION OF OV26, A NOVEL *ONCHOCERCA VOLVULUS* ANTIGEN. Lobos E*, Steiger S, Zahn R, Schneider D, Weiss N, and Chippaux JP. Swiss Tropical Institute, Basel, Switzerland; Centre Pasteur, Yaounde, Cameroon.

268 CURRENT STATUS OF ONCHOCERCIASIS IN COLOMBIA. Corredor A, Palma GI*, Nicholls RS, Granada JF, Alvarez CA, and Guderian R. Inst. Salud en el Trópico, Univ. Nacional, Bogotá, Colombia; Department Microbiología, Univ. del Valle/ CIDEIM, Cali, Colombia; Lab. Parasitología, Inst. Nacional de Salud, Bogotá, Colombia; Hospital Regional Guapi, Colombia; and Hospital VozAndes, Quito, Ecuador.

269 DEVELOPMENT OF A POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF *BRUGIA* MICROFILARIAE IN TISSUE SAMPLES. Lizotte MR*, Pearlman E, Kazura JW, and Williams SA. Department of Biological Sciences, Smith College, Northampton, MA; and Division of Geographic Medicine, Case Western Reserve University School of Medicine, Cleveland, OH.

270 IVERMECTINE AND DIETHYLCARBAMAZINE FOR THE TREATMENT OF HIGH MICROFILAREMIA *LOA LOA* AND *MANSONELLA PERSTANS*. Richard-Lenoble D*, Kombila M, Duong TH, Walker A. Department of Parasitology and Tropical Medicine, University School of Medicine Libreville, Gabon and Tours, France.

PARASITOLOGY

271 PARASITOLOGIC STUDIES OF *TAENIA SOLIUM* ADULT STAGE IN HAMSTERS. Aguilar L*, Avila G, and Flisser A. Departmento de Microbiologia y Parasitologia, Facultad de Medicina, UNAM, Mexico D.F.

272 EVALUATION OF HEALTH EDUCATION FOR CONTROL OF *TAENIA SOLIUM* IN A RURAL COMMUNITY IN MEXICO. Bronfman M, Flisser A, Sarti E, Schantz PM*, Gleizer M, Loya M, Plancarte A, Avila G, Allan J and Fineblum W. Direccion General de Coordinacion Sectorial e Internacional and Direccion General de Epidemiologia, SSa; Facultad de Medicina, UNAM, Mexico D.F.; and Division of Parasitic Diseases, NCID, CDC, Atlanta, GA

273 EPIDEMIOLOGICAL ASPECTS OF HUMAN INFECTIONS BY INTESTINAL CESTODES IN SANTIAGO, CHILE (1985-1994). Mercado R *, Arias B, Romero M, Peñaloza, and Castillo D. Departamento de Parasitología, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

274 ULTRASTRUCTURAL ANALYSIS OF ADULT *TAENIA SOLIUM*-INTESTINAL WALL INTERFACE IN HAMSTERS.. Willms K *, Avila G, Merchant MT, Aguilar L, and Flisser A. Department of Microbiology & Parasitology, Facultad de Medicina, Universidad Nacional Autónoma de México, México.

275 GRANULOMA CYTOKINES IN MURINE CYSTICERCOSIS. Robinson P*, Atmar RL, Lewis DE, and White, Jr AC. Baylor College of Medicine, Houston, TX.

276 *TAENIA SAGINATA* ONCOSPHERE PEPTIDASES. White, Jr. AC*, Baig S, and Robinson P. Baylor College of Medicine, Houston, TX.

277 ISOLATION AND CHARACTERIZATION OF A GENE FROM THE TAPEWORM *TAENIA SOLIUM* THAT ENCODES A PUTATIVE GLUCOSE TRANSPORTER. Rodriguez DD*, Shoemaker CB, and Laclette JP. Department of Immunology, Inst. de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico City; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

278 ARE BARREN FEMALE *STRONGYLOIDES STERCORALIS* FROM CHRONIC INFECTIONS REJUVENATED BY TRANSPLANTATION INTO PARASITE NAIVE RECIPIENT HOSTS? Schad GA*, Thompson F, Lee G, Lange AM, Nolan TJ, Holt D, and Bhopale VM. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

279 USING A BAYESIAN APPROACH TO ENHANCE THE ESTIMATION OF THE PREVALENCE OF INFECTION. Gyorkos TW*, and Joseph L. Division of Clinical Epidemiology, Montreal General Hospital, Montreal, Quebec, Canada and; and Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada.

280 *STRONGYLOIDES STERCORALIS*: EOSINOPHIL-MEDIATED, TH-2 DEPENDENT PROTECTIVE IMMUNITY TO INFECTIVE LARVAE IN BALB/CBYJ MICE. Rotman HL*, Gleich GJ, Nolan TJ, Schad GA, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA; Department of Immunology and Medicine, Mayo Clinic and Foundation, Rochester, MN; and Department of Pathobiology, University of Pennsylvania, Philadelphia, PA.

281 IDENTIFICATION OF NOVEL GENES USING A cDNA LIBRARY FROM FILARIFORM AND RHABDITIFORM STAGES OF *STRONGYLOIDES STERCORALIS*. Moore TA*, Ramachandran S, Gam A, Neva FA, Saunders L, Lu W, Williams SA, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Department of Biological Sciences, Smith College, Northampton, MA.

282 ROLE OF ANTIBODY ISOTYPE AND COMPLEMENT IN IMMUNITY TO *STRONGYLOIDES STERCORALIS* L3. Brigandi RA*, Rotman HL, Yutanawiboonchai W, Nolan TJ, Schad GA, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA; and Department of Pathobiology, School of Veterinary Medicine University of Pennsylvania, Philadelphia, PA.

283 ALBENDAZOLE PRODRUGS IN EXPERIMENTAL TRICHINELLOSIS. Yepez-Mulia L*, Morales-Hurtado R, Viveros N, Cedillo-Rivera R, Hernández F, Castillo R, Jung H, and Muñuz O. Unidad de Investigacion Medica en Enfermadades Infecciosas, Centro Medico Nacional, Instituto Mexicano del Seguro Social, Mexico City, Mexico.

284 THE PERSISTENCE OF ENZOOTIC DRACUNCULIASIS IN CENTRAL ASIA 50 YEARS AFTER THE ERADICATION OF GUINEA WORM DISEASE. Kliks MM*. Institute of Zoology, National Academy of Sciences, Akademgorok, Almaty, Republic of Kazakhstan.; CTS Foundation, Honolulu, Hawaii.

286 CD4+ CELLS REGULATE MUCUS PRODUCTION IN INTESTINAL NEMATODE INFECTION. Khan WI*, Abe T, and Yoshimura K. Department of Parasitology, Akita University School of Medicine, Akita, Japan.

287 EXPERIMENTAL *OESOPHAGOSTOMUM BIFURCUM* INFECTION IN MONKEYS. Eberhard ML*, Polderman J, Blokamp J, and Baeta S. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; Department of Parasitology, University of Leiden, Leiden, The Netherlands; and Department of Gynecology, University of Lome, Toga.

288 MOLECULAR IDENTIFICATION OF *ENTEROBIUS VERMICULARIS* AS A CAUSE OF HUMAN EOSINOPHILIC ENTEROCOLITIS. Liu LX*, Chi JY, Upton MP, and Ash LR. Beth Israel Hospital, Harvard Medical School, Boston, MA; and UCLA School of Public Health, Los Angeles, CA.

289 EVALUATION OF LMD ELISA KITS FOR DETECTION OF HUMAN ANTIBODIES TO *ECHINOCOCCUS GRANULOSIS* AND *ENTAMOEBA HISTOLYTICA*. Wilson M*, Schantz PM, and Ware DA. Division Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

290 CYSTIC ECHINOCOCCOSIS IN JORDAN: A SIGNIFICANT DISEASE OF STABLE ENDEMICITY. Abdel-Hafez SK*, and Kamhawi SA. Department of Biological Sciences, Yarmouk University, Irbid, Jordan.

KINETOPLASTS

291 CLINICAL AND PATHOLOGICAL ASPECTS OF *LEISHMANIA* STRAINS FROM NATURALLY INFECTED DOGS IN MOROCCO. Berrag B *, Sahibi H, Natami A, Lasri S, Rhalem A, Bichichi M, Riyad M, and Guessous-Idrissi N. Departement de Parasitologie, Institut Agronomique et Veterinaire Hassan II, Rabat, Morocco; Unite d'Etudes et de Recherches sur les Leishmanioses, Faculte de Medicine & Pharmacie, Casablanca, Morocco.

292 NO TITLE. Guessous-Idrissi N*, Hamdani A, Dehbi F, Bichihi M, Sahibi H, Berrag B, and Rhalem A. Unite d'Etudes et de Recherche sur les Leishmanioses, Faculte de Medicine etde Pharmacie, Casablanca, Morocco.

293 A SIMULATION MODEL OF THE INFECTION CYCLE OF *LEISHMANIA MEXICANA* IN *NEOTOMA MICR OPUS*. Kerr SF*, Grant WE, and Dronen NO. Biology Department, Incarnate Word College, San Antonio, TX; and Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX.

294 THERAPY OF CHAGAS DISEASE: ACTIVITY OF THIO-SEMICARBAZONES. Kinnamon KE*, Poon BT, Hanson WL, and Waits VB. Department of Preventive Medicine & Biometrics, F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA.

295 EFFECT OF VEPAPAMIL AND RELATED COMPOUNDS IN ACUTE MURINE *TRYPANOSOMA CRUZI* INFECTION. Tanowitz HB*, Chen B, Huang H, Wittner M, Weiss LM, and Morris SM. Albert Einstein College of Medicine, Bronx, NY.

296 NEW S-ADENOSYLMETHIONINE DECARBOXYLASE INHIBITORS ARE TRYPANOCIAL AGENTS. Bacchi CJ*, Brun R, and Croft SL. Haskins Laboratories and Department of Biology, Pace University, New York, NY; Swiss Tropical Institute, Basel, Swutzerland; and Department of Medical Parasitology, and London School of Hygiene and Tropical Medicine, London, U.K.

297 STRUCTURE FUNCTION ANALYSIS: A NEW APPROACH TO TRIFLURALIN ANALOGS YIELDS RESULTS. Kelley C, Callahan HL*, Pereira TR, and Grogl M. US Army Medical Research Unit - Brazil Unit, American Consulate-Rio; and Department of Medicinal Chemistry, WRAIR, Washington, DC.

298 DESIGN OF A SERO-PREVALENCE STUDY TO DETERMINE ANTIBODIES TO *TRYPANOSOMA CRUZI* IN LATIN-AMERICAN REFUGEES AND IMMIGRANTS TO CANADA. Steele LS*, MacPherson DW, Gushulak B, Gully P, and Blajchman M. Faculty of Medicine - M.D. Programme, McMaster University, Hamilton, ON, Canada; Parasitology, St.Joseph's Hospital and Pathology, McMaster University, Hamilton, ON, Canada; Quarantine Health Services, Health Protection Branch, Health Canada, Ottawa, ON, Canada; and Canadian Red Cross Society and Haematology & Pathology, McMaster University, Hamilton, ON, Canada.

299 THE DRUG SENSITIVITY PROFILE OF FREE AMASTIGOTES: DEVELOPMENT OF A NEW MODEL SYSTEM FOR SCREENING DRUGS. Grogl M, Portal AC, and Callahan HL. U.S.A. Medical Research Unit-Brazil, Walter Reed Army Institute of Research.

300 GOOD MANUFACTURING PRACTICES (GMP) PRODUCTION OF *LEISHMANIA* SKIN TEST ANTIGEN (LSTA): 2. PRODUCTION OF A MICROFLUIDIZED LYSATE (MFL) LSTA. Stiteler JM*, Ballou WR, Eckels KH, Wellde BT, Topper MJ, Rowton ED, and Magill AJ. Division of Communicable Diseases & Immunology, Walter Reed Army Instituteof Research, Washington, DC.

301 TREATMENT OF CUTANEOUS LEISHMANIASIS WITH LOCALIZED CONTROLLED HEAT (RADIO FREQUENCY) IN 213 PATIENTS IN LA CHONTALPA, TABASCO, MEXICO. Velasco-Castrejon O, Walton BC*, Rivas-Sanchez B, Garcia MF, Lazaro GJ, Hobart O, Roldan S, Floriani-Verdugo J, Munguia-Saldana A, and Berezaluce R. Instituto del Diagnostico y Recursos Epidemiologicas, Mexico, DF Mexico; Consultant, Gettysburg, PA, PanAmerican Health Organization (Retired); Secretaria de Salud del Estado de Tabasco; and Hospital General de Comalcalco, Tabasco, Mexico.

302 INHIBITION OF *TRYPANOSOMA CRUZI* WITH DISULFIRAM AND SODIUM DIETHYLAMINE-*N*-CARBODITHIOATE *IN VITRO* AND *IN VIVO*. Lane JE*, Ribeiro-Rodrigues R, Carter CE, Suarez CC, Jones MM, Singh PK, and Romanha AJ. Department of Biology, Vanderbilt University, Nashville, TN; Department of Chemistry, Vanderbilt University, Nashville, TN; and Centro de Pesquisas "Rene Rachou" Belo Horizonte, MG, Brazil.

303 KNOWLEDGE, ATTITUDES AND PRACTICES (KAP) SURVEY FOR CHAGAS' DISEASE IN AN ENDEMIC AREA OF GUATEMALA. Nix NA*, Hernandez B, Mendoza C, and Klein RE. Universidad de Valle de Guatemala, Guatemala City, Guatemala; Medical Entomology Research and Training Unit/Guatemala; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

MALARIA IMMUNOLOGY

304 ROLE OF MALARIA ANTIBODIES IN THE DEVELOPMENT OF HTLV-I IMMUNOREACTIVITY IN INDONESIAN TRANSMIGRANTS. Porter KR*, Anthony RL, Richards A, Sandjaya B, Ignatias H, Hadiputranto H, Wignall FS, and Hayes CG. Department of Infectious Diseases, Naval Medical Research Institute, Bethesda, Maryland; and Department of Immunology, Naval Medical Research Unit #2, Jakarta, Indonesia.

305 LYMPHOCYTE SUBSETS, MALARIA INFECTION, AND DISEASE AMONG MEN IN WESTERN KENYA. Mak'obongo M, McElroy PD, Guo WS, Scott LA, Sullivan AD, Orago AS, Oloo AJ, and Weiss WR*. Department of Zoology, Kenyatta University, Nairobi, Kenya; Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan; Vector Borne Disease Control and Research Center, Kenya Medical Research Institute, Kisumu, Kenya; and Malaria Program, Naval Medical Research Insititute, Bethesda, MD.

306 CHANGES IN RESPONSE AGAINST *PLASMODIUM FALCIPARUM* PEPTIDES OF T AND B LYMPHOCYTES FROM CHILDREN SUBMITTED TO ONE SEASON OF MALARIA TRANSMISSION IN HIGHLANDS OF MADAGASCAR. Rasamoel P*, Ralamboranto L, Ramambanirina L, Ranaivo L, Raharimalala L, Ollivier G, Druilhe P, Laventure S, Jambou R. Laboratoire du paludisme, and Unité d'entomologie, Institut Pasteur de Madagascar, Antananarivo, Madagascar; and Laboratoire de Parasitologie biomédicale, Institut Pasteur, Paris France.

307 LONGTERM ISOTYPE MODULATION OF B LYMPHOCYTE RESPONSES AGAINST *PLASMODIUM FALCIPARUM* PEPTIDES AFTER ARREST OF MALARIA TRANSMISSION IN A VILLAGE OF THE HIGHLANDS. Ralamboranto L*, Rasamoel P, Ramambanirina L, Ranaivo L, Raharimalala L, Ollivier G, Druilhe P, Laventure S, Jambou R. Laboratoire du paludisme, and Unité d'entomologie, Institut Pasteur de Madagascar, BAntananarivo, Madagascar; Laboratoire de Parasitologie biomédicale, Institut Pasteur, Paris France.

308 HUMAN IMMUNE RESPONSE TO SYNTHETIC PEPTIDES CONTAINING CTL MOTIVES ON THE *PLAS MODIUM VIVAX* CS PROTEIN. Herrera MA, Perlaza BL, Toala A, Ferro B, Reyes ME, Betterns F, Corradin G*, and Herrera S. Institute de Inmunologia del Valle, Universidad del Valle; Institute of Biochemistry, University of Lausanne, Lausanne, Switzerland.

309 CHARACTERIZATION OF SERA FACTORS CAUSING *IN VITRO* INHIBITION OF *PLASMODIUM FALCIPARUM*. Perlaza BL*, de Plata C, Zapata C, Clavijo P, Herrera MA, AND Herrera S. Instituto de Inmunologia del Valle, Universidad del Valle, Cali Colombia; and Department of Medical and Molecular Parasitology, New York University, NY.

310 SERA FROM PATIENTS WITH FALCIPARUM MALARIA INDUCE SUBSTANCE P GENE EXPRESSION IN CULTURED HUMAN BRAIN MICROVESSEL ENDOTHELIAL CELLS. Chiwakata CB, Hort G, Hemmer CJ, and Dietrich M*. Department of Medicine, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

311 CYTOKINES INDUCED BY PROTECTIVE VACCINATION IN THE *PLASMODIUM YOELII* BLOOD-STAGE MODEL. Patterson PS*, Udhayakumar V, Bosshardt SC, Kidd MR, Xiao L, Hunter RL, and Lal AA. Immunology Branch, DPD, Centers for Disease Control, Atlanta, GA; Department of Pathology, Emory University, Atlanta, GA; and School of Medicine, Vanderbilt University, Nashville, TN.

312 GUT IMMUNITY OF BALB/C MICE INFECTED WITH THE LETHAL STRAIN OF *PLASMODIUM CHABAUDI ADAMI*. Dimayuga FO*, Dimayuga ER, and Wei Y. Department of Biological Sciences, Ohio University, Athens, OH; and Edison Biotechnology Institute, Ohio University, Athens, OH.

313 ENHANCEMENT OF MALARIA IN MICE IMMUNIZED WITH PEPTIDE FORMULATIONS; A MODEL SYSTEM TO STUDY IMMUNOPATHOLOGY OF MALARIA. Jennings VM*, Udhayakumar V, Lal AA, and Hunter RL. Department of Pathology, Emory University, Atlanta, GA; and Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

MALARIA VACCINE

314 IDENTIFICATION OF T CELL EPITOPES IN CONSERVED AND SEMI-CONSERVED REGIONS OF ELEVEN *PLASMODIUM FALCIPARUM* ANTIGENS IN CONGENIC MICE. Parra ME*, Roberts T, Quakyi IA, Berzofsky JA, Miller LH, Houghten RA, and Taylor DW. Department of Biology, Georgetown University, Washington, DC; Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD; Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda MD; Torrey Pines Institute of Molecular Biology, San Diego, CA.

315 POPULATION EFFECTS OF STAGE-SPECIFIC MALARIA VACCINATION. Burke MA*, Halloran EM, and Lal AA. Rollins School of Public Health, Emory University, Atlanta, GA; and Division of Parasitic Disease, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

316 T CELL RESPONSES TO *PLASMODIUM FALCIPARUM* LSA- I IN IMMUNE ADULT RESIDENTS OF TH E WOSERA, PAPUA NEW GUINEA. Connelly M, Genton B, King C, Hollingdale M, Boykins R, Alpers M, and Kazura J*. Case Western Reserve University, Cleveland, OH; Papua New Guinea Institute of Medical Research, M aprik, Madang, and Goroka; and Food and Drug Administration, Bethesda, MD.

317 MALARIA VACCINE TRIALS AGAINST PRE-ERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM* IN CHIMPANZEES: 2) CORRELATION BETWEEN EFFECT ON PARASITEMIA AND T-CELL ACTIVATION AFTER CHALLENGE ELICITED BY A PRE-ERYTHROCYTIC STAGE MALARIA VACCINE BASED ON LSA-3. Luty A*, Millet P, Dubreuil G, LeRoy E, DeBels F, Tartar A, Eling WM, George AJ, and Druilhe P. Centre International de Recherche Médicale de Franceville (CIRMF), Franceville, Gabon; Institut Pasteur, Roux, Paris, France; and University Hospital Nijmogen, Department of Parasitology, Nijmegen, The Netherlands.

318 *PLASMODIUM VIVAX* INFECTIONS IN CHIMPANZEES FOR SPOROZOITE VACCINE CHALLENGE STUDIES IN MONKEYS. Sullivan JS*, Morris CL, McClure HM, Strobert EA, Richardson BB, Galland GG, Goldman IF, and Collins WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Yerkes Regional Primate research Center, Emory University, Atlanta, GA.

319 NATURAL IMMUNE RESPONSES TO THE C-TERMINAL DOMAIN OF *PLASMODIUM FALCIPARUM* MSP-1: CORRELATION OF ANTIBODY RESPONSES WITH PARASTEMIA AND ILLNESS. Branch OH*, Udhayakumar V, Bloland PB, Hightower AW, Oloo AJ, Hawley WA, Nahlen BL, Kaslow DC, and Lal AA. Division of Parasitic Diseases,NCID, CDC, Atlanta, GA; Vector Biology and Control Research Center, Kenya Medical Research Institute, Kissian, Kenya; Laboratory of Malaria Research, National Institute of Health, Bethesda, MD.

320 CHARACTERIZATION OF PROTECTIVE ANTIBODIES GENERATED BY IMMUNIZATION WITH BOTH EGF-LIKE DOMAINS OF MSP-1 FROM *PLASMODIUM YOELII*. Calvo PA*, and Long CA. Program in Molecular and Cell Biology; Department of Microbiology and Immunology, MCP-Hahnemann University, Philadelphia, PA.

321 AN ALTERNATIVE STRATEGY FOR VACCINATION AGAINST MALARIA: IMMUNIZATION WITH PLASMID DNA ENCODING THE C-TERMINAL REGION OF THE *PLASMODIUM YOELII* MSP-1. Kang Y, Farley PJ, Hedstrom R, Hoffman SL, and Long CA. Department of Microbiology and Immunology, Molecular Biology and Biotechnology Program, MCP-Hahnemann University, Philadelphia, PA; and Malaria Program, Naval Medical Research Institute, Bethesda, MD.

322 IMMUNOGENICITY OF PvMSP1 DNA VACCINES IN BALB/c MICE. del Portillo H*. Departamento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, Brasil

323 EFFECTS OF ADJUVANTS ON MURINE IMMUNE RESPONSES TO THE C-TERMINAL DOMAIN OF PV200 OF *PLASMODIUM VIVAX*. Yang C*, Collin WE, Reed RC, Hunter RL, Patterson P, Udhayakumar V, Kaslow DC, and Lal AA. Division of Parasitic Diseases, CDC, Atlanta, GA; Department of Pathology, Emory University, Atlanta, GA; and National Institute of Health, Bethesda, MD.

324 ANALYSIS OF POTENCY OF THE SPf66 MALARIA VACCINE. Ballou WR*, Scheumann D, Kolodny N, Williams JA, and Gordon DM. Department of Immunology, WRAIR, Washington DC.

325 CHARACTERIZATION OF THE PEPTIDE POLYMER SPf66 MALARIA VACCINE. Ballou WR*, Kolodny N, Lees RA, and Hagopian RA. Department of Immunology, WRAIR, Washington DC; and Multiple Peptide Systems, Inc. San Diego, CA.

326 IMMUNITY TO MALARIA ELICITED BY HYBRID HEPATITIS B VIRUS SURFACE ANTIGEN PARTICLES CARRYING EPITOPES OF *PLASMODIUM FALCIPARUM*. Chen A*. First Military Medical University, Molecular Biology Institute, Guangzhou, P.R. China

327 RABBIT ANTI-PFS25 SERA, THAT BLOCKS PARASITE INFECTION IN 6-8 DAY OLD ANOPHELES MOSQUITOES, ONLY SUPPRESSES OOCYST DEVELOPMENT IN YOUNGER MOSQUITOES. Keister DB*, Muratova OV, and Kaslow DC. Malaria Vaccine Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.

328 CLONING OF THE FUSED DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE (DHFR-TS) GENE OF *PLASMODIUM FALCIPARUM* IN THE SHUTTLE VECTOR PYES2. Tsai YL*, and Krogstad DJ. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

329 IMMUNOGENIC PROPERTIES OF THE N- AND C-TERMINAL REGIONS OF THE *PLASMODIUM VIVAX* MSP1 PROTEIN AFTER INJECTION OF DNA. Levitus GL*, Hoffman SL, and del Portillo HA. Departamento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, Brasil; and Malaria Program, Naval Medical Research Institute, Rockville, MD.

LATE BREAKERS IN MOLECULAR BIOLOGY

Monday, November 20, 1995 Pecos
9:00 - 11:30 AM
Chairs: K. Sim and A. James

SCIENTIFIC SESSION P: FILARIASIS - MOLECULAR BIOLOGY / CHEMOTHERAPY

Monday, November 20, 1995 Pecan
9:00 - 12:00 AM
Chairs: A. Scott and C. Maina

9:00 330 EST ANALYSIS AND ANTIGEN DISCOVERY FROM *BRUGIA MALAYI* L3 cDNA LIBRARIES. Scott AL*, Raghavan N, Ghosh I, Blaxter M, Lu W, and Williams SA. Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.; and Biology, Imperial College, London; Biological Sciences, Smith College, Northampton, MA.

9:15 331 MOLECULAR CLONING OF AN *ONCHOCERCA VOLVULUS* CYSTEINE PROTEASE ESSENTIAL FOR THE SUCCESSFUL MOLTING OF THIRD-STAGE LARVAE. Lustigman S *, Shah K, Huima T, Hough M, McKerrow JH, and Brotman B. Virology and Parasitology, The Lindsley F. Kimball Research Institute, New York, NY; Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia; Department of Pathology, University of California, San Francisco, CA.

9:30 332 A THIOL-SPECIFIC ANTIOXIDANT FROM *BRUGIA MALAYI* L3'S. Ghosh I*, Raghavan N, and Scott AL. Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

9:45 333 PROTECTIVE IMMUNITY IN ONCHOCERCIASIS: IDENTIFICATION OF NOVEL LARVAL ANTIGENS RECOGNIZED BY SERUM FROM INDIVIDUALS RESISTANT TO INFECTION. McCarthy JS*, Raghavan NK, Scott AL, Lu WH, Williams SA, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Johns Hopkins University, Baltimore MD; Smith College, Northampton MA.

10:00 Coffee Break

10:30 334 A POTENTIALLY PROTECTIVE *ONCHOCERCA VOLVULUS* ANTIGEN IS A NEURONAL PROTEIN. Erttmann KD*, BÅttner DW, and Gallin MY. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany.

10:45 335 A *DIROFILARIA IMMITIS* L3 cDNA CLONE WITH HOMOLOGY TO A VESPID VENOM ALLERGEN ANTIGEN 5. Tripp CA*, Mika-Grieve M, Frank GR, Rushlow MB, and Grieve RB. Paravax, Inc., Fort Collins, CO.

11:00 336 CLONING AND CHARACTERIZATION OF ECDYSONE RECEPTOR AND *ULTRASPIRICLE* HOMOLOGS FROM A FILARIAL PARASITE, *DIROFILARIA IMMITIS*. Shea C*, Richer J, Hough DM, and Maina CV. Molecular Parasitology Group, New England Biolabs, Inc., Beverly, MA.

11:15 285 CLONING AND EXPRESSION OF *ANCYLOSTOMA CANINUM* ANTICOAGULANT PEPTIDE-1 (ACAP-1): A NOVEL HOOKWORM-DERIVED INHIBITOR OF HUMAN COAGULATION FACTOR XA. Cappello M*, Hawdon JM, Jones BF, and Hotez PJ. Departments of Pediatrics and Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

11:30 337 PRACTICAL AND THEORETICAL IMPLICATIONS OF A REPEATED DNA SEQUENCE OF *WUCHERERIA BANCROFTI*. Hamburger J*, Abassi I, Ramzy R, Gad A, Anjilli C, Mbogo C, Agure R, Ochola J, Githure J, and Williams SA. Hebrew University, Jerusalem, Israel; Ain Shams University, Cairo, Egypt; Kenya Medical Research Institute, Kenya; and Smith College, Northampton, MA.

11:45 338 FURTHER CHARACTERIZATION OF THE FILARICIDE UMF-078, A BENZIMIDAZOLE CARBAMATE, AGAINST LYMPHATIC INFECTIONS OF *BRUGIA PAHANGI* IN DOGS. Dzimianski MT*, McCall JW, Supakorndej P, and Jun JJ. Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, GA.

SCIENTIFIC SESSION Q: PHYSIOLOGY OF VECTOR COMPETENCE

Monday, November 20, 1995 Rio Grande - E
9:00 AM - 12:00 N
Chairs: J. Beier and C. Lowenberger

9:00 339 MOSQUITO CELLS, GENES, AND TRANSFORMATION SYSTEMS. Fallon AM*. Department of Entomology, University of Minnesota, St. Paul, MN.

9:15 340 GUT-SPECIFIC TRANSCRIPTIONAL REGULATORY ELEMENTS ARE CONSERVED BETWEEN BLACK FLIES AND *DROSOPHILA*. Xiong B, and Jacobs-Lorena M*. Case Western Reserve University, School of Medicine, Department of Genetics, Cleveland, OH.

9:30 341 CHARACTERIZATION OF PHENOLOXIDASE IN HEMOLYMPH AND MIDGUT OF ADULT *ANOPHELES STEPHENSI* MOSQUITOES. Sidjanski S*, Mathews GV, and Vanderberg JP. Department of Medical & Molecular Parasitology, New York University School of Medicine, New York, NY.

9:45 342 BIOCHEMICAL PATHWAY OF CHORION HARDENING IN *AEDES AEGYPTI*. Li JY*, Hodgeman BA, and Christensen BM. Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI.

10:00 Coffee Break

10:30 343 DOPA DECARBOXYLASE EXPRESSION AND REGULATION: EVAULATION WITH RESPECT TO DIVERSE BIOLOGICAL FUNCTION IN *AEDES AEGYPTI.* Ferdig MT*, Li JY, Severson DW, and Christensen BM. Department of AHABS, University of Wisconsin-Madison, Madison, WI.

10:45 344 IDENTIFICATION AND TEMPORAL EXPRESSION OF THREE ANTIBACTERIAL DEFENSINS PRODUCED BY *AEDES AEGYPTI* IN RESPONSE TO BACTERIAL CHALLENGE. Lowenberger CA*, Ferdig MT, Bulet P, Hetru C, Hoffmann JA, and Christensen BM. AHABS, University of Wisconsin, Madison WI; and IBMC, Strasbourg, France.

11:00 345 EXPRESSION OF CECROPIN A: AN ANTI-TRYPANOSOMAL PEPTIDE, IN ENDOSYMBIOTIC BACTERIA OF *RHODNIUS PROLIXUS.* Durvasula RV*, Aksoy A, Beard CB, and Richards FF. Department of Internal Medicine, Yale University School of Medicine, New Haven, CT; Centers for Disease Control, Atlanta, GA.

11:15 346 AMPLIFICATION OF *PLASMODIUM FALCIPARUM* DNA FROM SPOROZOITE-INFECTED ANOPHELINES. Toure YT*, Koita O, Doumbo O, Cogswell FB, Kaslow DB, Keister DB, Beier JC, and Krogstad DJ. National School of Medicine and Pharmacy, Bamako, Mali; National Institutes of Health, Bethesda, MD; and Tulane University, Covington and New Orleans, LA.

11:30 347 INEFFICIENT SPOROGONIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN THE VECTOR *ANOPHELES GAMBIAE* IN MALI, WEST AFRICA. Vernick KD*, and Toure YT. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; and Ecole Nationale de Medecine et de Pharmacie, Bamako, Mali.

11:45 348 REGULAR PRODUCTION OF INFECTIVE MALARIA SPOROZOITE IN COLONIZED *ANOPHELES ALBIMANUS* MOSQUITOES. Hurtado S, Salas ML, Romero JF, Ortiz H, Herrera S, and Herrera MA*. Instituto de Inmunologia del Valle, Universidad del Valle, Cali, Colombia.

SCIENTIFIC SESSION R: MALARIA - CHEMOTHERAPY II

Monday, November 20, 1995 Rio Grande - C
9:00 AM - 12:00 N
Chairs: G. Posner and D. Wesche

9:00 349 EVIDENCE FOR Fe(IV)=O IN THE MOLECULAR MECHANISM OF ACTION OF THE TRIOXANE ANTIMALARIAL ARTEMISININ. Posner GH*, Cumming JN, Ploypradith P, and Oh CH. Department of Chemistry, The Johns Hopkins University, Baltimore, MD, USA; and Department of Chemistry, Inje University, Kimhae, Korea.

9:15 350 MECHANISM OF CARDIOTOXICITY OF HALOFANTRINE. Wesche DL*, Chen Y, Wang W, Schuster BG, and Woosley RL. Div. of Clinical Pharmacology, Department of Pharmacology, Georgetown University Medical Center, Washington, DC; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

9:30 351 A *PLASMODIUM FALCIPARUM* BIOASSAY FOR THE MEASUREMENT OF TOTAL ANTIMALARIAL ACTIVITY OF HALOFANTRINE AND ITS METABOLITE(S) IN PLASMA. Taamasri P, Kyle DE*, Schuster BG, and Wesche DL. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Phramongkutklao College of Medicine, Bangkok, Thailand.

9:45 352 PHARMACOKINETIC (PK) AND PHARMACODYNAMIC (PD) OF A SINGLE DOSE OF ARTEMETHER (AM) BY THREE ROUTES OF ADMINISTRATION IN HEALTHY CAUCASIAN VOLUNTEERS. Teja-Isavadharm P *, White NJ, Brewer TG, Peggins JO, Nosten F, and Kyle DE. Department of Immunology & Parasitology, Armed Forces Research Institute of Medical Sciences, Bangkok,Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; and Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford.

10:00 Coffee Break

10:30 353 THE EFFECTS OF ARTEMISININ DERIVATIVES ON MALARIA TRANSMISSABILITY. Price R*, Nosten F, Luxemburger T, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University Bangkok, Thailand.

10:45 354 ARTEMETHER EFFICACY IN THE CEREBRAL MALARIA MODEL: *PLASMODIUM COATNEYI* IN THE RHESUS MONKEY. Todd GD*, Morris CL, Sullivan JS, Aikawa M, and Collins WE. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA.

11:00 355 AZITHROMYCIN COMPARED TO DOXYCYCLINE FOR MALARIA CHEMOPROPHYLAXIS IN WESTERN KENYA. Andersen SL*, Oloo AJ, Gordon DM, Klotz FW, Aleman GM, Ogutu BO, Koech DK, Berman JD, and Shanks GD. US Army Medical Research Unit-Kenya; Kenya Medical Research Institute, Nairobi, Kenya; and Walter Reed Army Institute of Research, Washington, DC.

11:15 356 SAFETY, TOLERANCE, PHARMACOKINETICS AND PRELIMINARY ANTIMALARIAL EFFICACY OF WR 238605 IN MAN. Brueckner RP*, Coster T, Wesche DL, Shmuklarsky M, Lasseter KC, and Schuster BG. Division of Experimental Therapeutics, WRAIR, Washington, DC; Medical Division, USAMRIID, Ft Detrick, Frederick, MD; and Clinical Pharmacology Associates, Miami, FL.

11:30 357 USE OF ANTISENSE OLIGODEOXYNUCLEOTIDES AGAINST *PLASMODIUM FALCIPARUM*. Barker Jr. RH*, Metelev V, Rapaport E, Agrawal S, and Zamecnik PC. Worcester Foundation for Experimental Biology, Shrewsbury, MA; and Hybridon, Inc., Worcester MA.

11:45 358 THE RIBOSOMAL RNAS OF *PLASMODIUM FALCIPARUM* AS A DRUG TARGET. McConkey GA*, Rogers MJ, Li J, and McCutchan TF. Growth and Development Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.

SCIENTIFIC SESSION S: HEPATITIS

Monday, November 20, 1995 Nueces / Frio
9:00 - 11:45 AM
Chairs: B. Innis and L. Binn

9:00 359 SEROPREVALENCE OF HEPATITIS B AMONG SCHOOL-AGED CHILDREN IN THE STANN CREEK DISTRICK OF BELIZE, CENTRAL AMERICA. Chamberlin J*, Bryan JP, Jones DL, Reyes L, and Hakre S. Department of Preventive Medicine and Biometrics, Uniformed Services Univ of the Health Sciences, Bethesda, MD; and Epidemiology Research Center, Ministry of Health, Belize City, Belize.

9:15 360 PREVALENCE OF HEPATITIS C INFECTION AMONG PERUVIANS. Sanchez JL*, Sjogren MH, Watts DM, Chauca G, Callahan J, Hinostroza S, Ramos-Garcia S, Carrillo-Carrillo L, Cardenas R, Cabezas C, Rodriguez G, and Estacio-Rojas C. U.S. Army Medical Research Unit-Brazil, Rio de Janeiro, Brazil; Walter Reed Army Medical Center, Washington, DC; U.S. Navy Medical Research Institute Detachment-Lima, Lima, Peru; Clinica Santa Isabel, San Borja, Lima, Peru; and Hospital Edgardo Rebagliatti M.

9:30 361 EPIDEMIOLOGICAL EVIDENCE FOR FAMILIAL TRANSMISSION OF HEPATITIS C VIRUS IN RURAL EGYPT. Kamel MA, Miller FD*, Baraket R, Ghaffer Y, and Troonen H. School of Public Health, University of Hawaii, Honolulu, HI; Faculty of Medicine, Ain Shams University, Cairo, Egypt; Abbott GmbH Diagnostika, Wiesbaden, Germany; High Institute of Public Health, Alexandria University, Alexandria, Egypt; and Faculty of Medicine, Cairo University, Cairo, Egypt.

9:45 362 CROSS-SECTIONAL PROFILE OF EPIDEMIC/SPORADIC HEPATITIS E VIRUS TRANSMISSION WEST KALIMANTAN, BORNEO, 1994. Putri MP*, Lubis I, Jarot K, Orndorff G, Punjabi NH, Tan R, Wignall SF, Graham RR, and Corwin AL. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; National Institute of Health Research, Jakarta, Indonesia; and Dinas Kesehatan, Kabupaten Sintang, West Kalimantan, Indonesia.

10:00 Coffee Break

10:30 363 A WATER-BORNE OUTBREAK OF HEPATITIS E VIRUS TRANSMISSION IN THE SOUTHWEST OF VIETNAM. Corwin AL*, Ha BK, Clayson ET, Pham KS, Vo TT, Vu TY, Cao TT, Vaughn D, Merven J, Richie TL, Wignall FS, and Hyams KC. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; Pasteur Institute, Ho Chi Minh City, Republic of Vietnam; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Abbott Laboratories (Singapore), PTE Ltd; and U.S. Naval Medical Research Institute , Bethesda, MD.

10:45 364 ACUTE HEPATITIS IN HANOI, VIETNAM, 1993-1995. Dao DD*, Tran CD, Hoang TN, Clayson E, Vaughn D, Kanti L, Widjaja S, Punjabi NH, Graham RR, Wignall FS, Hyams KC, and Corwin AL. Institute of Clinical Research in Tropical Medicine, Hanoi, Vietnam; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; and Naval Medical Research Institute, Bethesda MD.

11:00 365 MIXED VIRUS INFECTIONS IN PATIENTS WITH HEPATITIS E. Binn LN*, Asher LV, Longer CF, Martinez E, Miele T, Ticehurst J, Hoke C, and Innis BL. The Walter Reed Army Institute of Research, Department of Virus Diseases, Washington DC; and Johns Hopkins University School of Medicine, Department of Pathology, Baltimore, MD.

11:15 366 VIROLOGIC AND IMMUNOLOGIC EVENTS DURING ACUTE HEPATITIS E. Clayson ET*, Myint KS, Snitbhan R, Vaughn DW, Innis BL, Chan L, Cheung P, and Shrestha MP. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC; Genelabs Diagnostics, Singapore; Infectious Disease Hospital, Kathmandu, Nepal.

11:30 367 BACULOVIRUS EXPRESSION OF THE HEPATITIS E VIRUS (HEV) GENE SEGMENT CONTAINING THE FULL LENGTH COMBINED OPEN READING FRAME (ORF) 2 AND 3. Zhang HY*, Burrous J, Zhao BT, Caudill JD, Gandre HC, Putnak R, Warren R, and Longer CF. Dept of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

SYMPOSIUM:
BACTERIAL SYMBIONTS AND ARTHROPOD GENETIC MANIPULATIONS

Monday, November 20, 1995 Chula Vista
9:00 - 12:00
Chairs: A. Azad and S. O'Neill

9:00 SYMBIONTS OF FLEAS: MOLECULAR INTERACTIONS WITH INVERTEBRATE AND VERTEBRATE HOSTS. Higgins JA and Azad AF. University of Maryland School of Medicine, Baltimore, MD.

9:30 BACTERIAL SYMBIONTS OF THE CHAGAS' DISEASE VECTOR *RHODNIUS PROLIXUS* AS VEHICLES FOR EXPRESSING AND SECRETING POTENTIAL ANTI-TRYPANOSOMAL GENES. Beard CB and Durvasula R. CDC, Atlanta, GA.

10:00 INHERITED *WOLBACHIA* INFECTIONS IN ARTHROPODS. Braig H and O'Neill S. Yale University School of Medicine, New Haven, CT.

10:30 Coffee Break.

11:00 THE ENDOSYMBIONTS OF TSETSE FLIES AND THEIR INTERACTIONS WITH TRYPANOSOMES. Aksoy S, Chow A, Pourhouseini A and Narasimhan S. Yale University School of Medicine, New Haven, CT.

11:30 SYMBIONTS THAT ALTER REPRODUCTION IN HOSTS. Werren JH. University of Rochester, NY.

SYMPOSIUM:
NEW TARGET *PLASMODIUM FALCIPARUM* ANTIGENS FOR VACCINE DEVELOPMENT: A PANEL DISCUSSION

Monday, November 20, 1995 Rio Grande W
9:00 - 11:30 AM
Chair: C. Diggs

During the last decade, a number of clinical trials have been conducted of vaccines based on the circumsporozoite protein of *Plasmodium falciparum* and on the hybrid antigen peptide SPf66. In spite of some successes, most authorities agree that substantial progress must be made before deployment of malaria vaccines is practical. Two years ago the UNDP/World Bank/WHO/Special Programme for Tropical Disease Research and Training (TDR) held a meeting of experts to identify blood stage antigens that were sufficiently characterized to warrant development of experimental vaccines for human trials. Initiatives to test vaccines based on a number of the antigens selected (MSP-1, AMA-1, SERA) are now in progress. Among the most urgent questions to be answered

now is which among the putative target antigens not yet being prepared for testing in humans demonstrate the greatest potential as vaccine constituents. An updated assessment of antigens from all stages of the parasite will be very useful to vaccine developers engaged in planning next initiatives. This Panel Discussion will be designed to provide such an assessment.

Panel members will introduce antigens they consider most promising for vaccine development efforts. Additional suggestions will be sought from the audience. After the brief presentations, there will be a guided discussion in which each of the antigens introduced will be assessed according to criteria designed to assess their merit and readiness.

SYMPOSIUM:
ALLEN CHEEVER SYMPOSIUM ON THE QUANTITATIVE PATHOPHYSIOLOGY OF SCHISTOSOMIASIS

Monday, November 20, 1995 Blanco/Llano
9:00 - 12:00 N
Chairs: F. von Lichtenberg and F. Neva

9:00 INTRODUCTION. Neva FA. NIH, Bethesda, MD.

9:10 HOW IT ALL BEGAN. Weller TH. Harvard University School of Public Health, Boston, MA.

9:20 HEPATIC EXTRACELLULAR MATRIX CHANGES IN SCHISTOSOMIASIS. Andrade Z. Federal University of Bahia, Bahia, Brazil.

9:40 EXPERIMENTAL SCHISTOSOMIASIS IN PRIMATES AND MICE. von Lichtenberg F. Harvard University Medical School, Boston, MA.

10:00 CHEEVER IN EGYPT. Smith JH. University of Texas, Galveston, TX.

10:15 Coffee Break.

10:45 INTRODUCTION OF DR. BARRAL. Cheever L. Johns Hopkins School of Medicine, Baltimore, MD.

10:55 CYTOKINE REGULATION IN HUMAN LEISHMANIASIS AND COMPARISON TO RESPONSES IN SCHISTOSOMIASIS. Barral-Netto M. University of Bahia, Bahia, Brazil.

11:15 INTRODUCTION OF DRS. TOM WYNN AND TED NASH. Sher A. NIH, Bethesda, MD.

11:25 CHEEVER, GRANULOMAS, CYTOKINES, FIBROSIS AND ME. Wynn T. NIH, Bethesda, MD.

11:45 WHAT I LEARNED ABOUT SCHISTOSOMIASIS FROM CHEEVER-SCIENCE PLUS MORE. Nash T. NIH, Bethesda, MD.

SOPER LECTURE

Monday, November 20, 1995 Regency Ballroom East/Center
1:30 - 2:15
Chair: G. Hill

LEISHMANIASIS: NEW LESSONS FROM AN OLD DISEASE.

W. Johnson
Cornell University, Ithaca, NY.

SYMPOSIUM:
AMERICAN COMMITTEE ON CLINICAL TROPICAL MEDICINE AND TRAVELERS HEALTH

Monday, November 20, 1995 Rio Grande C
2:30 - 6:00 PM
Chair: S. Hoffman

2:30 ***Vincenzo Marcolongo Memorial Lecture:*** TUBERCULOSIS: DEVELOPMENTS IN EPIDEMIOLOGY, DIAGNOSIS, TREATMENT AND PREVENTION. Ellner JJ. Case Western Reserve University, Cleveland, OH.

3:15 Question Period.

3:30 Coffee Break.

3:45 Update and Roundtable on Malaria Prophylaxis. Zucker J, CDC, Atlanta, GA; Keystone J, Toronto General Hospital, Toronto, Canada; Steffen R, ISPM, Zurich, Switzerland.

4:30 Plague into the 21st Century. Dennis D. CDC, Fort Collins, CO.

5:00 Business Meeting.

SYMPOSIUM: Spf66 SYNTHETIC PEPTIDE MALARIA VACCINE

Monday, November 20, 1995 Rio Grande W
2:30 - 6:00 PM
Chair: W. Ballou and M. Patarroyo

2:30 MOLECULAR CHARACTERIZATION OF THE Spf66 SYNTHETIC PEPTIDE VACCINE. Hagopian R. Multiple Peptide Systems, San Diego, CA.

2:50 PHASE III STUDIES OF Spf66 IN LA TOLA AND RIO ROSARIO, COLOMBIA. Valero MV. Instituto de Inmunologia, Colombia.

3:10 PHASE I SAFETY AND IMMUNOGENICITY STUDIES OF Spf66 IN THE GAMBIA. D'Alessandro U. Medical Research Council Laboratories, The Gambia.

3:30 SAFETY AND IMMUNOGENICITY OF Spf66 IN MALARIA NAIVE AND MALARIA IMMUNE ADULTS AND CHILDREN IN THAILAND. Heppner DG. AFRIMS, Bangkok, Thailand.

3:50 EPIDEMIOLOGIC CONSIDERATIONS FOR A PHASE IIB EFFICACY TRIAL OF Spf66 IN SHOKLO, THAILAND. Luxemburger C. Shoklo Malaria Research Unit, Thailand.

4:10 PHASE IIB TRIAL OF THE EFFICACY OF Spf66 IN KAREN CHILDREN (SHOKLO, THAILAND). Nosten F. Shoklo Malaria Research Unit, Thailand.

4:40 PHASE III TRIAL OF THE EFFICACY OF Spf66 IN GAMBIAN INFANTS (THE GAMBIA). D'Alessandro U. Medical Research Council Laboratories, The Gambia.

5:10 EXPANDED PHASE III TRIALS OF Spf66 IN TANZANIA: RATIONALE AND PLANS.

5:40 DISCUSSION

SYMPOSIUM:
BURROUGHS WELLCOME MOLECULAR PARASITOLOGY SYMPOSIUM
"GENETIC ANALYSIS OF HOST-PARASITE INTERACTIONS"

Monday, November 20, 1995 Chula/Vista
2:30 - 6:00 PM
Chair: S. Beverley

2:30 MAPPING VIRULENCE GENES OF *TOXOPLASMA*. Sibley D. Washington University, St. Louis, MO.

3:15 GENETIC ANALYSIS OF GAMETOGENESIS IN *PLASMODIUM FALCIPARUM*. Vaidya A. Hahnemann University, Philadelphia, PA.

4:00 Coffee Break.

4:30 IDENTIFICATION OF *LEISHMANIA* LIPOPHOSPHOGLYCAN (LPG) BIOSYNTHETIC GENES RELEVANT TO SURVIVAL IN THE MAMMALIAN AND SAND FLY HOSTS. Beverley S. Harvard Medical School, Boston, MA.

5:15 TGF-B SIGNALING IS REQUIRED FOR INVASION BY *TRYPANOSOMA CRUZI*. Pereira M. Tufts-New England Medical Center, Boston, MA.

AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES SYMPOSIUM: EBOLA: THE RETURN OF THE DEADLY VIRUS

Monday, November 20, 1995 Regency Ballroom-Center
2:30 PM - 6:30 PM
Chairs: T. Ksiazek and G. Clark

2:30 Business Meeting

3:30 Scientific Session

3:30 INTRODUCTION. Johnson KM.

3:50 THE EXPERIENCE IN THE IVORY COAST. Leguenno B.

4:10 AN OVERVIEW OF ZAIRE. Rollin P.

4:30 Coffee Break.

4:45 THE VIRUSES FROM IVORY COAST AND ZAIRE. Sanchez A.

5:05 CLINICAL MANIFESTATIONS. Calain P.

5:25 ECOLOGICAL INVESTIGATIONS. Ksiazek T.

5:45 FROM ZAIRE TO _____. Peters CJ.

6:05 DISCUSSION.

SCIENTIFIC SESSION T: MALARIA BIOLOGY / MOLECULAR BIOLOGY

Monday, November 20, 1995 Rio Grande E
2:30 - 6:30 PM
Chairs: U. Frevert and M. Galinski

2:30 368 CELL SURFACE GLYCOSAMINOGLYCANS ARE NOT REQUIRED FOR *PLASMODIUM BERGHEI* SPOROZOITE INVASION *IN VITRO*. Frevert U*, Sinnis P, and Nussenzweig V. Department of Molecular and Medical Parasitology, New York University Medical Center, New York, NY; and Department of Pathology, New York University Medical Center, New York, NY.

2:45 369 *PLASMODIUM BERGHEI* SPOROZOITES RELEASE CS PROTEIN INTO THE HOST CELL CYTOPLASM. HÅgel FU*, Pradel G, and Frevert U. Department of Molecular and Medical Parasitology, New York University Medical Center, New York, NY.

3:00 370 INCREASED EXPRESSION OF ICAM-1 AND VCAM-1 IN THE BRAINS OF MICE INFECTED WITH *PLASMODIUM YOELII* 17XL A MODEL OF CEREBRAL MALARIA IN MICE. Das B, Berman JW,

Buchwald D, Nagel RL, and Shear HL*. Division Hematology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; and Department Pathology, Albert Einstein College of Medicine, Bronx NY.

3:15 371 STUCTURE-FUNCTION OF THE PfEMP1 DOMAIN MEDIATING THE BINDING OF *PLASMODIUM FALCIPARUM* PARASITIZED ERYTHROCYTES TO CD36. Baruch DI*, Singh HB, Ma XC, Bi X, Pasloske BL, and Howard RJ. Affymax Research Institute, Santa Clara, CA.

3:30 Coffee Break

4:00 372 SWITCHES IN EXPRESSION OF *PLASMODIUM FALCIPARUM VAR* GENES CORRELATE WITH CHANGES IN ANTIGENIC AND CYTOADHERENT PHENOTYPES OF INFECTED ERYTHROCYTES. Smith JD*, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson DS, Pinches R, Newbold CI, and Miller LH. Laboratory of Parasitic Diseases, National Institute of Health, Bethesda, MD; and Molecular Parasitology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

4:15 373 GENES ENCODING VARIANT ANTIGENS EXPRESSED ON THE SURFACE OF *PLASMODIUM KNOWLESI*-SCHIZONT-INFECTED ERYTHROCYTES. Al-Khedery B*, Barnwell JW, Galinski MR. New York University School of Medicine, Department Medical and Molecular Parasitology, New York, NY.

4:30 374 CONSERVATION OF STRUCTURE AND FUNCTION OF THE ERYTHROCYTE BINDING DOMAIN OF *PLASMODIUM FALCIPARUM* EBA-175. Liang H *, and Sim BK. EntreMed, Rockville, MD.

4:45 375 POST-TRANSLATIONAL MODIFICATION OF THE *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTE MEMBRANE CORRELATES WITH EXPRESSION OF MESA. Magowan CC*, Coppel RL, Esperanza RG, and Narla M. Lawrence Berkeley Laboratory, Berkeley CA; and Monash University, Department of Microbiology, Clayton, Victoria, Australia.

5:00 376 IDENTIFICATION AND CLONING OF ERYTHROCYTE BINDING PROTEIN GENE HOMOLOGUES IN THE RODENT MALARIA SPECIES *PLASMODIUM YOELII* AND *PLASMODIUM BERGHEI*. Kappe SH*, Curley GP, Dalton JP, and and Adams JH. Department of Biological Sciences, University of Notre Dame, Notre Dame,IN; and School of Biological Sciences, Dublin City University, Glasnevin, Dublin, Republic of Ireland.

5:15 377 PCR CHARACTERIZATION OF *PLASMODIUM VIVAX* ISOLATES FROM COLOMBIA, SOUTH AMERICA. Alger J*, Acosta MC, Saravia NG, and Krogstad DJ. Tulane School of Public Health and Tropical Medicine, New Orleans, LA; and Fundacion CIDEIM, Cali, Colombia.

5:30 378 A CLONE OF AN ANTIGEN CONTAINING EPITOPES THAT ARE COMMON TO *PLASMODIUM FALCIPARUM* AND *PLASMODIUM YOELII*. Kironde FA*, Ma H, Ray P, Sahoo N, Patra LK, Paliwal S, and Singh B. International Centre for Genetic Engineering and Biotechnolology, New Delhi, India; and Vector Control Research Centre, Medical Complex, Pondicherry, India.

5:45 379 STABLE TRANSFORMATION OF *PLASMODIUM FALCIPARUM* WITHIN HUMAN ERYTHROCYTES: REPLICATING EPISOMES AND HOMOLOGOUS INTEGRATION. Wu Y*, and Wellems TE. Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD.

6:00 380 A NOVEL PROTECTIVE 67-KDA INTERSPECIES-CONSERVED INTEGRAL MEMBRANE ANTIGEN OF *PLASMODIUM FALCIPARUM*. Ma H*, Ray P, Paliwal S, Patra KK, Sahoo N, Singh B, and Kironde FA. International Centre for Genetic Engineering and Biotechnolology, New Delhi, India; and Vector Control Research Centre, Medical Complex, Pondicherry, India.

6:15 381 IDENTIFICATION OF PUTATIVE GENE EXPRESSION CONTROL ELEMENTS IN OOKINETTE SURFACE PROTEIN PGS28 OF *PLASMODIUM GALLINACEUM*. Mbacham WF*, Budge E, Golightly LM, Ruel K, Sullivan TJ, and Wirth DF. Department of Tropical Public Health, Harvard School of Tropical Health, Boston, MA.

SCIENTIFIC SESSION U:
EPIDEMIOLOGY AND CHEMOTHERAPY OF THE KINETIPLASTIDAE

Monday, November 20, 1995 Blanco / Llano
2:30 - 6:15 PM
Chairs: S.F. Kerr and J.E. Jackson

2:30 *KEYNOTE ADDRESS:* LEISHMANIASIS IN TEXAS: EPIDEMIOLOGY AND ECOLOGY. McHugh C.

3:00 383 CRITERIA FOR PRIMARY RESERVOIR ADAPTED TO WILD HOSTS OF LEISHMANIASES IN THE NEW WORLD. Van Wynsberghe NR*, Canto-Lara SB, Andrade-Narvaez FJ, and Itza-Ortiz MF. Department of Immunology, Center of Regional Research (CIR), University of Yucatan (UADY), Merida, Yucatan, Mexico.

3:15 384 IS CUTANEOUS LEISHMANIASIS DUE TO *LEISMANIA TROPICA* A ZOONOSIS IN NORTHERN JORDAN? Kamhawi SA*, Abdel-Hafez SK, Al-Daher HA, and Qadoumi MZ.

3:30 Coffee Break

4:00 385 DETECTION OF *LEISHMANIA* INFECTED RESERVOIR HOSTS USING THE POLYMERASE CHAIN REACTION. Carrion R*, Melby PC, McHugh CP, Alvarez RA, and Kerr SF. Department of Biology, Incarnate Word College, San Antonio, TX; and The University of Texas Health Science Center, San Antonio, TX; Armstrong Laboratory, Brooks Air Force Base, TX.

4:15 386 PREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION IN A SOUTHEASTERN REGION OF GUATEMALA. Powell MR*, Nix NA, Hernandez B, Arana FE, Ramirez L, and Greer GJ. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Medical Entomology Research and Training Unit (MERTU/G), USEMB/HHS/MERTU, Guatemala City, Guatemala.

4:30 387 SEROPREVALENCE OF *TRYPANOSOMA CRUZI*-ANTIBODIES IN A SUBPOPULATION OF AMERICAN RED CROSS BLOOD DONORS. Leiby DA*, Read EJ, Lenes BA, Pan AA, Stumpf RJ, and Dodd RY. Transmissible Diseases Department, American Red Cross, Rockville, MD; Southern California Region, American Red Cross, Los Angeles, CA; South Florida Region, American Red Cross, Miami, FL; and Abbott Laboratories, Abbott Park, IL.

4:45 388 NEW FINDINGS AND SIMPLIFIED CLINICAL STAGING OF AFRICAN TRYPANOSOMIASIS. McGovern TW*, Williams W, Fitzpatrick JE, Cetron MS, Hepburn BC, and Gentry RH. Dermatology Service, Fitzsimons Army Medical Center, Aurora, CO; Infectious Disease Service, Fitzsimons Army Medical Center, Aurora, CO; Division of Parasitic Diseases, National Center for Infectious Diseases, Atlanta, GA; and Department of Family Practice, US Air Force Academy, CO.

5:00 389 DEVELOPMENT OF AN *IN VITRO LEISHMANIA* TEMPERATURE SENSITIVITY MODEL: PRELIMINARY STUDIES WITH STRAINS WITH ABERRANT TROPISM *IN VIVO*. Callahan HL, Portal IF, and Grogl M. U.S.A. Medical Research Unit-Brazil, Walter Reed Army Institute of Research.

5:15 390 REINVENTING ANTILEISHMANIALS: MODERN ANTILEISHMANIALS FROM TRADITIONAL HERBAL THERAPY. Jackson JE*, Okunji CO, Tally JD, Iwu MM, Hanson WL, Waits VB, Nolan LL, and Schuster BG. Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria; College of Veterinary Medicine, University of Georgia, Athens, GA; and Division of Public Health, University of Massachusetts, Amherst, MA.

5:30 391 PARASITICIDAL MECHANISMS IN MACROPHAGES INFECTED BY *LEISHMANIA DONOVANI* FOLLOWING TREATMENT *IN VITRO* WITH LAK CELLS AND/OR PENTOSTAM. Eslami Z*, and Tanner CE. Institute of Parasitology, McGill University, Quebec, Canada.

5:45 392 INTERFERON-γ AND INTERLEUKIN-5 PRODUCTION FOLLOWING CHEMOTHERAPY OF MURINE VISCERAL LEISHMANIASIS WITH THE 8-AMINOQUINOLINE, WR6026. Shin SS*, and Hanson WL. Department of Parasitology, College of Veterinary Medicine, The College of Georgia, Athens, GA.

6:00 393 ALLOPURINOL IN THE TREATMENT OF CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS: A SUMMARY OF CLINICAL STUDIES. Marr JJ, Modabber F, Martinez S, and Llanos-Cuentas. Ribozyme Pharmaceuticals, Boulder, Colorado; World Health Organization, Geneva, Switzerland; University of Popayan, Popayan, Colombia; Alexander von Humboldt Institute, Lima, Peru; and the Latin American Investigative Group.

SCIENTIFIC SESSION V: SCHISTOSOMIASIS: DIAGNOSIS, CHEMOTHERAPY, EPIDEMIOLOGY

Monday, November 20, 1995 Nueces / Frio
2:30 - 6:15 PM
Chairs: V. Tsang and S. McGovern

2:30 *KEYNOTE ADDRESS:* DRUG ACTION AND DRUG RESISTANCE IN SCHISTOSOMIASIS. Brindley P. Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

3:00 394 APPLICATION OF IMMUNODIAGNOSIS ASSAYS IN FIELD STUDIES: I. DETECTION OF ANTIBODIES TO MAMA AND HAMA IN HUMAN SCHISTOSOMIASIS STUDY SURVEY. Osman AM*, Abdelfatah M, Abdelmoneim E, Al-Sherbiny MM, Galal N, and Tsang VC. Immunology Division, Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt; Egyptian Reference Diagnostic Center, VACSERA, Cairo, Egypt; Division of Parasitic Diseases, Centers of Disease Control, Atlanta, GA; and Schistosomiasis Research Project, Ministry of Health, Cairo, Egypt.

3:15 395 FIELD APPLICABLE METHOD FOR DETECTION OF ANTIBODIES TO *SCHISTOSOMA* SPECIES AND GENUS SPECIFIC ANTIGENS USING DIPSTICKS. Al-Sherbiny MM*. Egyptian Reference Diagnostic Center, VACSERA, Cairo, Egypt.

3:30 Coffee Break

3:45 396 DETECTION OF CIRCULATING ANTIGENS IN PATIENTS WITH ACTIVE *SCHISTOSOMA HAEMATOBIUM* INFECTION. Hassan MM*, Medhat A, Shata MT, and Strand M. Parasitology Department, Zagazig Faculty of Medicine; Tropical Medicine Department, Assuit Faculty of Medicine; Microbiology Department, Assuit Faculty of Medicine, Egypt; and Pharmacology and Molecular Sciences, The Johns Hopkins University, School of Medicine, Baltimore, MD.

4:00 397 CORRELATIONS BETWEEN ANTIBODY RESPONSES, CELL MEDIATED IMMUNITY,AND CIRCULATING ANODIC ANTIGEN LEVELS IN A RURAL COMMUNITY IN ZIMBABWE. Ndhlovu PD*, Cadman H, Chidimu M, Vennervald B, Christensen NO, Chandiwana SK, Gundersen SG, and Deelder AM. Blair Research Laboratory, Zimbabwe; University of Zimbabwe, Harare Zimbabwe; Danish Bilharziasis Laboratory, Charlottenlund, Denmark; Department of Infectious Diseases and Department of Microbiology, Ullevaal Center for International Medicine, Research Forum, Ullevaal University Hospital, Oslo, Norway; and Laboratory of Parasitology, Medical Faculty, University of Leiden, Leiden, The Netherlands.

4:15 398 PRAZIQUANTEL RESISTANCE TO *SCHISTOSOMA MANSONI* IN EGYPT. Ismail MM*, Metwally AA, Farghaly AM, Benntt J, Coleman R, and Tao LF. Parasitology Department, Zagazig Faculty of Medicine, Zagazig, Egypt; Theodor Bilharz Institute, Cairo, Egypt; Pharmacology Department, Michigan State University, East Lansing, MI.; and Center for Tropical Diseases, University of Massachusetts, Lowell, MA.

4:30 399 EVALUATION OF SCHOOL BASED CONTROL PROGRAMS ON *SCHISTOSOMA HAEMATOBIUM* AND *S. MANSONI* PREVALENCE AND INTENSITY OF INFECTION IN EGYPT. Husein MH, Talaat M*, El-Sayed MK, El-Badawi A, and Evans D. Theodor Bilharz Research Institute, Cairo, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Zagazig University, Zagazig, Egypt; Special Program for Reserach and Training, WHO, Geneva; and Faculty of Medicine, Cairo University, Cairo, Egypt.

4:45 400 CHANGING PATTERNS OF *SCHISTOSOMA MANSONI* AFTER SELECTIVE POPULATION CHEMOTHERAPY WITH PRAZIQUANTEL IN THE NILE DELTA, EGYPT. Barakat R*, Farghaly A, El Masry AG, ElSayed MK, Husein MH, and Miller FD. High Institute of Public Health, Alexandria University, Alexandria, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Cairo University, Cairo, Egypt; and School of Public Health, University of Hawaii, HI.

5:00 401 THE IMPACT OF CONTROL MEASURES ON URINARY SCHISTOSOMIASIS IN SCHOOL CHILDREN IN NORTHERN CAMEROON: A UNIQUE OPPORTUNITY FOR CONTROLLED OBSERVATIONS. Bausch DG, and Cline BL. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

5:15 402 SEROLOGIC PREVALENCE FOR SCHISTOSOMIASIS IN PUERTO RICO (1995) -- FIRST REPORT OF THE BILHARZIA COMMISSION, PR. Tsang VC*, Hillyer GV, Noh JC, Vivas-Gonzales BE, Ahn LH, Pilcher JB, Hightower AW, Deseda C, Feliciano de and Melicio C. Divison of Parasitic Diseases, NCID, Centers for Disease Control & Prevention, Atlanta, GA; Pathology Department, University of Puerto Rico School of Medicine, San Juan, PR; Office of the Secretary, Department of Health, Commonwealth of Puerto Rico, San Juan, PR.

5:30 403 GEOGRAPHIC INFORMATION SYSTEMS FOR CONTROL OF SCHISTOSOMIASIS IN BAHIA, BRAZIL. Bavia M*, Hale L, and Malone JB. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA; and College of Engineering, Louisiana State University, Baton Rouge, LA.

5:45 404 LONG-TERM AND ANNUAL COMMUNITY CHANGES IN HEPATIC FIBROSIS WITH ANTI-SCHISTOSOMAL TREATMENT IN NORTHEASTERN LEYTE, THE PHILIPPINES. McGarvey ST*, Olveda RM, Wiest PM, Aligui G, and Olds GR. International Health Institute and Department of Medicine, Brown University, Providence, RI; The Research Institute for Tropical Medicine, Alabang, Muntinlupa, Philippines; and Department of Medicine, MetroHealth Medical Center, Cleveland, OH.

6:00 405 HOW OFTEN SHOULD PRAZIQUANTEL (PZQ) THERAPY BE ADMINISTERED TO PATIENTS OF SCHISTOSOMIASIS MANSONI TO REDUCE INFECTION RATE AND LIVER MORBIDITY? Ali Homeida MM*. Faculty of Medicine, University of Khartoum, Khartoum, Sudan.

SCIENTIFIC SESSION W: GIARDIA AND TRICHOMONAS

Monday, November 20, 1995 Pecan
2:30 - 5:00 PM
Chairs: J. Yee and S. Das

2:30 406 IDENTIFICATION OF REGULATORY ELEMENTS WITHIN THE UPSTREAM SEQUENCE OF THE *GIARDIA LAMBLIA* GLUTAMATE DEHYDROGENASE GENE BY TRANSIENT TRANSFECTION. Yee J*, and Nash TE. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

2:45 407 IMMUNE RESPONSES OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS INFECTED WITH *GIARDIA LAMBLIA*. Soliman M, Taghi-Kilani R*, Hegazi MM, and Belosevic M. Departments of Biological Sciences and Immunology, University of Alberta, Edmonton, Canada; and Department of Medicine, Mansoura School of Medicine, Mansoura, Egypt.

3:00 408 STUDIES ON LIPID UPTAKE BY *GIARDIA* USING FLUORESCENT LIPID ANALOGS. Stevens TL*, Allison ML, Ellzey JT, Das S. Department of Biological Sciences, University of Texas at El Paso, El Paso, Texas.

3:15 409 RESISTANCE OF NATIVE TSA 417, A *GIARDIA LAMBLIA* VARIANT SURFACE PROTEIN, TO PROTEOLYSIS AND REDUCTION. Reiner DS*, Aley SB, and Gillin FD. Department of Pathology, University of California at San Diego, Medical Center, San Diego, CA.

3:30 Coffee Break

4:00 410 IDENTIFICATION OF A SARCOPLASMA-ENDOLASMIC RETICULUM CALCIUM ATPASE GENE IN *TRICHOMONAS VAGINALIS*. Li C*, Moate ME, Lushbaugh WB, Finley RW, and Meade JC. Division of Infectious Diseases, Department of Medicine, University of Mississippi Medical Center, Jackson, MS; and Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

4:15 411 GENETIC VARIATION IN *GIARDIA DUODENALIS*. Meloni BP*, Lymbery AJ, and Thompson RC. WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, Institute for Molecular Genetics and Animal Disease, School of Veterinary Studies, Murdoch University, Western Australia.

4:30 412 GIARDIASIS AMONG SHANTY-TOWN CHILDREN OF MOROCCO: EVALUATION OF HEALTH AND NUTRITIONAL STATUS. El Kadioui F *, Guessous-Idrissi N, Soubhi H, and Dehbi F. Laboratoire de Parasitologie-Mycologie, Hôpital Ibn Rochd Casablanca, Morocco; Service de Médecine Sociale, Faculté de Médecine, et de Pharmacie, Casablanca, Morocco; and Service de Pédiatrie IV, Hôpital d'Enfants, Casablanca, Morocco.

4:45 413 ENTERIC PARASITE PREVALENCE AND INTER-PARASITE ASSOCIATIONS, HAMILTON, ONTARIO. MacPherson DW*, McQueen RA, MacQueen W, Soo and Lum Y. Regional Parasitology Lab, St. Joseph's Hospital, McMaster University, Hamilton, Ontariao, Canada.

SCIENTIFIC SESSION X: OPPORTUNISTIC INFECTIONS

Monday, November 20, 1995 Ballroom East 1 & 2
2:30 - 6:15 PM
Chairs: S. Vermund and P. Walzer

2:30 414 PARTIAL DESCRIPTION OF THE LIFE CYCLE OF *CYCLOSPORA* AND HUMORAL RESPONSE IN ACUTE INFECTIONS. Ortega YR*, Miyagui J, Watanabe J, Kanagusuku P, Gilman RH, and Sterling CR. Veterinary Sciences Department, University of Arizona, Tucson, AZ; Policlinico Peruano-Japones, Lima, Peru; and Johns Hopkins University, Baltimore, MD.

2:45 415 AN OUTBREAK OF CRYPTOSPORIDIOSIS ASSOCIATED WITH WELL WATER, WASHINGTON. Dworkin MS*, Goldman D, Herwaldt BL, and Kobayashi J. CDC, Atlanta, GA; Washington State Department of Health, Seattle, WA; and U. S. Army Medical Corps, Tacoma, WA

3:00 416 DIRECT IMMUNIZATION OF SHEEP WITH DNA ENCODING A *CRYPTOSPORIDIUM PARVUM* ANTIGEN ELICITS PARASITE-SPECIFIC SERUM AND COLOSTRUM ANTIBODIES. Jenkins MC*, Kerr D, Fayer R, and Wall R. Parasite Immunobiology Laboratory, ARS, USDA, Beltsville, MD; AltraBio, Inc., Arden Hills, MN; and Gene Evaluation and Mapping Laboratory, ARS, USDA.

3:15 417 THE INFLUENCE OF ANTI-*CRYPTOSPORIDIUM* ANTIBODIES ON THE OUTCOME OF INFECTION IN HUMANS. Lammie PJ*, Moss DM, Hightower AW, Arrowood MJ, Chappell CL, and DuPont HL. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Center for Infectious Diseases, University of Texas School of Public Health, Houston TX.

3:30 622 ASSESSMENT OF ANTI-*CRYPTOSPORIDIUM* ANTIBODIES IN VOLUNTEERS BEFORE AND AFTER EXPERIMENTAL EXPOSURE TO OOCYSTS. Moss DM*, Lammie PJ, Hightower AW, Arrowood MJ, Chappell CL, and DuPont HL. Parasitic Diseases Division, CDC, Atlanta, GA; and Center for Infectious Diseases, School of Public Health, University of Texas at Houston, Houston, TX.

3:45 Coffee Break

4:00 418 GLUTATHIONE-S-TRANSFERASE ACTIVITY IN *CRYPTOSPORIDIUM PARVUM* OOCYSTS. Okhuysen PC*, Chappell CL, Chakravarthy S, Dang H, and Ali-Osman F. Center for Infectious Diseases, The University of Texas Health Science Center; Experimental Pediatrics, M.D. Anderson Cancer Center, and The University of Texas, Houston, TX.

4:15 419 AN *IN VITRO* MODEL 0F *CRYPTOSPORIDIUM PARVUM* INFECTION IN HUMAN BILIARY CELLS. Verdon R *, Pereira ME, Tzipori S, Keusch GT, Jefferson DM, and Ward HD. Division of Geographic Medicine and Infectious Diseases, New England Medical Center; Department of Cellular and Molecular Physiology, Tufts University School of Medicine; Division of Infectious Diseases, Tufts University School of Veterinary Medicine.

4:30 420 DOGS AS POTENTIAL MECHANICAL VECTORS OF *TOXOPLASMA GONDII*. Frenkel JK*, Lindsay DS, Parker BB. Department of Biology, University Of New Mexico, Albuquerque, NM; Department of Pathobiology, Auburn University, AL; and Arrighetti Animal Hospital, Santa Fe, NM.

5:45 421 INDUCTION OF IL-12 BY *TOXOPLASMA GONDII* IN INTERFERON-γ KNOCKOUT MICE. Scharton-Kersten TM*, Denkers EY, Gazzinelli RT, Grunvald E, Hieny S, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

5:30 422 EFFECT OF DINITROANILINES ON *TOXOPLASMA GONDII*. Stokkermans TW*, Roos DS, and Schwartzman JD. Dartmouth Medical School, Hanover NH; and University of Pennsylvania, Philadelphia PA.

5:45 423 MOLECULAR MONITORING OF *BABESIA MICROTI* PARASITEMIA: EVIDENCE OF CHRONIC INFECTION IN HUMANS. Persing DH*, Krause PJ, Telford SR, Sikand VJ, Christianson D , Cartter M , Magera J , and Spielman A. Hartford Hospital, Hartford, CT; Harvard School of Public Health, Boston, MA; Mayo Clinic, Rochester, MN; and Connecticut Department of Health, Hartford, CT

6:00 424 COMPARATIVE PATHOGENESIS OF A NEW HUMAN *BABESIA* (WA1) AND *BABESIA MICROTI* IN A SYRIAN HAMSTER MODEL. Wozniak EJ, Lowenstine L, Hemmer R, Robinson TW, and Conrad PA*. Department of Pathology, Immunology, and Microbiology, School of Veterinary Medicine, University CA, Davis, CA.

SCIENTIFIC SESSION Y:
TICK-BORNE DISEASES: LYME, BABESIA, ERLICHIA, AND RICKETTSIA

Monday, November 20, 1995 Ballroom East 3
2:30 - 5:45 PM
Chairs: D. Walker and S.R. Telford

2:30 425 INCREASED SEVERITY OF LYME DISEASE ILLNESS DUE TO CONCURRENT BABESIOSIS. Krause PJ*, Telford SR, Spielman A, Sikand VJ, Ryan R, Christianson D, Brassard P, Pollack R, Burke G, and Persing DH. Hartford Hospital, Hartford, CT; Harvard School of Public Health, Boston, MA; University of Connecticut Health Center, Farmington, CT; and Mayo Clinic, Rochester, MN.

2:45 426 IMMUNIZATION WITH RECOMBINANT OSPA PROTECTS WHITE-FOOTED MOUSE RESERVOIRS AGAINST THE AGENT OF LYME DISEASE. Rosa Brunet LC*, Katavolos P, Spielman A, and Telford SR. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

3:00 427 MACROGEOGRAPHIC ABUNDANCE OF *IXODES SCAPULARIS* (=*I. DAMMINI*) RELATED TO LYME DISEASE INCIDENCE BUT NOT TO DENSITY OF DEER. Wilson ML*, Bertrand MR, Kilpatrick HJ, and Cartter ML. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; Department of Environmental Protection, State of Connecticut, Franklin, CT; and Department of Public Health and Addiction Services, State of Connecticut, Hartford, CT.

3:15 428 *BORRELIA BURGDORERI* OspA EXPRESSION IN *IXODES SCAPULARIS*: SIGNIFICANT DIFFERENCES IN TICK LINES WITHIN AND AMONG DIFFERENT GEOGRAPHIC POPULATIONS. Burkot TR*, and Schriefer M. Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, CO.

3:30 Coffee Break

4:00 429 POSSIBLE ROLE OF A SPIROCHETE FROM LONE STAR TICKS IN AN OUTBREAK OF A LYME DISEASE-LIKE ILLNESS IN MARYLAND. Armstrong PM*, Rich SM, Spielman A, and Telford III SR. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

4:15 430 NEW RISK FACTORS ASSOCIATED WITH *RICKETTSIA TSUTSUGAMUSHI* INFECTIONS IN NORTHERN THAILAND. Linthicum KJ, Tanskul P, Gordon SW, Suwanabun N, and Prachumsri J. Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

4:30 431 PCR DETECTION OF *RICKETTSIA TSUTSUGAMUSHI* IN BLOOD SAMPLES COLLECTED ON FILTER PAPER. A COMPARISON WITH MOUSE ISOLATION AND SEROLOGIC DIAGNOSIS. Gordon SW*, Choksajjawatee N, Rongnoparut P, Linthicum KJ, Watt G, Watcharapichat P, and Eamsila C. Department of Entomology, USAMC, AFRIMS, Bangkok, Thailand; Department of Medicine, USAMC, AFRIMS; Department of Veterinary Medicine, Royal Thai Army Component, AFRIMS; and Department of Epidemiology, Royal Thai Army Component, AFRIMS, Bangkok, Thailand.

4:45 432 TICK CELL CULTURE SYSTEM FOR PHAGOSOSOMAL RICKETTSIAE. Munderloh UG*, Kurtti TJ, Blouin EF, Kocan KM, Ewing SA, Dumler JS, and Madigan J. University of Minnesota, Department

of Entomology, St. Paul, MN; Oklahoma State University, College of Veterinary Medicine, Stillwater, OK; University of Maryland, School of Medicine, Baltimore, MD; and University of California, College of Veterinary Medicine, Davis, CA.

5:00 433 PERPETUATION OF THE AGENT OF HUMAN GRANULOCYTIC EHRLICHIOSIS IN A DEER TICK-RODENT CYCLE. Telford III SR*, Dawson JE, Katavolos P, Warner CK, Kolbert CP, and Persing DH. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Department of Laboratory Medicine and Pathology, Mayo Foundation, Rochester, MN.

5:15 434 NATURAL KILLER CELL AS AN EFFECTOR OF NON-SPECIFICIMMUNITY TO RICKETTSIAE. Billings AN*, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX.

5:30 435 EXPRESSION OF *RICKETTSIA RICKETTSII* rOmpA RECOMBINANT FRAGMENT IN MYCOBACTERIA. Crocquet-Valdes PA*, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX.

SCIENTIFIC SESSION Z: HANTAVIRUSES AND ARENAVIRUSES

Tuesday, November 21, 1995 Rio Grande East
8:00 - 11:00 AM
Chairs: T. Ksiazek and R. Tesh

7:30 436 PRELIMINARY RESULTS OF LONGITUDINAL STUDIES OF SIN NOMBRE HANTAVIRUS IN DIVERSE ECOSYSTEMS IN COLORADO, 1994-95. Beaty BJ, Calisher CH*, Sweeney W, Canestorp KM, Davis T, and Mills JN Colorado State University, Fort Collins, CO; U.S. Fish and Wildlife Service, Model, CO; Colorado Department of Health, Denver, CO; and DVRD, NCID, CDC, Atlanta, GA.

7:45 437 DISTRIBUTION AND PREVALENCE OF ANTIBODY REACTIVE WITH SIN NOMBRE VIRUS AMONG RODENTS IN THE MAJOR HABITAT TYPES IN THE SOUTHWESTERN UNITED STATES. Mills JN*, Ksiazek TG, Rollin PE, Nichol ST, Ellis BA, Yates TL, Gannon WL, Levy CE, Engelthaler DM, Davis T, Tanda D, and Frampton W. Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Department of Molecular Microbiology and Immunology, Johns Hopkins University, Baltimore, MD; Department of Biology, University of New Mexico, Albuquerque, NM; Vector-Borne and Zoonotic Disease Section, Arizona Department of Health Services, Phoenix, AZ; Colorado Department of Public Health and Environment, Denver, CO; and Bureau of Epidemiology, Utah Department of Health, Salt Lake City, UT.

8:00 438 SURVEILLANCE FOR HANTAVIRUS ANTIBODY IN TEXAS RODENTS. Rawlings JA*, Tabony LJ, Hunt PR, and Regner GD. Infectious Disease Epidemiology and Surveillance Division, Texas Department of Health, Austin, TX; and Bureau of Laboratories, Texas Department of Health, Austin, TX.

8:15 439 SEROLOGICAL SURVEY ON *HANTAVIRUS* HUMAN INFECTION IN JUQUITIBA STATE OF SAO PAULO, BRAZIL. Iversson LB*, Branquinho MS, and Rosa MD. School of Public Health/University of Sao Paulo, Brazil; Department of Endemic Control(SUCEN)/Health Department, Sao Paulo, Brazil.

8:30 440 INVESTIGATION ON CASE-CONTACTS OF HUMAN DISEASE CAUSED BY *HANTAVIRUS* IN JUQUITIBA, STATE OF SAO PAULO, BRAZIL. Zaparoli MA, Iversson LB*, Rosa MD, Travassos da Rosa E, Pereira LE, Rollin P, and Peters CJ. Health Department of the State of Sao Paulo; School of Public Health/University of Sao Paulo; Evandro Chagas Institute; and Centers for Disease Control and Prevention, Atlanta, GA.

8:45 441 EMERGENCE OF HANTAVIRUS PULMONARY SYNDROME IN ARGENTINA. Levis SC, Briggiler AM, Cacass M, Peters CJ, Ksiazek TG, Cortes J, Lazaro ME, Resa A, Rollin PE, Pinheiro FP, and Enriz D. Instituto Nacional de Enfermes Virales Humanes (INEVH), pergamino, Argentina; Hospital de Oran, Salta, Argentina; Centers for Disease Control and Prevention, Atlanta, GA; Hospital de El Bolson, Rio Negro, Argentina; and HCP/HCT-Pan American Health Organization.

9:00 442 TOTAL SERUM (S.) CHOLESTEROL (CHOL), HDL CHOLESTEROL AND TRIGLYCERIDES (TG) AS PREDICTORS OF CLINICAL SEVERITY IN HANTAVIRUS (HTV) INFECTION. Clement J*, Colson P, Mc Kenna P, and Heyman P. Belgian Zoonosis Workgroup, Queen Astrid Military Hospital, Brussels, Belgium; and Centre de Santé des Fagnes, Chimay, Belgium.

9:15 443 SABIÁ VIRUS GENOME STRUCTURE AND PHYLOGENY. Gonzalez JP*, Bowen MD, Nichol ST, and Rico-Hesse R. Institut Franais de Recherche Scientifique pour le Développement en Coopération, Paris, France; Special Pathogens Branch, Centers for Disease Control & Prevention, Atlanta, GA; and Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

9:30 Coffee Break

10:00 444 EFFECT OF INFECTION WITH GUANARITO VIRUS (ARENAVIRIDAE) ON FECUNDITY IN *ZYGODONTOMYS BREVICAUDA*. Fulhorst CF*, Tesh RB, Ksiazek TG, Salas RA, and Peters CJ. The University of Texas Medical Branch at Galveston, Texas; National Center for Infectious Diseases, Atlanta, Georgia; and Instituto Nacional de Higiene, Caracas, Venezuela.

10:15 445 INVESTIGATION OF A FAMILIAL OUTBREAK OF VENEZUELAN HEMORRHAGIC FEVER. Salas R*, Tesh R, Manzione N, Fulhorst C, Utrera A, Duno G, Aranson J, Miller E, and Ksiazek T. Instituto Nacional de Higiene, Caracas, Venezuela; University of Texas Medical Branch at Galveston, Texas; Universidad de los Llanos, Guanare, Venezuela; and National Center for Infectious Diseases, Atlanta, GA.

10:30 446 BOLIVIAN HEMORRHAGIC FEVER: INTRAFAMILIAL OUTBREAK IN THE ABSENCE OF EPIDEMIC DISEASE. Kilgore PE, Ksiazek TG, Mills JN, Rollin PE, Peters CJ, Pinheiro FP, Enria DA, McKee KT, Glass RI, Villagra MR, Pozo SI, and Arce RB. Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Instituto Nacional de Enfermedades Virales Humanas (INEVH), Pergamino, Argentina; Division of Communicable Disease Division, Pan American Health Organization, Washington, DC; Communicable Disease Unit, Womack Army Medical Center, Fort Bragg, NC; National Health Secretary for Bolivia, La Paz, Bolivia; and University of San Simon de Major Medical School, Cochabamba, Bolivia; Magdalena Hospital, Magdalena, Beni Department, Bolivia.

10:45 447 CASE CONTACT TRACING DURING AN EPIDEMIOLOGIC INVESTIGATION OF AN ARENAVIRUS INFECTION. Armstrong LR*, Khan AS, Russi MB, Rollin PE, and Peters CJ. Special Pathogens Branch, Division of Viral and Rickettsial Diseases, National Centers for Infectious Disease, Centers for Disease Control and Prevention (CDC), Atlanta, GA; Epidemology Program Office, Division of Training, CDC, Atlanta, GA; and Occupational Medicine Program, Internal Medicine, Yale University, New Haven, CT.

SCIENTIFIC SESSION AA: MALARIA EPIDEMIOLOGY & DIAGNOSIS

Tuesday, November 21, 1995 Rio Grande C
7:30 - 11:15 AM
Chairs: T. Jones and C.C. Campbell

7:30 448 ABRUPT ONSET OF MALARIA AMONG THE YANOMAMI AMERINDIANS. Laserson KF*, Petralanda I, Almera R, Alvarez R, Matos A, Bolivar M, Gonzalez M, Jank M, Lopez A, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Centro Amaz. para la Invest. y Control de Enferm. Trop., "Simon Bolivar", C.A.I.C.E.T., Pto. Ayacucho, VZ; SNEM, Santo Domingo, Rep. Dominicana; New Tribes Mission, Pto. Ayacucho, VZ.

7:45 449 CONGENITAL MALARIA: A PAN-AFRICAN SURVEY. Fischer PR*. Department of Pediatrics, University of Utah, Salt Lake City, UT.

8:00 450 EFFECT OF NEW AND PERSISTENT MALARIA INFECTIONS ON THE HEMATOLOGIC STATUS OF CHILDREN AND WOMEN IN WESTERN KENYA. Bloland PB*, Boriga DA, Ruebush TK, Oloo AJ, McCormick JB, Lal AA, Nahlen BL, and Campbell CC. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

8:15 451 PARASITOLOGIC AND HEMATOLOGIC VARIABLES ASSOCIATED WITH MORTALITY AMONG INFANTS IN WESTERN KENYA. Koumans EH*, Hightower AW, Bloland PB, Lal AA, Oloo AJ, and Nahlen BL. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

8:30 452 *PLASMODIUM FALCIPARUM* GAMETOCYTEMIA IN KENYAN CHILDREN. Jones TR*, McElroy PD, Oster CN, Beier JC, Oloo AJ, Onyango FK, Chumo DK, Sherwood JA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; U.S. Army Medical Research Unit-Kenya; School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA; and Kenya Medical Research Institute, Kisumu, Kenya.

8:45 453 FIELD EVALUATION OF A POLYMERASE CHAIN REACTION-BASED NONISOTOPIC LIQUID HYBRIDIZATION FOR MALARIA DIAGNOSIS. Oliveira DA*, Shi YP, Oloo A, Anyona D, Nahlen B, Hawley W, Holloway BP, and Lal AA. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Vector Biology and Control Research Center, Kenya Medical Research Institute, Kisumu, Kenya; and Biotechnology Core Facility, Scientific Resources Program, CDC, Atlanta, GA.

9:00 454 GIS/DGPS APPLIED TO THE ASEMBO BAY COHORT PROJECT. Hightower AW*, Hawley WA, Nahlen BL, Koumans EH, Lal AA, and Oloo A. Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; Division of Parasitic Diseases, NCID, CDC, Nairobi, Kenya; Division of Parasitic Diseases, NCID, CDC, Kisumu, Kenya; Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

9:15 455 MALARIA AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AMONG SUGAR ESTATE WORKERS, M ALAWI, 1994. Nwanyanwu OC*, Kumwneda N, Jemu S, Ziba C, Kazembe PN, and Redd SC. Malawi Ministry of Health, Lilongwe, Malawi; and Centers for Disease Control and Prevention, Atlanta, GA.

9:30 Coffee Break

10:00 456 SEROLOGIC INVESTIGATION OF HUMAN *PLASMODIUM VIVAX*-LIKE MALARIA IN SEVERAL LOCALITIES IN THE STATE OF SAO PAULO. Curado I, Duarte AM, Lal AA, Nussenzweig RS, Oliveira DA, Oliveira S, and Kloetzel JK*. Instituto Butantan de Sao Paulo, Sao Paulo, Brazil; Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Department of Medical and Molecular Parasitology, New York, University School of Medicine, New York, NY; Instituto Evandro Chagas, Belem, PA, Brazil; and Department of Parasitology, Institute of Medical Sciences, Sao Paulo University, Sao Paulo, Brazil.

10:15 457 PREVALENCE OF *PLASMODIUM VIVAX*-LIKE HUMAN MALARIA PARASITE IN A MALARIA ENDEMIC REGION OF BRAZIL. Oliveira DA, Qari S*, Machado RL, Oliveira S, Povoa MM, Collins W, and Lal AA. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; and Instituto Evandro Chagas, Belem, PA, Brazil.

10:30 458 EFFICACY OF λ-CYHALOTHRIN TREATED BED NETS IN PREVENTING MALARIA IN A COHORT OF RADICALLY CURED VOLUNTEERS. Church CJ, Richie TL, Ohrt C, Tjitra E, Subianto B, Sandjaya B, Gomez E*, Baird JK, Fryauff DJ, and Richards AL. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; Walter Reed Army Institute of Research, Washington DC; National Institute of Health Research and Development, Jakarta, Indonesia; and Provincial Health Service, Irian Jaya, Indonesia.

10:45 459 EPIDEMIOLOGY OF MALARIA VECTORS IN THE LACANDON FOREST OF CHIAPAS, MEXICO. Arredondo-Jiménez JI*, Danis-Lozano R, González-Cerón L, Rodríguez MH, Hernández-Avila M, and Washino RK. CIP-INSP, Chiapas, Mexico; CISP-INSP, Cuernavaca, Morelos, Mexico; and Department of Entomology, University of California, Davis, CA.

11:00 460 ABANDONING DDT: A BURGEONING GLOBAL MALARIA CONTROL CRISIS. Roberts D*, Sherman S, and Vanzie E. Uniformed Services University of the Health Sciences, Bethesda, MD; and Ministry of Health, Belize City, Belize C.A.

SCIENTIFIC SESSION BB: CLINICAL TROPICAL MEDICINE II

Tuesday, November 21, 1995 Chula Vista
8:00 - 10:45 AM
Chairs: R. Steffen and N. Punjabi

8:00 461 *KEYNOTE ADDRESS:* EPIDEMIOLOGY OF TRAVELER'S DIARRHEA IN JAMAICA. Steffen R*, Stucki A, DuPont HL, Mathewson JJ, Ashley DV, Ashley D, and Campbell-Forrester S. Division of Epidemiology and Prevention of Communicable Diseases, University of Zurich, Switzerland; St. Lukes Episcopal Hospital, Texas Medical Center, Houston, TX; and Ministry of Health, Epidemiology Unit, Kingston, Jamaica.

8:30 462 REVIEW OF THE BACTERICIDAL AND VIRICIDAL EFFICACY OF IODINATED RESINS. Hart PE*, and Hembree D. Recovery Engineering, Inc. Minneapolis, MN.

8:45 463 A DOUBLE BLIND CONTROLLED TRIAL COMPARING STANDARD AND LOW OSMOLARITY ORAL REHYDRATION SOLUTION FOR MAINTENANCE THERAPY OF ADULT CHOLERA PATIENTS. Punjabi NH*, Pulungsih SP, Rifajati A, Kumala S, O'Hanley P, Simanjuntak CH, Juwono, and Lesmana M. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Prof. Dr. Sulianto Saroso Infectious Diseases Hospital, Jakarta; and National Institute of Health Research and Development, Jakarta, Indonesia.

9:00 464 LEPTOSPIROSIS: AN ENDEMIC DISEASE IN BALTIMORE, MARYLAND. Vinetz JM*, Glass GG, Bragg S, Mueller P, and Kaslow DC. Johns Hopkins School of Medicine, Baltimore, MD; Johns Hopkins School of Public Health and Hygiene, Baltimore, MD; Centers for Disease Control and Prevention, Atlanta, GA; and National Institute of Allergy and Infectious Diseases, Bethesda, MD.

9:15 465 LEPTOSPIROSIS IN HAWAII, 1971-1990: CLINICAL AND EPIDEMIOLOGIC ANALYSIS OF 187 LABORATORY CONFIRMED CASES. Katz AR*, Sasaki DM, Odello LM, and Ansdell VE. Department of Public Health Sciences, University of Hawaii School of Public Health, Honolulu, HI; Communicable Disease Division, State of Hawaii Department of Health, Honolulu, HI; and Kaiser Permanente Medical Center, Honolulu, HI.

9:30 Coffee Break

10:00 466 PLAGUE *YERSINIA PESTIS* IN INDIA, 1994. Bhattacharjee SJ, Biswas G, Chu MC, Datta KK, Dennis DT, Gage KL, Khera AK, Kumar K, Orloski KA*, and Sehgal S. National Institute of Communicable Diseases, New Delhi, India; and Division of Vector-Borne Infectious Diseases, NCID, Centers for Disease Control & Prevention, Ft. Collins, CO.

10:15 467 EVALUATION OF THE TOLERANCE OF ANTIVENIN IN TROPICAL AFRICA. Rage V, Chippaux JP*, Amadi-Eddine S, Fagot P, and Lang J. Centre Pasteur du Cameroun, Yaounde, Cameroun; Centre Pasteur du Cameroun, Garoua, Cameroun; Hopital Provincial, Garoua, Cameroun; and Pasteur Merieux Serums & Vaccins, Lyon, France.

10:30 468 HANSEN'S DISEASE- THE TEXAS EXPERIENCE. Joyce MP*, and Longfield RN. Texas Center for Infectious Disease, Texas Department of Health, San Antonio, TX.

SYMPOSIUM:
CYTOKINES IN INFECTION
Sponsored by Corixa Corp. and Paravax, Inc.

Tuesday, November 21, 1995 Rio Grande W
8:00 AM - 11:30 AM
Chairs: S. Reed and A. Sher

8:00 IMMUNOLOGIC REGULATION BY CD40 LIGAND. Maliszewski C. Immunex.

8:30 THE ROLE OF IL-10 PRODUCING B AND B-1 CELLS IN TH1/TH2 DOMINANCE. Harn D. Harvard School of Public Health, Boston, MA.

9:00 THE ROLE OF IL-10 IN THE REGULATION OF FILARIAL SPECIFIC IMMUNE RESPONSES. Nutman T. NIH, Bethesda, MD.

9:30 Coffee Break.

10:00 IL-12 REGULATED PROTECTIVE TH1-ASSOCIATED RESPONSES IN THE SPLEEN DURING BLOOD-STAGE MALARIA. Stevenson M. Montreal General Hospital Research Institute.

10:30 CYTOKINE PRODUCTION AT THE SITE OF DISEASE IN EXPERIMENTAL CHAGAS' DISEASE. Tarleton R. University of Georgia.

11:00 CYTOKINE REGULATION OF INTESTINAL PARASITISM: A MODEL FOR NOVEL INTERACTIONS AND EFFECTORS. Urban J. Parasitic Immunobiology Laboratory, USDA.

SYMPOSIUM:
LIPID MEDIATION OF HOST PATHOGEN INTERACTIONS

Tuesday, November 21, 1995 Blanco/Llano
7:30 AM - 11:00 AM
Chairs: S. Furlong and P. Weller

7:30 FORMATION OF EICOSANOID LIPIDS BY FILARIA. Weller P. Beth Israel Hospital, Boston, MA.

8:10 ECDYSONE RECEPTORS FROM *C. ELEGANS*. Maina C. New England Bio. Labs, Beverly, MA.

8:50 STEROLS AS POTENTIAL DRUG TARGETS. Nes D. Texas Tech University, Lubbock, TX.

9:30 Coffee Break.

9:40 LIPOPHOSPHOGLYCAN FROM *LEISHMANIA*. Sacks D. NIH, Bethesda, MD.

10:20 MYCOLIC ACIDS AS T CELL ANTIGENS. Furlong S. Brigham & Women's Hospital, Boston, MA.

SYMPOSIUM:
CRYPTOSPORIDIOSIS: THE PROBLEM AND STRATEGIES FOR CONTROL

Tuesday, November 21, 1995 Nueces/Frio
8:00 AM - 11:00 AM
Chair: R. Fayer

8:00 OVERVIEW AND INTRODUCTION. Fayer R. USDA, Beltsville, MD.

8:10 THE BIOLOGY OF *CRYPTOSPORIDIUM PARVUM*. Sterling CR. University of Arizona, Tucson, AZ.

8:35 EXPERIMENTAL INFECTION OF HUMAN VOLUNTEERS, DOSE RESPONSE. Chappell C. University of Texas, Houston, TX.

9:00 Coffee Break.

9:20 MEDICATION OF AIDS PATIENTS FOR CRYPTOSPORIDIOSIS. Soave R. Cornell Medical Center, New York, NY.

9:45 OUTBREAKS, SOURCES OF INFECTION, ROUTES OF TRANSMISSION. Juranek D. CDC, Atlanta, GA.

10:10 WORKING GROUP ON WATERBORNE CRYPTOSPORIDIOSIS. Colley D. CDC, Atlanta, GA.

POSTER SESSION II

Tuesday, November 21, 1995
11:00 AM - 12:30 PM

Regency Ballroom East/Center/West

VIROLOGY

469 EFFECTS OF THE VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION IN THE BIOGENIC AMINES CONCENTRATIONS OF MOUSE BRAIN. Estévez J*, Singh R, Arrieta A, and Teruel-López M. Instituto de Investigaciones Clínicas, Universidad del Zulia, Maracaibo, Venezuela.

470 THE GENERATION OF MOSQUITOES RESISTANT TO FLAVIVIRUS INFECTION VIA EXPRESSION OF FLAVIVIRUS SEQUENCES USING SINDBIS VIRUS VECTORS. Higgs S*, Olson KE, Gaines PJ, Powers AM, Beaty BJ, and Blair CB. Arthropod-borne and Infectious Diseases Laboratory, Microbiology Department, Colorado State University, Ft. Collins, CO.

471 NATURAL IMMUNITY OF ROTAVIRUS INFECTION. Mahmud MA*, Hossain MM, Mathewson JJ, Habib M, and DuPont HL. Beth Israel Medical Center, Division of Infectious Disease, New York; United Arab Emirates University; University of Texas School of Public Health; The Center for Applied Research, Ministry of Health, Cairo, Egypt; Baylor College of Medicine.

472 TOWARDS THE UTILIZATION OF CHAPERONIN 60 AS AN ANTIVIRAL TARGET: A STRUCTURAL STUDY. Braig K*, and Braig HR. Department of Genetics, Yale University School of Medicine, New Haven, CT; and Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

473 SILENT TRANSMISSION OF DENGUE VIRUS IN SOUTHERN TAIWAN. Chen WJ*, Chen SL, Chien LJ, and King CC. Department of Parasitology, Chang Gung College of Medicine and Technology, Kwei-San, Tao-Yuan, Taiwan; and School of Public Health, National Taiwan University, Taipei, Taiwan.

474 EXPERIMENTAL BUNYAVIRUS INFECTIONS OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*). Blackmore CG*, and Grimstad PR. Laboratory for Arbovirus Research and Surveillance, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

475 THE EFFECT OF GLOBAL WARMING ON THE GEOGRAPHIC AND SEASONAL DISTRIBUTION OF *PHLEBOTOMUS PAPATASI* IN SOUTHWEST ASIA. Cross ER*, and Hyams KC. Infectious Diseases Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD.

476 DEVELOPMENT OF A PURIFIED, INACTIVATED, DENGUE-2 VIRUS VACCINE PROTOTYPE IN VERO CELLS: IMMUNOGENICITY IN MICE AND RHESUS MONKEYS. Putnak JR*, Barvir D, Burrous MJ, D'Andrea VM, Dubois D, Hoke CH, Sadoff J, and Eckels KH. Division of Communicable Diseases and Immunology, The Walter Reed Army Institute of Research, Washington DC; Division of Clinical Laboratory Devices, The United States Food and Drug Administration, Rockville, MD.

477 DETECTION OF ANTI-DENGUE VIRUS ANTIBODY USING RECOMBINANT FUSION PROTEINS CONTAINING THE B DOMAIN OF DENGUE ENVELOPE. Simmons M*, Porter KR, Escamilla J, Shope RE, Eckels KH, Watts DM, Burans JP, and Hayes CG. Naval Medical Research Institute, Bethesda, MD; NEPMU-6, Pearl Harbor, HI; Naval Medical Research Institute Detachment, Lima, Peru; Walter Reed Army Institute of Research, Washington, DC; and Yale Arbovirus Research Unit, New Haven, CT.

478 EVALUATION OF DENGUE VIRUS ANTIGENS FOR USE IN A RAPID DIP-STICK IMMUNOASSAY. Wu SL*, Hanson B, Paxton H, Simmons M, and Hayes CG. Infectious Diseases Department, Naval Medical Research Institute, Bethesda, MD; Integrated Diagnostics, Inc., Baltimore, MD.

479 PURIFICATION OF BACULOVIRUS PRODUCED DENGUE-2 ENVELOPE GLYCOPROTEIN. Kelly EP*, King, AD and Bailey SL. Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

480 EFFECTS OF FOLLICULAR QUIESCENCE ON REPLICATION OF LA CROSSE VIRUS IN *AEDES TRISERIATUS* OVARIES. Chandler LJ*, Wasieloski LP, and Beaty BJ. Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

481 MISSOURI POST FLOOD MOSQUITO SURVEILLANCE - 1994. Frazier CL*, and Robins JH. Southeast Missouri State University, Cape Girardeau, MO; and Southeast Missouri State University, Cape Girardeau, MO.

482 PANTROPIC RETROVIRAL INFECTION OF MOSQUITO CELL LINES. Matsubara T*, Beeman RW, Besansky NJ, Mukabayire O, Higgs S, Burns JC, and James AA. Department of Pediatrics, UCSD School of Medicine, La Jolla, CA; USDA, US Grain Marketing Research Laboratory, Manhattan, KS; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; AIDL, Colorado State University, Fort Collins, CO; and Department of Molecular Biology and Biochemistry, UC Irvine, Irvine, CA.

483 NEW VEE VACCINE CANDIDATE INDUCES PE2-SPECIFIC ANTIBODIES AND RETAINS CONFORMATION OF A DOMINANT NEUTRALIZING EPITOPE. Buckley MJ*, Ludwig GV, Davis NL, Johnston RE, and Hart MK. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC.

484 EVALUATION OF A NEUTRALIZATION-ELISA ASSAY (NT-ELISA) TO MEASURE NEUTRALIZING ANTIBODIES TO HANTAAN VIRUS. Summers PL*, and McClain DJ. Virology Division, U.S. Army Medical Research Institute of Research, Ft Detrick, MD.

485 CLINICAL AND EPIDEMIOLOGICAL FEATURES OF DENGUE FEVER INFECTIONS AMONG US TROOPS IN HAITI. Smoak BL*, DeFraites RF, Trofa AF, Kanesa-thasan N, King AD, Burrous JM, MacArthy PO, Putnak R, Quan J, Scheutte J, Longacre J, and Hoke, Jr. CH. Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC; Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC; and 28th Combat Support Hospital, Port-au-Prince, Haiti.

486 A COLORIMETRIC MICROPLATE ASSAY FOR DETECTING PCR-AMPLIFIED DENGUE VIRUS RNA. Ibrahim MS*, Baird JB, Lofts RS, Roberts LW, and Henchal EA. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

487 RECOGNITION OF DENGUE-2 VIRUS E PROTEIN BY MURINE CD8+ CYTOTOXIC T LYMPHOCYTES. Rothman AL*, Kurane I, lai CJ, Bray M, and Ennis FA. University of Massachusetts Medical Center, Worcester, MA; and Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD.

KINETOPLASTS

488 A RECOMBINANT BCG VACCINE EXPRESSING THE *LEISHMANIA CHAGASI* ANTIGEN Lcr1. Streit JA*, Donelson JE, and Wilson ME. Department of Internal Medicine, University of Iowa, and VAMC, Iowa City, IA; Department of Biochemistry, University of Iowa, and Howard Hughes Medical Institute, Iowa City, IA; and Departments of Internal Medicine and Microbiology, University Iowa and VAMC, Iowa City, IA.

489 EVIDENCE OF GENETIC EXCHANGE DURING SILVATIC TRANSMISSION OF *TRYPANOSOMA CRUZI*. Carrasco HJ*, Frame IA, Valente SA, and Miles MA. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, UK; and Chagas' Disease Control Division, Institute Evandro Chagas, Belem, Para, Brazil.

490 IMMUNIZATION OF MICE WITH MOLECULAR ANTIGENS OF *LEISHMANIA (LEISHMANIA) DONOVANI* AND EFFECTS ON ANTIBODY AND LYMPHOKINE PRODUCTION. Shin SS*, Hanson WL, Russell DG, and McMaster WR. Department of Parasitology, College of Veterinary Medicine, The College of Georgia, Athens, GA; Department of Molecular Microbiology, Washington University Medical School, St. Louis, MO; and Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia.

491 EVOLUTIONARY GENETICS OF PARASITIC PROTOZOA AND OTHER PATHOGENIC MICROORGANISMS. IMPLICATIONS FOR STRAIN IDENTIFICATION AND MOLECULAR TAXONOMY. Tibayrenc M*. ORSTOM, Montpellier Cedex 01, France.

492 MURINE MODEL OF *LEISHMANIA INFANTUM* DISSEMINATED VISCERAL LEISHMANIASIS. Garin YJ*, Sulahian A, and Derouin F. Hôpital St-Louis, Paris, France.

493 THE INOCULATION SITE CONDITIONS RESPONSE ON THE HAMSTER MODEL TO *LEISHMANIA VIANNIA* SPP. Travi BL, Osorio Y, Valderrama L, and Guarin N. Centro Internacional de Entrenamiento e Investigaciones Medicas-CIDEIM, Cali, Colombia; and Universidad del Valle, Cali, Colombia.

494 NO TITLE. Traub-Cseko.

495 A RAPID DIAGNOSTIC ASSAY FOR VISCERALIZING *LEISHMANIA TROPICA*. Alwen A*, Francies WM, Campbell JR, Hall E, and Mansour M. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt.

496 IMMUNE RESPONSE TO *TRYPANOSOMA CRUZI* GRP78 DELIVERED INTRAMUSCULARLY AS NAKED cDNA IN A EUCARYOTIC EXPRESSION VECTOR. Chen Z, and Rowland EC*. Department of Biological Sciences, College of Osteopathic Medicine, Ohio University, Athens, OH.

497 CHARACTERIZATION OF INTRALESIONAL CYTOKINE PRODUCTION IN LOCALIZED CUTANEOUS LEISHMANIASIS. Isaza DM*, Restrepo MI, Restrepo M, and Melby PC. Instituto de Colombiano de Medicina Tropical, Medellin, Colombia; The University of Texas Health Science Center, San Antonio, TX.

498 PCR AMPLIFICATION OF SERCA LIKE ATPase MOLECULES IN *LEISHMANIA*. Xue L*, and Meade JC. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

499 RESERVOSOMES OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES AND THE *IN VITRO* EFFECTS OF EXTRACELLULAR ATP ON THEIR SEQUESTRATION OF HORSERADISH PEROXIDASE. Ribeiro-Rodrigues R*, Carter CE, and Bogitsh BJ. Department of Biology, Vanderbilt University, Nashville, TN.

500 EXPRESSION AND LOCALIZATION OF THE 24 KDA FLAGELLAR CALCIUM BINDING PROTEIN OF *TRYPANOSOMA CRUZI*. Godsel LM*, Olson CL, Maldonado RA, Goldenberg S, and Engman DM. Departments of Pathology and Microbiology-Immunology, Northwestern University Medical School, Chicago, IL; and Department of Biochemistry and Molecular Biology, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil.

501 IDENTIFICATION OF AN ENHANCER-LIKE ELEMENT IN THE *TRYPANOSOMA CRUZI* RIBOSOMAL RNA GENE PROMOTER. Harris-Kerr CL*, and Buck GA. Department of Microbiology and Immunology, Medical College of Virginia Campus, V.C.U., Richmond, VA.

502 DIFFERENTIAL EXPRESSION OF ATPase 1B PROTON PUMP IN *LEISHMANIA DONOVANI*. Hicock PI*, Kong L, Stiles JK, and Meade JC. Department of Clinical Laboratory Sciences, School of Health Related Professions, University of Mississippi Medical Center Jackson MS; and Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

503 TRANS-SPLICING AND POLYADENYLATION AT NASCENT RNA TRANSCRIPTS FROM CATION TRANSLOCATING ATPASE 1A LOCUS IN *LEISHMANIA DONOVANI*. Stiles J*, Kong L, Hicock P, Xue L, and Meade JC. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

504 THE USE OF *scid*-SOURCE OF *LEISHMANIA MAJOR* TO STUDY THE HOST CELL-PARASITE INTERFACE. Guy RA*, and Belosevic M. Departments of Biological Sciences and Immunology, University of Alberta, Edmonton, Canada.

505 EVIDENCE THAT A UNIQUE GENE IS CONTAINED IN THE REGION BETWEEN STATIONARY MSP (GP63) GENES OF *LEISHMANIA CHAGASI*. McCoy JJ*, Donelson JE, Wilson ME. Department of Internal Medicine, University of Iowa, Iowa City, IA; Department of Biochemistry, University of Iowa, and Howard Highes Med Institute, Iowa City, IA; Departments of Internal Medicine and Microbiology, University of Iowa, and VA Medical Center, Iowa City, IA.

506 INFECTIVITY OF PROCYCLIC AND METACYCLIC PROMASTIGOTES OF AN HUMAN VISCERAL STRAIN OF *LEISHMANIA INFANTUM*. Louassini M*, Adroher FJ, and Benítez R. Department of Parasitology, Faculty of Pharmacy, University of Granada, Granada, Spain.

507 CANINE LEISHMANIASIS: ESTABLISHMENT OF A DOG *LEISHMANIA* MODEL AND STUDY OF THE VIRULENCE OF PARASITES. Rhalem A *, Sahibi H, Guessous-Idrissi N, Natami A, Lasri S, and Berrag B. Departement de Parasitologie, Institut Agronomique et Veterinaire Hassan II, Rabat, Morocco; and Unite d'Etudes et de Recherche sur les Leishmanioses, Faculte de Medicine et Pharmacie de Casablanca, Morocco.

508 CLONING AND OVEREXPRESSION OF CASEIN KINASE II α SUBUNIT FROM *LEISHMANIA DONOVANI CHAGASI*. Bhatia A*, Ismail SO, Paramchuk W, Gedamu L. Dept. of Biological Science, University of Calgary, Canada.

509 MOLECULAR CLONING AND EXPRESSION OF A *TRYPANOSOMA BRUCEI* RIBONUCLEASE H (RNase H2) GENE. Campbell AG*, and Bodnick JS. Division of Biology and Medicine, Brown University, Providence, RI.

510 INTERFERON-γ AND INTERLEUKIN-5 PRODUCTION IN MICE IMMUNIZED WITH FORMALIN-KILLED PROMASTIGOTES OF *LEISHMANIA (LEISHMANIA) DONOVANI* AND CHALLENGED. Shin SS*, and Hanson WL. Department of Parasitology, College of Veterinary Medicine, The College of Georgia, Athens, GA.

511 ALTERATIONS OF G-PROTEINS IN ACUTE MURINE CHAGAS' DISEASE. Huang H *, Tanowitz HB, Chen B, Wittner M, and Morris SA. Albert Einstein College of Medicine, Bronx, N.Y.

FILARIASIS

512 MOLECULAR CLONING AND CHARACTERIZATION OF AN IMMUNODOMINANT *ONCHOCERCA VOLVULUS* LARVAL ANTIGEN RECOGNIZED BY PUTATIVELY IMMUNE INDIVIDUALS. Joseph GT*, Mair KF, Kass PH, Huima T, and Lustigman S. Virology and Parasitology, The Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY; and Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA.

513 CHARACTERIZATION OF FILARIAL RETINOL-BINDING PROTEINS. Gundlapalli AV*, and Rajan TV. Department of Pathology, University of Connecticut Health Center, CT.

514 CONSTRUCTION AND ANALYSIS OF A cDNA LIBRARY OF *ONCHOCERCA VOLVULUS* INFECTIVE STAGE (L3) LARVAE. Lu W*, and Williams SA. Molecular and Cellular Biology, University of Massachusetts at Amherst, MA; and Department of Biological Sciences, Smith College, Northampton, MA.

515 CHARACTERIZATION OF ECDYSONE RECEPTOR TARGET GENES IN A FILARIAL PARASITE, *DIROFILARIA IMMITIS*. Crossgrove K*, and Maina CV. Molecular Parasitology Group, New England Biolabs, Inc., Beverly, MA.

516 ANTIBODY RESPONSES TO RECOMBINANT PARASITE ANTIGENS IN PATIENTS WITH ONCHOCERCAL DERMATITIS. Chandrashekar R*, Murdoch ME, Weil GJ, Hay RJ, Maizels RM, Jones BR, and Abiose A. Kaduna/London/St. Louis Collaboration for Research on Onchocerciasis.

517 HELMINTH INFECTION WITH EOSINOPHILIA IS ASSOCIATED WITH INCREASED SERUM PHOSPHOLIPASE A_2 LEVELS. Mawhorter SD*, Bernton EW, Hershey J, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Allergy/Immunology Service, Walter Reed Army Medical Center, Washington, DC.

518 EFFECT OF GAMMA RADIATION ON *BRUGIA* L3 DEVELOPMENT *IN VIVO* AND THE KINETICS OF GRANULOMATOUS INFLAMMATION INDUCED BY THESE PARASITES. Nasarre C*, Coleman SU, Rao UR, and Klei TR. Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton, Rouge, LA.

519 DOWN REGULATION OF T-CELL PROLIFERATIVE RESPONSES BY *BRUGIA MALAYI* ADULT ANTIGEN IN RHESUS MONKEYS. Giambartolomei GH*, Dennis VA, Lasater BL, Grab DJ, and Philipp MT. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

520 IMMUNE RESPONSE STUDIES IN BANCROFTIAN FILARIASIS. Regunathan J, and Kaliraj P*. Center for Biotechnology, Anna; University, Madras, India.

521 RECOMBINANT *ONCHOCERCA VOLVULUS* ANTIGENS AND THE DIAGNOSIS OF PREPATENCY IN CHILDREN FROM AN ENDEMIC AREA. Gbakima AA*, and Scott AL. Tropical Diseases Research Unit, Njala University College, University of Sierra Leone, Sierra Leone; and Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

522 MULTIPLEX PCR AND ELISA-BASED DETECTION STRATEGY FOR THE DIAGNOSIS OF *LOA LOA*. Kubofcik J*, Williams SA, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD; and Department of Biological Sciences, Smith College, Northampton, MA.

523 DIAGNOSIS AND NINE YEAR IMMUNOMONITORING OF FILARIAL PATIENTS DURING DEC THERAPY IN FILARIA ENDEMIC AREA IN INDIA. Padigel UM, Devi KK, Chenthamarakshan V, Reddy

MV, and Harinath BC*. Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram, India.

524 EFFECT OF REPEATED LARGE SCALE IVERMECTIN TREATMENT ON THE TRANSMISSION OF *LOA LOA* IN SOUTHERN CAMEROON. Bouchite B, Demanou M, Prud'hom JM, Boussinesq M, and Chippaux JP*. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon.

525 SAFETY OF IVERMECTIN FOR TREATING ONCHOCERCIASIS IN POPULATIONS WHERE LOIASIS IS CO-ENDEMIC (CENTRAL AFRICA - GABON). Kombila M*, Duong TI, and Richard-Lenoble D. Department of Parasitology and Tropical Medicine, University School of Medicine, Libreville, Gabon and Tours, France.

526 SEASONAL SHIFTS IN CIRCULATING *WUCHERERIA BANCROFTI* ANTIFILARIAL IgG ANTIBODY ISOTYPES IN AN ENDEMIC AREA: KWALE DISTRICT, KENYA. Wamae CN*, and Lammie PJ. Center for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya; Parasitic Diseases Division, Centers for Disease Control and Prevention, Atlanta, GA.

SCHISTOSOMIASIS AND OTHER HELMINTHS

527 DETECTION OF A 26-28 KD COPROANTIGEN OF *FASCIOLA HEPATICA* USING A CAPTURE ELISA. Abdel-Rahman SM*, O'Reilly KL, and Malone JB. Department of Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, MD.

528 RECOMBINANT ANTIGENS FOR DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* IN TURKANA, KENYA. Blanton RE*, Aman RA, Wachira TM, and Zeyhle E. Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH; Department of Molecular Genetics, National Museums of Cleveland, Nairobi, Kenya; and Nomadic Health Unit, African Medical and Research Foundation, Nairobi, Kenya.

529 ESTABLISHMENT AND APPLICATION OF DOT COLLOIDAL IMMUNOASSAY (DIA) FOR ANTIBODY DETECTION IN PATIENTS OF SCHISTOSOMIASIS JAPONICA. Zhu YC*, Yu CX, Yin XR, Liu YJ, and Xu YL. Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu, P.R. China.

530 ANTIBODY REACTIVITY OF SMB, A RECOMBINANT *SCHISTOSOMA MANSONI* PROTEIN. Hancock K, Noh JC, and Tsang V. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

531 RATES OF *SCHISTOSOMA MANSONI* INFECTION AND REINFECTION IN THE NILE DELTA. El-Morshedy H, Barakat R*, El Masry AG, ElSayed MK, Farghaly A, Husein MH, and Miller FD. High Institute of Public Health, Alexandria University, Alexandria, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Cairo University, Cairo, Egypt; and School of Public Health, University of Hawaii, HI.

532 DETAILED AGE SEX SPECIFIC PREVALENCE OF *SCHISTOSOMA MANSONI* IN THE NILE DELTA FROM A LARGE SAMPLE. El-Sayed MK, Barakat R, El Masry AG, Husein MH, Farghaly A, and Miller FD*. High Institute of Public Health, Alexandria University, Alexandria, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Cairo University, Cairo, Egypt; and School of Public Health, University of Hawaii, HI.

533 COINFECTION WITH *SCHISTOSOMA MANSONI* AND INTESTINAL HELMINTHS IN BOA UNIAO, MINAS GERAIS, BRAZIL. Correa-Oliveira R, Viana IR, Addiss DG*, Prata A, Silveira A, Carvalho O, Colley DG, and Gazzinelli G. Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, Brazil; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

534 THE IMPACT OF PASSIVE CHEMOTHERAPY ON *SCHISTOSOMA MANSONI* PREVALENCE AND INTENSITY OF INFECTION IN THE EGYPTIAN NILE DELTA. Miller FD*, El-Sayed MK, Farghaly A, El-Badway A, Soliman NK, Husein MH, and Barakat R. School of Public Health, University of Hawaii, Honolulu, HI; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Zagazig University, Zagazig, Egypt; High Institute of Public Health, Alexandria University,Alexandria, Egypt; and Faculty of Medicine, Cairo University, Cairo, Egypt.

535 TWO YEARS EPIDEMIOLOGICAL FOLLOW UP STUDIES ON *SCHISTOSOMA HAEMATOBIUM* INFECTION IN EL-MINIA GOVERNORATE, EL-MINIA, EGYPT. Gabr NS*, Hussein MH, Hammad TH,

Aboel-Einin M, Orieby AO, Showky EM, and Saad A. Parasitology Department,Faculty of medicine, El-Minia University,El-Minia,Egypt; Department of Medicine, Faculty of Medicine, El-Minia University,El-Minia, Egypt; and Public Health Department, Faculty of Medicine, Cairo University, Egypt.

536 GEOGRAPHIC INFORMATION SYSTEMS AND RISK OF SCHISTOSOMIASIS IN EGYPT. Malone JB*, Huh OK, Soliman MS, El Bahy MM, and Shafik M. School of Veterinary Medicine, Louisiana State University; Coastal Studies Institute, Louisiana State University; and Cairo University, Cairo, Egypt; Ministry of Health, Cairo, Egypt.

537 SEA-SPECIFIC ISOTYPE LEVELS DIFFER IN THE DISTINCT PATHOLOGIC SYNDROMES OF CHRONIC EXPERIMENTAL *SCHISTOSOMA MANSONI* INFECTIONS. Montesano MA*, Secor WE, and Colley DG. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Universidade Federal de Juiz de Fora, Minas Gerais, Brazil.

538 INDUCTION OF SPECIFIC *SCHISTOSOMA MANSONI* EGG ANTIGEN HYPORESPONSIVENESS IN UNSENSITIZED AND SENSITIZED MURINE MODELS. Hassanein HI*, Botros SS, Akl M, El-Ghorab N, and Doughty BL. Theodar Bilharz Research Institute, NAMRU-3, Cairo, Egypt; and Texas A&M University, College Station, TX.

539 ELEVATED INNATE PERIPHERAL BLOOD EOSINOPHILIA FAILS TO AUGMENT IRRADIATED CERCARIAL VACCINE-INDUCED RESISTANCE TO *SCHISTOSOMA MANSONI* IN IL-5 TRANSGENIC MICE. Freeman GL*, Tominaga A, Takatsu K, Secor WE, and Colley DG. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Kochi Medical School, Okoh-cho, Nankokushi, Kochi, Japan; and Department of Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

540 ANTISENSE OLIGONUCLEOTIDES AGAINST SCHISTOSOMIASIS: A PRELIMINARY STUDY. Tao LF*, Marx KA, Wongwit W, Jiang ZW, Agrawal S, and Coleman RM. Center for Tropical Diseases, University of Massachusetts-Lowell, Lowell, MA; Department of Chemistry, University of Massachusetts-Lowell, Lowell, MA; and Hybridon, Inc., Worcester, MA.

541 *SCHISTOSOMA MANSONI* ALDOLASE: AN IMMUNODOMINANT T AND B CELL CANDIDATE VACCINE ANTIGEN IN HUMANS WITH SCHISTOSOMIASIS. Gamal El-Din N, Al-Sherbiny M*, and El Ridi R. Biomedical Research Center for Infetious Diseases, VACSERA, Cairo, Egypt.

542 MULTIPLE EPITOPE CONFORMATIONS OF A SCHISTOSOME ANTIGEN. Bungiro RD*, Petzke MM, Goldberg M, Suri PK, McCray Jr. JW, and Knopf PM. Department of Molecular Microbiology & Immunology, Brown University, Providence, RI; and Department of Biology, Morehouse College, Altlanta, GA.

543 A PUTATIVE CALCIUM-TRANSPORTING ATPASE GENE FROM *SCHISTOSOMA MANSONI*. De Mendonca RL*, Beck E, Rumjanek FD, and Goffeau A. Universite Catholique de Louvain, Unite de Biochimie Physiologique, Louvain-la-Neuve, Belgium; Der Justus Liebig Universitat, Biochemisches Inst., Giessen, Germany; and Universidade Federal do Rio de Janeiro, Department de Bioquimica Medica, ICB/CCS, Rio de Janeiro, Brazil.

MALARIA EPIDEMIOLOGY AND DIAGNOSIS

544 CHARACTERIZATION OF NON-CS PROTEIN REPEAT *PLASMODIUM VIVAX*-210 SPOROZOITE MONOCLONAL ANTIBODIES. Gonzalez-Ceron L*, Wirtz RA, Sina BJ, Hall T, Sattabongkot J, Tsutsumi V, and Rodriguez MH. Center for Malaria Research, Tapachula, Mexico; Walter Reed Army Institute of Research, Washington DC; University of Maryland, College Park, MD; US Army Medical Component, Bangkok, Thailand.; and Center for Research and Advance Studies, Mexico D.F.

545 MYOCARDIAL SEQUESTRATION IN RHESUS MONKEYS INFECTED WITH *PLASMODIUM FRAGILE*. Didier PJ*, Campeau RJ, Cogswell FB, Blanchard JL, Bohm RP, Ratterree MS, Peyman CA, Spurlock JP, Dupepe L, Krogstad FM, Collins WE, and Krogstad DJ. Tulane University, New Orleans, LA; Centers for Disease Control, Atlanta, GA; and Louisiana State University, New Orleans, LA.

546 *PLASMODIUM OVALE* IN THE SPLENECTOMIZED CHIMPANZEE. Morris CL*, Sullivan JS, McClure HM, Strobert EA, Richardson BB, Galland GG, Goldman IF, and Collins WE. Div. of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Yerkes Regional Primate research Center, Emory University, Atlanta, GA.

547 MOLECULAR AND MORPHOLOGICAL RESOLUTON OF CRYPTIC SPECIES OF MALARIA VECTORS FROM SOUTH AMERICA. Lounibos LP*, Conn J, Hribar LJ, Wilkerson RC, and Fritz GN. Florida Medical Entomology Laboratory, University of Florida, Vero Beach FL; Department of Biology, University of Vermont, Burlington VT; Walter Reed Biosystematics Unit, Smithsonian Institution, Washington DC; and Department of Zoology, Eastern Illinois University, Charleston IL.

548 MALARIA IN INFANTS AND ITS RELATIONSHIP WITH MATERNAL MALARIA HISTORY. Luxemburger C*, Nosten F, Neminn K, McGready R, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

549 CLINICAL PREDICTORS OF MALARIA DIAGNOSIS IN AN AREA OF LOW AND UNSTABLE TRANSMISSION OF RESISTANT *PLASMODIUM FALCIPARUM*. Luxemburger C*, Nosten F, Heh Wah T, Slight S, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

550 POOLING STRATEGY FOR ESTIMATION OF SPOROZOITE RATES. Gu WD*. Department of Parasitology, Second Military Medical University, Shanghai, People's Republic of China.

551 PATTERNS OF MALARIA EPIDEMICS IN THE HIGHLAND AREAS OF KENYA. Ouma JH*, Rapuoda B, and Beier JC. Division of Vector-Borne Diseases, Ministry of Health, Kenya; and Department of Tropical Medicine, Tulane University, New Orleans, LA.

552 OVERVIEW OF MALARIA IN NIGER. Mounkaila AB*. Ministäre de la Santé Publique, Niger.

553 SEASONAL VARIATION OF ANTI-*PLASMODIUM FALCIPARUM* ANTIBODIES DIRECTED AGAINST GAMETOCYTE ANTIGEN PFS2400 IN THREE LOCALITIES IN AMAPA, BRAZIL. Marrelli MT, Malafronte RS, Nussenzweig RS, Kloetzel JK. Instituto de Medicina Tropical de Sao Paulo, Sao Paulo, Brazil; Department of Molecular and Biochemical Parasitology, New York University School of Medicine, New York, NY: and Department of Parasitology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

554 MALARIA IN HAITI: EPIDEMIOLOGIC AND CLINICAL ASSESSMENTS. Nicolas E*, Jean-Francois V, Rogers HM, Bloland PB, Ruebush TK, and Nguyen-Dinh P. Ministry of Public Health and Population, Haiti; Pan American Health Organization, Haiti; Hopital Le Bon Samaritain, Limbe, Haiti; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

555 THE VILLAGE OF DIENGA AS A FIELD BASE FOR VACCINE TESTING IN EASTERN GABON-FREQUENCY OF MALARIA ASSOCIATED SYMPTOMS IN SCHOOLCHILDREN AND PARASITOLOGICAL SURVEY. Millet P*, Luty A, Reltien J, Renaut A, Tshipamba P, Lekoulou F, Mayombo J, DeLoron P, and Georges AJ. Centre International de Recherche Médicale de Franceville (CIRMF), Franceville, Gabon.

MALARIA BIOLOGY AND MOLECULAR BIOLOGY

556 COMPARISON OF A PUTATIVE MULTI-DRUG RESISTANCE GENE IN SENSITIVE AND RESISTANT STRAINS OF *PLASMODIUM BERGHEI*. Gervais GW, Trujillo-Nevarez K, Serrano AE*. Department of Microbiology and Medical Zoology, University of Puerto Rico School of Medicine, San Juan, PR.

557 HEMOZOIN CATALYZED OXIDATION OF ARACHIDONIC ACID. Green MD*, Xiao L, Udhayakumar V, Lal AA, and Todd GD. Division of Parasitic Diseases, Centers for Disease Control and Prevention. Atlanta, GA.

558 HEMOZOIN (β-HEMATIN) INHIBITS CYTOKINE RELEASE AND ADHESION MOLECULE EXPRESSION OF STIMULATED HUMAN ENDOTHELIAL CELLS. Taramelli D*, Basilico N, Saresella M, Pagani E, Ferrante P, Chione M, Olliaro P. Institute of Medical Microbiology, University of Milan, Milan, Italy; Laboratory of Biology, Don Gnocchi Foundation, Milan, Italy; Sieroterapico, Soc Coop., Milan, Italy; and UNDP/World Bank/WHO Special Program for Research and Training (TDR), Geneva, Switzerland.

559 CLONING AND SEQUENCING OF A PROTO-ONCOPROTEIN INVOLVED IN SIGNAL TRANSDUCTION IN *PLASMODIUM FALCIPARUM*. Hatin I*, Hernandez-Rivas, Hernandez-Rivas R, and Jaureguiberry G. Institut National de la Santé et de la Recherche Médicale, Paris, France.

560 CYTOADHERENCE CHARACTERISTICS OF *PLASMODIUM FALCIPARUM* ISOLATES IN THAILAND: EVIDENCE FOR CHONDROITIN SULFATE A AS A CYTOADHERENCE RECEPTOR. Chaiyaroj SC*, Buranakiti A, Angkasekwinai P, Looareesuwan S, Rogerson SJ, and Brown GV. Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; and Immunoparasitology Unit, The Walter and Eliza Hall Institute, Victoria, Australia.

561 STUDIES ON THE MECHANISMS OF CYTOADHERENCE AND ANTIGENIC VARIATION IN *PLASMODIUM FALCIPARUM*. Reeder JC*, Rogerson SJ, Davern KM, and Brown GV. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

562 ANALYSIS OF VARIABILITY IN THE MEROZOITE SURFACE PROTEIN OF *PLASMODIUM BERGHEI*. Jennings GJ*, van Belkum A, van Doorn LJ, and Wiser MF. Department of Tropical Medicine, Tulane University Medical Center, New Orleans LA; and TNO Primate Center, Rijswijk, The Netherlands.

563 CHARACTERIZATION OF TWO CLONES EXPRESSING FUSION PROTEINS RECOGNIZED BY MAB RAISED AGAINST A GLU-RICH PROTEIN ASSOCIATED WITH THE ERYTHROCYTE MEMBRANE. Giraldo LE*, Jennings G, Schmitt-Wrede HP, Wunderlich F, and Wiser MF. Department of Tropical Medicine, School of Public Health & Tropical Medicine, Tulane University, New Orleans, LA; and Division of Molecular Parasitology, Universitat Dusseldorf, Dusseldorf, Germany.

564 SICKLE CELL TRAIT AND PLACENTAL MALARIA INFECTION IN TWO AREAS OF KENYA WITH DEFFERENT TRANSMISSION PRESSURE. Nahlen BL*, Udhayakumar V, Parise ME, Ayisi JG, Bloland PB, Oloo AJ, and Lal AA. Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; Vector Biology and Control Research Center, KEMRI, Kisumu, Kenya.

565 THE NOD/LTSZ-*SCID* MOUSE MODEL FOR *PLASMODIUM FALCIPARUM* INFECTION: POTENTIAL AND LIMITATIONS. Moore JM*, Schultz LD, and Rajan TV. Department of Pathology, University of Connecticut Health Center, CT; and Jackson Laboratory, Bar Harbor, ME.

566 DEVELOPMENT OF AN IN VITRO MODEL FOR INVASION OF MOSQUITO SALIVARY GLANDS BY MALARIA SPOROZOITES. Suwanabun N*, Sattabongkot J, Linthicum KJ, and Rosenberg RM. Department of Entomology, U.S. Army Medical Component, AFRIMS, Bangkok, Thailand; and Department of Entomology, Walter Reed Army Institute of Research, Washington DC.

567 PCR-BASED NON-ISOTOPIC LIQUID HYBRIDIZATION VERSUS MICROSCOPY IN MONITORING SPOROZOITE-INDUCED *PLASMODIUM VIVAX* INFECTIONS IN *SAIMIRI* MONKEYS. Grady KK*, Oliveira DA, Sullivan JS, Lal AA, and Collins WE. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

568 EXPRESSION OF THE *PLASMODIUM FALCIPARUM* NUTRIENT-PERMEABLE CHANNEL ON OOCYTES. Desai SA*. Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, NC.

569 HIGH RESOLUTION X-RAY MICROSCOPY, A NEW TOOL TO INVESTIGATE INTACT PARASITES. Meyer-Ilse W*, Magowan CC, and Moronne MM. Center for X-ray Optics, Lawrence Berkeley Laboratory, Berkeley, CA.

571 OPTIMIZATION OF ELECTROPORATION PARAMETERS FOR *IN VITRO* TRANSFECTION OF *PLASMODIUM FALCIPARUM*. Bell CA*, Mbachem WF, Miller RE, Kesler JC, Glassic MA, Wirth DF, and Nuzum EO. Walter Reed Army Institute of Research, Department of Parasitology, Division of Experimental Therapeutics, Washington, DC; and Harvard School of Public Health, Department of Tropical Public Health, Boston, MA.

572 IDENTIFICATION OF PUTATIVE 3' GENE FLANKING ELEMENTS NECESSARY FOR GENE EXPRESSION IN MALARIA. Golightly LM*, Budge E, Mbacham WF, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

573 ANALYSIS OF MUTATIONS IN *pfmdr1* ASSOCIATED WITH DRUG RESISTANT PARASITES EXPRESSED IN *SACCHAROMYCES CEREVISIAE*. Volkman SK*, Woodcock S, Cowman AF, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

MALARIA CHEMOTHERAPY

574 HEMOZOIN CATALYZED OXIDATION OF ARACHIDONIC ACID. Green MD*, Xiao L, Udhayakumar V, Lal AA, and Todd GD. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA.

575 ASSESSMENT OF THE EFFECT OF THE ORAL IRON CHELATOR DEFERIPRONE ON MILD *PLASMODIUM FALCIPARUM* PARASITEMIA IN HUMANS. Thuma PE*, Olivieri NF, Mabeza GF, Biemba G, Parry D, Zulu S, Fassos FF, Koren G, Brittenham GM, and Gordeuk VR. Division of General Pediatrics, Pennsylvania State University College of Medicine, Hershey, PA; Division of Haematology/Oncology, The Hospital for Sick Children and University of Toronto, Toronto, Canada; Macha Mission Hospital, Choma, Zambia; Division of Clinical Pharmacology, The Hospital for Sick Children and University of Toronto, Toronto, Canada; Division of Hematology/Oncology, MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH; and Division of Hematology/Oncology, The George Washington University Medical Center, Washington, DC.

576 EFFECT OF ORALLY ACTIVE IRON CHELATORS IN COMBINATION WITH ANTIMALARIALS ON THE GROWTH IN VITRO OF *PLASMODIUM FALCIPARUM*. Pattanapanyasat K *, Kotipun K, Yongvnithchit K, Hider RC, Kyle DE, and Heppner DG. Department of Immunology and Parasitology, AFRIMS, Bangkok, Thailand; Department of Pharmacy, King's College, University of London, UK; Division of Experimental Therapeutics, WRAIR, Washington, D.C.; Department of Tropical Medicine, Mahidol University, Bangkok, Thailand.

577 CHARACTERIZATION OF CHLOROQUINE-FERRIPROTOPORPHYRIN-IX BINDING USING ISOTHERMAL TITRATION MICROCALORIMETRY. Vippagunta SR*, and Vennerstrom JL. College of Pharmacy, University of Nebraska Medical Center, Omaha, NE.

578 THE CRYSTAL STRUCTURE OF THE TRIAZINE ANTIMALARIAL AGENT WR 99,210 AS A MODEL FOR ITS BIOLOGICAL INTERACTION. Karle JM*. Department of Pharmacology, Walter Reed Army Institute of Research, Washington, DC.

579 ERADICATION OF *PLASMODIUM VIVAX* LIVER-STAGE PARASITES BY ANTIMALARIAL DRUGS IN A TISSUE CULTURE MODEL. Karnasuta C*, Aikawa M, Fujioka H, Chantakulkij S, and Watt G. Department of Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Institute of Pathology, Case Western Reserve University, Cleveland, OH.

580 MANNICH BASES WITH GREATER ANTIMALARIAL ACTIVITY THAN PYRONARIDINE. Kotecka BM*, Barlin GB, and Rieckmann KH. Army Malaria Research Unit, Sydney, Australia; John Curtin School of Medical Research, Canberra, Australia.

581 THE EFFECTS OF BISTRATENE A ON THE DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN CULTURE. Mann VH*, Law MH, Watters D, and Saul A. Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

582 EVALUATION OF ANTIMALARIAL ACTIVITY OF MEDICINAL HERBS. Obih*. College of Pharmacy, Xavier University of Louisiana, New Orleans, LA.

583 STRUCTURE-BASED DEVELOPMENT OF NOVEL MALARIA CYSTEINE PROTEASE INHIBITORS. Miller R*, Nuzum E, Li R, Chen X, Gong B, Dominguez J, Rosenthal P, Kenyon G, Kuntz I, and Cohen F. Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA.

584 MOLECULAR MODELING STUDIES OF POLYCYCLIC PEROXIDES AND THE SEARCH FOR DESCRIPTORS OF ANTIMALARIAL ACTIVITY. Jefford CW*, Weber J, Grigorov M, Thomson C, Suter HU, and Maric D. Departments of Organic and Physical Chemistry, University of Geneva, Geneva; Department of Chemistry, University of St. Andrews, St. Andrews, Scotland; and Swiss Scientific Computer Center, Manno, Switzerland.

586 ARTEMISININ DERIVATIVES AS INDUCERS OF CELL DIFFERENTIATION. Wongpanich V*, Suh N, Angerhofer CK, El-Feraly FS, and Pezzuto JM. Program for Collaborative Research in the Pharmaceutical Sciences, Department Medicinal Chemistry and Pharmacognosy; and Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

587 METABOLISM OF ARTEETHER TO DIHYDROQINGHAOSU BY ISOZYMES OF HUMAN AND RAT CYTOCHROME P-450. Grace JM*, Peggins JO, Aguilar AJ, and Brewer TG. Division of Experimental Therapeutics, WRAIR, Washington, DC; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

588 ARTEETHER PHARMACOKINETICS IN RATS AFTER 7 DAILY INTRAMUSCULAR DOSES. Li QG*, Brueckner RP, Peggins JO, Masonic KJ, Trotman KM, and Brewer TG. Department of Pharmacology, Division of Experimental TherapeuticsWalter Reed Army Institute of Research, Washington, DC.

589 ANORECTIC TOXICITY OF DIHYDROARTEMISININ, ARTEMETHER AND ARTEETHER IN RATS FOLLOWING MULTIPLE INTRAMUSCULAR DOSES. Peggins JO*, Li QG, and Brewer TG. Division of Experimental Therapeutics, WRAIR, Washington, DC; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

590 THE STATE OF PYRIMETHAMINE-RESISTANT *PLASMODIUM FALCIPARUM* PARASITES AMONG TANZANIAN CHILDREN; A FACILITY-BASED STUDY USING PCR. Edoh D *, Mshinda H, Kitua A, Jenkins J, Burger M, and Tanner M. Ifakara Centre, Ifakara, Tanzania; Swiss Tropical Institute, Basel, Switzerland; Friedrich Miescher-Institut, Basel, Switzerland.

591 SUSTAINED EFFICACY OF SULFADOXINE-PYRIMETHAMINE AS FIRST-LINE TREATMENT FOR MALARIA IN UNDER FIVE MALAWIAN CHILDREN. Ziba C*, Nwanyanwu OC, Kazembe PN, Wirima, and Redd SC. Ministry of Health, Lilongwe, Malawi; College of Medicine, University of Malawi, Blantyre, Malawi; and Centers for Disease Control and Prevention, Atlanta, GA.

592 A SYSTEMATIC REVIEW OF PUBLISHED AND UNPUBLISHED DATA ON ORAL AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA. Olliaro P*, LeBras J, Nevill C, Ringwald P, Mussano P, Garner P, and Brasseur P. WHO/TDR, Geneva, Switzerland; Hopital Bichat-C. Bernard, Paris, France; AMREF, Nairobi, Kenya; OCEAC, Yaounde, Cameroon; Cochrane Parasitic Diseases Group, Geneva, Switzerland; Cochrane Parasitic Diseases Group, Liverpool School of Tropical Medicine, UK; and Hopital C. Nicolle, Rouen, France.

593 VARIABLE PROGUANIL METABOLISM IN DIFFERENT ETHNIC GROUPS HAS CLINICAL IMPLICATIONS FOR PROPHYLAXIS OR TREATMENT OF MALARIA. Edstein MD*, Looareesuwan S, Kyle DE, Canfield CJ, Hutchinson DB, and Rieckmann KH. Army Malaria Research Unit, Sydney, Australia; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Experimental Therapeutics, WRAIR, Washington, DC; Pharmaceutical Systems Inc, Gaithersburg, MD; Wellcome Research Laboratories, Beckenham, UK.

594 *IN VIVO* SAFETY AND ANTIPLASMODIAL ACTIVITY OF CHLOROQUINE (CQ) ANALOGS ACTIVE AGAINST CQ-RESISTANT *PLASMODIUM FALCIPARUM IN VITRO*. Cogswell FB*, Preslan J, Spurlock JP, Mack PA, Krogstad FM, De D, George WJ, and Krogstad DJ. Tulane University, New Orleans, LA.

595 *IN VITRO* SENSITIVITY OF *PLASMODIUM FALCIPARUM* ISOLATES IN THAILAND, 1990-1994. Wongsrichanalai C*, Wimonwattrawatee T, Sookto P, Laoboonchai A, Heppner DG, and Wernsdorfer WH. Department of Immunology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Institute of Specific Prophylaxis and Tropical Medicine, University of Vienna, Vienna, Austria.

596 CHLOROQUINE-SENSITIVE FALCIPARUM MALARIA IN LAOS. Watt G*, Jongsakul K, Chum, and Karnasuta C. Department of Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Medical Director, Ministry of Public Health, Khammouang Province, Laos.

597 ANTIMALARIAL CHEMOPROPHYLAXIS IN AFRICA: EXTRAPOLATION FROM *IN VITRO* ACTIVITY OF CHLOROQUINE, CYCLOGUANIL, AND MEFLOQUINE. Basco LK*, Ringwald P, Thor R, Doury JC, and Le Bras J. Laboratoire de Parasitologie, Hopital Bichat-Claude Bernard, Paris; and Institut de Médecine Tropicale du Service de Santé des Armées, Le Pharo, Marseille, France.

598 EVALUATION OF SELECTED ANTIMALARIAL DRUGS IN THE *BABESIA MICROTI*-HAMSTER MODEL. Marley SE*, Eberhard ML, Stuerer FJ, Ellis WY, McGreevy PB, and Ruebush TK. Parasitic Diseases Division, Centers for Disease Control, Atlanta, GA; Zoology Department, University of Georgia, Athens, GA; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

OPPORTUNISTIC INFECTIONS

599 SHORT-TERM CULTURE OF *PNEUMOCYSTIS CARINII* FROM INOCULATED MOUSE. Shaw MM*, Bartlett MS, Durant PJ, Queener SF, and Smith JW. Department of Pathology and Laboratory Medicine, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

600 A BACTERIOPHAGE P1 GENOMIC LIBRARY OF *PNEUMOCYSTIS CARINII*. CONSTRUCTION AND CHARACTERIZATION. Metcheva IS*, and Buck GA. Medical College of Virginia, Virginia Commonwealth University, Richmond,VA.

601 HUMAN BABESIOSIS IN TAIWAN: FIRST CASE OF BABESIAL INFECTION IN A NATIVE TAIWANESE. Shih CM*, and Chung WC. Department of Parasitology and Tropical Medicine, National Defense Medical Center, Taipei, Taiwan, R. O. C.; and Department of Parasitology, Taipei Medical College, Taipei, Taiwan, R. O. C.

602 CYCLOSPORIASIS IN ONTARIO - A LABORATORY REVIEW AND ESTIMATE OF PREVALENCE AND CLINICAL DESCRIPTION OF CASES. Keystone JS*, MacPherson DW, and Palmer J. Tropical Disease Unit, The Toronto Hospital (General Division), University of Toronto, Toronto, ON; Regional Parasitology Lab, St. Joseph's Hospital, McMaster University, Hamilton, ON; and Technical Services, MDS Laboratories, Etobicoke, ON.

603 IMPROVED RECOVERY OF *CRYPTOSPORIDIUM* OOCYSTS FROM DRINKING WATER USING VORTEX-FLOW FILTRATION COMBINED WITH IMMUNOMAGNETIC SEPARATION. Fricker CR, Turner NB, Rolchigo PM, Margolin AB, and Crabb JH*. Thames Water Utilities, Reading, UK; ImmuCell Corporation, Portland, ME; Membrex Incorporated, Fairfield, NJ; Department of Microbiology, University of New Hampshire, Durham, NH.

604 USE OF ELISA-BASED ASSAYS TO STUDY *CRYPTOSPORIDIUM PARVUM*-HOST CELL INTERACTION *IN VITRO*. Ward HD*, Joe A, Kelley MA, Verdon R, Tzipori S, Pereira ME, and Keusch GT. Division of Geographic Medicine and Infectious Diseases, Tufts University-New England Medical Center; Division of Infectious Diseases, Tufts University School of Veterinary Medicine.

605 EFFECTS OF GAMMA IRRADIATION ON VIABILITY AND IMMUNOGENICITY OF *CRYPTOSPORIDIUM* OOCYSTS. McLain SD, and Crabb JH*. ImmuCell Corporation, Portland ME.

606 EVALUATION OF COMMERCIAL TEST KITS FOR DETECTION OF *CRYPTOSPORIDIUM* OOCYSTS OF SPECIES OTHER THAN *CRYPTOSPORIDIUM PARVUM*. Graczyk TK*, Cranfield CR, and Fayer R. Department of Molecular Microbiology and Immunology, The Johns Hopkins University, SHPH, Baltimore, MD; Division of Comparative Medicine, The Johns Hopkins University, Baltimore, MD; and Parasite Immunobiology Laboratory, LPSI, ARS, U.S. Department of Agriculture, Beltsville, MD.

607 EFFECT OF VITAMIN A AND VITAMIN A DEFICIENCY ON EXPERIMENTAL CRYPTOPORIDIOSIS. Leitch GJ*, He Q, Russell SA, and Rawls RA. Department of Physiology, Morehouse School of Medicine, Atlanta, GA.

608 *CRYPTOSPORIDIUM PARVUM*IN CHILDREN WITH DIARRHEA IN ZULIA STATE, VENEZUELA. Chacín-Bonilla L*, Bonilla MC, Soto de Torres L, Rios de Cándida Y, Sardiña M, Enmanuels C, Parra AM, and Sánchez-Chávez Y. Instituto de Investigaciones Clínicas, Universidad del Zulia, Maracaibo, Venezuela.

609 COMPARISON OF THREE STAINING METHODS FOR DETECTING MICROSPORIDIA IN FLUIDS. Didier ES*, Orenstein JM, Aldras AM, Bertucci DB, Rogers LB, and Janney FA. Department of Microbiology, Tulane Regional Primate Research Center, Covington, LA; Department of Pathology, George Washington Univ. Med. Center, Washington, DC; and Department of Pathology, Louisiana State Univ., New Orleans, LA.

610 CULTURE AND ANTIGENIC ANALYSIS OF FIVE ISOLATES OF *ENCEPHALITOZOON (SEPTATA) INTESTINALIS* ORIGINATING FROM SPUTUM AND URINE OF 3 AIDS PATIENTS. del Aguila C*, Croppo GP, Moss DM, Leitch GJ, Wallace S, and Visvesvara GS. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; and Department of Physiology, Morehouse School of Medicine, Atlanta, GA.

611 RECOVERY OF MICROSPORIDIA IN SAF-FIXED FECAL SAMPLES BY LIGHT MICROSCOPY AND CORRELATION WITH ELECTRON MICROSCOPY. Carter PL*, MacPherson DW, McKenzie RA. Regional Parasitology, St. Joseph's Hospital, Hamilton, Ontario, Canada; Parasitology, St.Joseph's Hospital and

Pathology, McMaster University, Hamilton, Ontario, Canada; and Electron Microscopy, McMaster University, Hamilton, Ontario, Canada.

612 IDENTIFICATION OF *NEOSPORA CANINUM* cDNA CLONES EXPRESSING RECOMBINANT ANTIGENS USEFUL FOR THE DIAGNOSIS OF BOVINE NEOSPOROSIS. Lally NC*, Jenkins MC, and Dubey JP. USDA, ARS, Parasite Immunbiology Laboratory, Beltsville, MD; and USDA, ARS, Parasite Biology and Epidemiology Laboratory, Beltsville MD.

613 POLYMERASE CHAIN REACTION FOR THE DETECTION OF *TOXOPLASMA GONDII* IN FORMALIN FIXED, PARAFFIN EMBEDDED TISSUE FROM AN INFECTED CAT. Soleimani Z*, Luinstra K, Mahony JB, and MacPherson DW. Department of Parasitology, Iran University of Medical Sciences, Tehran, Iran; and Medical Microbiology, St. Joseph's Hospital, McMaster University, Hamilton, Ontario, Canada.

614 EPIDEMIOLOGY OF BRUCELLOSIS AT A UNIVERSITY HOSPITAL IN SOUTH TEXAS, 1985-1995. Dib JC*, Miller D, and Patterson JE. Department of Med. Infectious Diseases, University of Texas Health Science Center at San Antonio, San Antonio TX.

615 A NEW MONOCLONAL ANTIBODY AGAINST *TOXOPLASMA GONDII* FOR DIAGNOSTIC USE WITH TISSUE SECTIONS. Sundermann CA*, Estridge BH, Branton M, and Lindsay DS. Department of Zoology & Wildlife Science, Auburn University, AL; Department of Biology, Stillman College, Tuscaloosa, AL; and Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL.

616 INTERACTION OF *ACANTHAMOEBA* SPP. WITH MACROPHAGES. Toney DM, Cabral GA, and Marciano-Cabral F. Department Microbiology & Immunology, VA Commonwealth University, Medical College of VA, Richmond, VA.

617 AN H^+-ATPase REGULATES CYTOPLASMIC pH IN PNEUMOCYSTIS TROPHOZOITES. McLaughlin G, Shaw M, Durant P, Bartlett MS, VanDerHeyden N, Moreno SNJ, and Docampo R. Indiana University School of Medicine, Indianapolis, IN; and Univerisy of Illinois, Urbana, IL.

618 INTESTINAL OPPORTUNISTIC PARASITES AMONG DIFFERENT GROUPS OF IMMUNOCOMPROMISED HOSTS. Abaza SM*, Makhlouf LM, El-Shewy KA, and El-Moamly AA. Parasitology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

619 A SIMPLE METHOD FOR SCREENING DRUGS AGAINST *CRYPTOSPORIDIUM PARVUM IN VITRO.* Meloni BP*, and Thompson RC. WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary Studies, Murdoch University, Western Australia.

620 DEVELOPMENT OF PCR PRIMERS FOR THE DIAGNOSIS OF *ENTEROCYTOZOON BIENEUSI* INFECTIONS, BASED ON REGION CODING FOR THE SSU-RRNA. Da Silva AJ*, Slemenda SB, Visvesvara GS, Moura H, Schwartz DA, Wilcox CM, and Pieniazek NP. Division of Parasitic Diseases NCID, Centers for Disease Control and Prevention, Atlanta, Georgia; Estate University of Rio de Janeiro, Rio de Janeiro, Brazil; and Grady Memorial Hospital, Emory University School of Medicine, Atlanta, GA.

621 THE USE OF RAPD ANALYSIS IN THE DEVELOPMENT OF DIAGNOSTIC PCR PRIMERS FOR *CRYPTOSPORIDIUM*. Morgan UM*, O'Brien PA, and Thompson RC. School of Veterinary Studies and Biotechnology Programme, Murdoch University, Western Australia.

623 MUCOSAL IMMUNOTHERAPY OF CRYPTOSPORIDIOSIS USING IgA MABS TO NEUTRALIZING ANTIGENS. Enriquez FJ*, Riggs MW, Palting J, and Hensel J. Department of Veterinary Science, University of Arizona, Tucson, AZ.

624 IMMUNOLOCALIZATION AND CHARACTERIZATION OF A *CRYPTOSPORIDIUM PARVUM* SECRETORY GLYCOPROTEIN ASSOCIATED WITH SPOROZOITE. El-Shewy KA*, Kilani RT, Sherburne R, and Wenman WM. Departments of Pediatrics and Medical Microbiology, Division of Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada.

ABSTRACTS

1 WORLD-WIDE CONTROLLED CLINICAL TRIALS WITH ATOVAQUONE AND PROGUANIL FOR TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA. Canfield CJ*, Boudreau EF, Altstatt LB, Dausel LL, and Hutchinson DB. Pharmaceutical Systems Inc., Gaithersburg, MD; and Glaxo Wellcome, UK.

Atovaquone is a new hydroxynaphthoquinone antimalarial discovered by Wellcome Research Laboratories. Use of the drug alone for treatment of acute falciparum malaria gave prompt clinical and parasitologic responses, but was associated with a high rate of recrudescences. In addition, the recrudescent parasites had become markedly resistant to atovaquone. *In vitro* studies of atovaquone and proguanil showed potentiation. Subsequent dose-ranging studies in Thailand of these two drugs administered concurrently confirmed the potentiation and resulted in a 100% cure rate in 24 patients using an optimized dose regimen of 1 g atovaquone plus 400 mg proguanil administered every 24 hours for three doses. This regimen has now been further evaluated in a series of comparative clinical trials involving more than 700 patients (adults and children) in Thailand, Zambia, Kenya, Gabon and the Philippines; all studies had 28-day followup. The comparator drugs were mefloquine, Fansidar, halofantrine, amodiaquine and chloroquine respectively. Three additional studies are ongoing, two in South America and one in France. The results of the completed studies showed that the atovaquone/proguanil combination gave a radical cure rate in excess of 98%; in all cases the cure rate was superior to the comparator drug. The drug combination was also well tolerated. Results from the individual centers will be presented.

2 TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA WITH ATOVAQUONE PLUS PROGUANIL IN BRAZILIAN ADULTS. Cerutti C*, Alencar F, Piovesan-Alves F, Milhous WK, and Pang LW. U.S. Army Medical Research Unit-Brazil, Rio de Janeiro, Brazil; and University of Sao Paulo, Sao Paulo, Brazil.

In a randomized, open labeled study we compared 24 patients with atovaquone (1000 mg single dose) + proguanil (400 mg single dose) for three consecutive days (AP) to 19 subjects on a standard antimalarial regimen (QT) of 7 days of quinine (600 mg TID) + tetracycline (250 mg QID). Patients were adult males presenting with symptoms of uncomplicated malaria with *Plasmodium falciparum* parasite counts between 1,000 and 100,000 per µl. Patients were administered all mediciations by investigators and hospitalized for 28 days on a study ward in a malaria free area. The AP and QT groups, respectively, were comparable for age (mean ± SD), 30 ± 9 vs 30 ± 10; number of falciparum malaria episodes during previous two years, 9 ± 11 vs 11 ± 9; years living in malarious area, 10 ± 8 vs 9 ± 8; percent with fever on admission, 63% vs 68%; proportion with spenomegaly, 24/24 vs 17/19. By day 28 the falciparum cure rates were 100% for both groups. Comparing the AP and QT groups there was a shorter mean parasite clearance time, 59 hr vs 71 hrs (P = .02). Of the 15 and 13 that had fever on admission in the AP and QT groups, respectively, the mean fever clearance times were 13 hrs vs 22 hrs, (P = .19). The commonest side effects were: dizziness (17%), nausea (17%), tinnitus (42%), dizziness (32%), abdominal pain (21%), nausea (21%), diarrhea (16%), vomiting (11%), and weakness (11%) in the QT group. Three patients had vivax malaria while on the ward in the AP group, compared with 6 patients in the QT group. We conclude that the efficacy of AP is high and possibly faster than that of QT and the ease of administration make it an effective regimen in this area of multidrug resistant *P. falciparum*.

3 HYDROXY-ANTHRAQUINONES AS ANTIMALARIAL AGENTS. Riscoe MK*, Winter RW, Cornell KA, Ignatushchenko M, and Hinrichs DJ. Medical Research Service, Department of Veterans Affairs Medical Center, Portland, OR; Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR; and Interlab Inc., Lake Oswego, OR.

A recent addition to the arsenal of drugs for malaria treatment is the hydroxynaphthoquinone, 2-[trans-4-(4-chlorophenyl-cyclohexyl)-3-hydroxy-1, 4- naphthoquinone (BW 566C80/Atovaquone®). Because of the success of this compound, we hypothesized that hydroxylated anthraquinones, acting as analogs of the naphthoquinones, could exert potent antimalarial effects. In this study we present structure-activity relationships for a series of hydroxy-anthraquinones. Accordingly, hydroxy- and polyhydroxy-anthraquinones were screened for inhibitory activity against the malarial parasite, *Plasmodium falciparum* (clone D6). Rufigallol demonstrated the most potent effects with a 50% inhibitory concentration (IC_{50}) value of ~10.5 ng/ml (~35 nM). Taking into account the known chemical properties of quinones in general and of rufigallol in particular, we speculate that rufigallol functions as a redox-active iron chelator. Therefore we consider rufigallol to be in the class of so-called "oxidant drugs" which exert their antimalarial effects through the enhanced production of oxygen radicals in parasitized red blood cells. Our discovery of a compound which potentiates the antimalarial activity of rufigallol by 300 to 500-fold supports the notion that rufigallol acts via a pro-oxidant mechanism. Details of this discovery will be presented including our insight into the mechanisms underlying this drug potentiation.

4 THERAPEUTIC CONCENTRATIONS OF CHLOROQUINE INDUCE CHANGES IN THE CELLULAR ROUTAGE OF CATHEPSIN D-LIKE PROTEIN IN *PLASMODIUM FALCIPARUM*. Jambou R*, Bailly E, Rabe T, Benedetti EL, and Jaureguiberry G. INSERM U13, Hopital Claude Bernard, Paris France; Institut Jacques Monod du CNRS, Université Paris VII, Paris France; Institut Pasteur de Madagascar, Antananarivo, Madagascar.

During the erythrocytic step of the *Plasmodium falciparum* life cycle, haemoglobin proteolysis provides the aminoacids required for parasite protein biosynthesis. Cathepsin D appears to be very important in this process, that occurs in the food vacuole. In order to follow the routage of this enzyme in the parasite, we have used a polyclonal affinity-purified anticathepsin D serum in immuno-electron microscopy characterization. As previously reported, the anti-serum recognize a parasite encoded 55-58kDa protein in parasite extracts. Cathepsin D was found in small vesicles in the parasite cytoplasm, in late endosoms and in the food vacuole. After two-hour treatment with chloroquine (10 to 30 nM), this enzyme was found both in small vesicles around enlarged endosomes and clustered with electron dense material spread in the red blood cell cytoplam. After four to six hours, it has disappeared from the food vacuole. This aspect of lysosomal enzyme missorting was similar to those described for mammalian cells treated with high chloroquine concentration (0.1 to 1mM). The presence of cathepsin D (particularly the 55-58kDa species) in the food vacuole supports its role in hemoglobin proteolysis, while this excretion process could explain part of chloroquine induced inhibition of proteolysis in the parasite.

5 SURVEYS OF RESISTANCE TO CHLOROQUINE BY *PLASMODIUM VIVAX* IN INDONESIA AND THE PHILIPPINES. Baird JK*, Masbar S, Fryauff DJ, Basri H, Tjitra E, Nalim MF, and Canete-Miguel E. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; National Institute of Health Research, Jakarta, Indonesia; and ALYKA, Puerto Princesa City, Palawan, Philippines.

Resistance to chloroquine by *Plasmodium vivax*, first reported from Papua New Guinea in 1989, has been confirmed by longitudinal survey of indigenous populations only in the Arso region of Irian Jaya, at the eastern edge of Indonesia. A single case was reportedly acquired at the far western island of Nias in Indonesia by an American tourist, and two cases from Myanmar (Burma) have been reported. These latter cases, over 5000 km from New Guinea, suggest that resistance to chloroquine by *P. vivax* may extend the length of the Indonesian archipelago. This laboratory conducted longitudinal surveys of resistance among residents infected by *P. vivax* from the following areas: 1) Arso, on the northeastern coast of Irian Jaya; 2) Sekotong, on the southwestern coast of the island of Lombok near Bali; 3) Kokap, near the south central coast of the island of Java; 4) Teluk Dalam, on the southern coast of the island of Nias near Sumatra; and 5) at Puerto Princesa City, on the east coast of the island of Palawan, the Philippines. The table below summarizes the results. All recurrent parasitemias appeared with >100 ng/ml chloroquine + desethylchloroquine in whole blood. These studies confirm resistance to chloroquine by *P. vivax* at Nias near Sumatra, but no evidence of resistance was found on Java or Lombok in Indonesia nor at Palawan, the Philippines. Other surveys currently in progress in Southeast Asia shall be described.

Cumulative Subjects Positive for P. vivax Trophozoites

Day	Irian Jaya	Lombok	Java	Sumatra	Palawan
0	50	24	14	21	21
7	4	0	0	0	0
14	24	0	0	1	0
21	33	0	0	3	0
28	37	0	0	3	0
% +	78%	0%	0%	14%	0%

6 TREATMENT OF UNCOMPLICATED *IN VITRO* CHLOROQUINE RESISTANT FALCIPARUM MALARIA IN IRIAN JAYA. Tjitra E*, Pribadi W, Budiono W, Arbani PR, Naibaho P, Supriyanto S, Romzan A, and Dewi RM. National Institute of Health Research and Development, Jakarta, Indonesia; Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; Freeport Hospital, Tembagapura, Timika, Irian Jaya, Indonesia; and Directorate General of Communicable Diseases Control and Environmental Health, Ministry of Health, Jakarta, Indonesia

Falciparum malaria resistant to chloroquine is a serious malaria control problem in Indonesia especially in Irian Jaya, Artemether is a new antimalarial drug, very effective and safe for clinical study. This drug is not yet available in Indonesia. To obtain an alternative antimalarial drug for treatment of chloroquine resistant falciparum malaria cases, a trial of artemether treatment for uncomplicated *in vitro* chloroquine resistant falciparum malaria cases was conducted at Freeport Hospital, Tembagapura, Irian Jaya, Indonesia, in April - November 1994. The objective of the

study was to assess the efficacy and safety of artemether. Sixty-six out of 307 uncomplicated falciparum malaria cases were eligible for *in vitro* sensitivity tests. Only 44 of the 54 uncomplicated *in vitro* chloroquine resistant falciparum malaria patients could be included in this study. They were treated orally with 1.6 mg/kg/BW/dose 12 hourly on D0 and followed by a single dose daily on D1-4. All patients were hospitalized until clinically and parasitologically cured. The most frequent symptoms of these malaria patients were headache (82%), paleness (55%), nausea (55%), fever (41%) and chills (39%). The parasite clearance rate of artemether was 100% on D7 (38/38), D14 (38/38), and D21 (31/31). However it was 90.3% (28/31) on D28 because of the presence of 3 (9.7%) recrudescence cases. The mean fever clearance time and parasite clearance time were as follows: 9 ± 10 hours and 31 ± 10 hours (D7, D14), 8 ± 10 hours and 30 ± 9 hours (D21), 8 ± 10 hours and 29 ± 9 hours (D28) respectively. Abdominal pain (7.9%) and diarrhea (5.3%) were noted as side effects of artemether which were mild and self- limiting. Artemether is effective and safe for treatment of uncomplicated *in vitro* chloroquine resistant falciparum malaria in Timika, Irian Jaya. A sequential dose of artemether or combination of artemether and other antimalarial drugs should be studied to achieve a radical cure.* This study was funded by PT Freeport Indonesia, Tembagapura, Irian Jaya.

7 MEFLOQUINE IN THE TREATMENT OF *PLAMODIUM FALCIPARUM* MALARIA IN PREGNANCY. Nosten F*, McGready R, Thein Cho T, Chongsupadjaisiddhi T, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Pregnant women are very vulnerable to infections by *Plasmodium falciparum*. Along the Thai-Burmese border populations are exposed to the most drug resistant strains of *P. falciparum* in the world. The failure rates associated with high dose mefloquine now exceeds 50%. The only two antimalarial drugs available for the treatment of uncomplicated cases in pregnancy are quinine and mefloquine. Between 1991 and 1994 we have treated over 200 Karen pregnant women with a primary attack of *P. falciparum* malaria. The majority (136) received mefloquine (25 mg/kg)and the remaining was treated with quinine sulphate (30 mg/kg/day for 7days). Symptoms and side effects were recorded at baseline and then weekly for 28 days. At each of the visit a blood film and an haematocrit were obtained. Results indicate that mefloquine is associated with a higher incidence of gastro-intestinal adverse effects. Quinine is associated with tinnitus in particular hearing loss. Cure rates were comparable in the two treatment groups and one third of the patients failed and were positive again for *P. falciparum*within 28 days. Second line treatment includes artesunate one of the artemisinin derivatives increasingly used in South-East Asia. There were no detectable differences in the outcome of the pregnancies in terms of birthweight, gestational age and fetal wastage when comparing mefloquine and quinine treated groups. However the babies born to mothers treated for multiple parasitaemic episodes were lighter than the babies born to women treated only once: 2606 g vs 2766 g, (p=0.03). Thirty five women received artesunate without apparent toxicity.

8 INTRARECTAL QUININE ADMINISTERED THROUGH A NEW GALENIC FORMULATION TO *PLASMODIUM FALCIPARUM* MALARIA CHILDREN IN NIGER. Barennes H*, Mahaman Sani A, Pussard E, Clavier F, Kahiatani F, Granic G, Ravinet L, and Verdier F. Coopération franaise; Hôpital de Niamey, Niger; INSERM U13/IMEA, Hôp.C. Bernard, Paris, France; and Sanofi-Winthrop, Gentilly, France.

For severe malaria treatment, intravenous (iv) quinine (Q) is not always suitable in the field and intramuscular (im) Q is often associated with infectious or neurologic complications. The intrarectal (ir) route, commonly used in paediatric practice, has been proposed as an alternative to parenteral administration. We compared the efficacy and the pharmacokinetics of a new Q ir formulation, to the im and iv administrations. Three groups of 7 *Plasmodium falciparum* malaria children each, randomly received either 8 mg kg^{1} Q base (cream) by the ir route or 8 mg kg-1 Quinimax® (a Cinchona alkaloids combination) by the im route or by an iv perfusion over 4 h, every 8 h for 3 days. Similar decrease in temperature and parasitaemia was obtained in the 3 groups and times required for a 50% fall in initial parasitaemia were 12.3 ± 5.4, 18.2 ± 6.1 and 14.5 ± 4.2 h for the ir, im and iv perfusion groups respectively. All patients were aparasitaemic by day 7. Tolerability of the 3 routes of administration was good and no rectal irritation was reported. Tmax appeared later with ir administration (4.1 ± 2.4 h) and with iv infusion (3.8 ± 0.5 h) than with im administration (1.6 ± 1.3 h). Cmax values were lower with the ir route (3.0 ± 1.0 mg l-1) and with the im route (3.2 ± 0.7 mg l-1) than with the iv infusion (5.1 ± 1.4 mg l-1). Areas under the curves (AUC) 0-8 h obtained with the ir route (17.0 ± 7. mg l-1 h) and with the im route (19.4 ± 4.8 mg l-1 h) were lower than with the iv infusion (27.8 ± 8.2 mg l-1 h). The bioavailability of ir Q was 36% vs iv Q and 51% vs im Q. Nevertheless, this Q formulation might be a safe and effective alternative to im Q for the treatment of *P. falciparum* malaria in children in the field.

9 SURVEILLANCE OF DRUG RESISTANT MALARIA IN BRAZIL. Zalis MG*, Cruz CM, Alcencar FE, Cerutti CJ, Pang LB, Wirth DF, and Milhous WK. Instituto de Biofisica Carlos Chagas Filo, Federal University of Rio de Janeiro, Brazil; US Army Medical Research Unit-Brazil, American Consulate, Rio de Janeiro, Brazil; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The prevalence and severity of drug resistant falciparum malaria continues to increase in the Amazon basin of Brazil. In support of clinical trials using the new antimalarial drug combination of Atovaquone and Proguanil, we are using rigorous clinical, therapeutic and parasitologic criteria to isolate and characterize drug resistant falciparum malaria using state of the art molecular techniques. Prior to the start of clinical trials, random surveys have been performed on parasites isolates from patients with uncomplicated malaria who were referred at our trial site. *In vitro* drug susceptibility testing was performed and the strains were found to be very resistant to chloroquine and quinine, but susceptible to arteminisin, Atovaquone and mefloquine and halofantrine (drugs very recently introduced into this region for treatment). One strain exhibited a modest amount of resistance (decreased susceptibility) to mefloquine similar to that observed with our control parasite strain from West Africa. In order to characterize the putative role of P-glycoprotein homologues (Pgh) in antimalarial drug resistance, the transmembrane domain (TM11) was amplified by polymerase chain reaction (PCR) and the product was sequenced. All the patient samples tested exhibited a pattern with Cys in position 1034 and Asp in position 1042, similar to that previously described in other Brazilian isolates. Southern and Northern blots will be performed with these parasites and other bona fide clinically resistant specimens to determine both gene copy number and expression of the *pfmdr1* and *pfmdr2* gene to correlate molecular changes associated with the emergence of clinical resistance.

10 PRIMAQUINE-RESISTANT STRAIN OF *PLASMODIUM VIVAX* MALARIA FROM BRAZIL. Nayar JK*, Collins W, Baker RH, and Knight JW. Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences, University of Florida, Vero Beach, FL; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

A non-immune American entomologist acquired an infection of *Plasmodium vivax*, Type-1, malaria during a mosquito collecting trip in Brazil in the summer of 1994, even though he was on Lariam (Mefloquine hydrochloride) prophylaxis. While in Brazil, he fed field-collected *Anopheles darlingi* on himself. After his return to the U.S., he had an attack of *P. vivax* malaria 24 days after he took his last dose of Lariam prophylaxis. During the next seven months he had three relapses of malaria. The first relapse occurred 30 days after the initial attack, the second relapse occurred 75 days after the first, and the third relapse occurred after another 115 days. The initial episode and all three relapses were microscopically confirmed by detection of malarial parasites in blood smears. He was treated with a standard chloroquine-primaquine regimen (an initial total dose of 1,500 mg of chloroquine-base given over a 48-hr period followed by 15 mg of primaquine a day for 14 days) after each of the first two relapses, but after the third relapse he was given 15 mg primaquine a day for 28 days. Three days after the beginning of his first relapse, colonized *Anopheles quadrimaculatus* mosquitoes were fed on him for subsequent three days during the treatment with chloroquine-primaquine regimen. Hundred percent of the mosquitoes blood-fed on him during these 3 days developed normal oocysts and sporozoites (Ten females were dissected from each days' blood-feeding). The sporozoites from the remaining infected mosquitoes were inoculated in three monkeys (Two *Saimiri boliviensis* and one *Aotus lemurinus griseimembra*), which were splenectomized 12-14 days after inoculation of sporozoites. All three monkeys became patent with asexual parasitemia with prepatent periods between 17-23 days.

11 ANTIMALARIAL DRUG METABOLISM IN A RAT DISEASED LIVER MODEL. Leo KU*, Grace JM, Peggins JO, Aguilar AJ, and Brewer TG. Division of Experimental Therapeutics, WRAIR, Washington, DC; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Drug metabolism is frequently effected by disease states. The effect of malaria infection on the metabolism of antimalarial agents has been minimally studied in the past. Studies which evaluated the disease effect on cytochrome P-450 metabolic pathways in rats have previously pooled multiple animals thereby eradicating any potential inter individual differences in metabolism. We have used a paired randomized block design, controlling for age, weight, and sex. Infant rats (100-120 gm) were inoculated via intraperitoneal route with the ANKA strain of P. berghei. Infection uniformly peaked at 9-11 days post infection. Animals were subsequently euthanized at peak parasitemia and livers were harvested for isolation and preparation of hepatic microsomes. Compared to control (CTR) rats, malaria infection (MI) resulted in 38% decrease in total protein (CTR, 10.97; MI, 6.83 mg/gm liver), 32% decrease in total P-450 content (CTR, 1.00; MI, 0.68 nmol of P-450/mg protein), and 30% decrease in total reductase activity (CTR, 0.020; MI, 0.014 nmol of reduced cytochrome c/minute/mg protein). Markers for isozyme specific hepatic metabolism are being evaluated. Preliminary data using arteether suggest a decrease in metabolic turnover in MI. However, using phenacetin, a specific CYP1A2 probe, indicated enhanced activity in malaria infection for this specific isozyme. Inhibition of phenacetin metabolism by arteether yielded an IC50 of 14 μM in MI compared to 27 μM in CTR. Drug-disease interactions may be manifest by specific isozyme effects. Delineating these effects may be crucial to predicting antimalarial drug metabolism.

12 DOES IRON THERAPY ENHANCE HEMATOLOGIC RECOVERY IN CHILDREN TREATED FOR MALARIA WITH SULFADOXINE-PYRIMETHAMINE? Kazembe PN*, Nwanyanwu OC, Ziba C, Gamadzi D, and Redd SC. Ministry of Health, Lilongwe, Malawi; and Centers for Disease Control and Prevention, Atlanta, GA.

In sub-Saharan African countries, malaria and malaria associated anemia are major public health problems. The usefulness of supplementary iron treatment(ferrous sulfate) for children with malaria associated anemia is unknown. During a six week period in the 1995 rainy season, 222 children under 5 years old seeking treatment for malaria who had parasite density ≥ 500 parasites/ L whose parents consented were enrolled in a study comparing the efficacy of sulfadoxine/pyrimethamine (SP), SP plus daily iron, and SP plus weekly iron as treatment for malaria associated anemia. Treatment regimen was assigned randomly. Study participants had hemoglobin concentration measured on days 0, 3, 7, 14, 21, and 28, and 215 (97%) completed the 28 day follow-up. These data suggest that children who have malaria and hemoglobin concentration below 8 g/dL benefit from daily iron therapy. Children with higher hemoglobin concentrations derive no benefit. Improvement in hemoglobin depended most on whether the child was anemic before treatment. Treatment with an effective antimalarial drug resulted in rapid resolution of anemia.

Increase in hemoglobin, concentration at enrollment ≤ 8 g/dl					
	n	Day 7	Day 14	Day 21	Day 28
SP	25	1.0	1.7	2.6	3.5
SP + Daily Fe	28	1.6	2.9	3.6	4.2
SP + Weekly Fe	19	1.7	2.4	2.7	3.7
Increase in hemoglobin, concentration at enrollment > 8 g/dl					
	n	Day 7	Day 14	Day 21	Day 28
SP	50	0.2	0.6	1.0	2.2
SP + Daily Fe	49	0.0	0.8	1.8	2.2
SP + Weekly Fe	44	0.0	0.6	0.8	2.2

13 THE EFFICACY OF SULFADOXINE-PYRIMETHAMINE (SP) IN THE PREVENTION OF PLACENTAL MALARIA IN WESTERN KENYA. Parise ME*, Schultz LJ, Ayisi JG, Nahlen BL, Oloo AJ, and Steketee RW. Malaria Section, Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

In an area of moderate malaria transmission in Malawi, 2 intermittent treatment doses of sulfadoxine-pyrimethamine (SP) during pregnancy significantly decreased the prevalence of placental malaria. To test the efficacy of SP in a hyperendemic area, we compared 3 regimens– a 2-dose SP regimen (SP/SP), a SP- monthly regimen, and a fever case management (CM) approach in western Kenya for their effect on placental parasitemia and low birth weight (LBW) in hospital-based deliveries. Primi- and secundigravidae attending antenatal clinic were assigned to 1 of the regimens based on day of enrollment; treatment groups were comparable on parity and prevalence of parasitemia at enrollment (42% overall, 51% in primigravidas and 25% in secundigravidas). Fever illness requiring treatment was observed in 10% of women in the CM group. Of 119 women who have delivered to date, the prevalence of placental parasitemia was highest in the CM group (25%) and significantly lower in the SP/SP (4%, p=0.02) and the SP- monthly groups (0%, p=0.005). LBW was most common in the CM group (32%) and lower in the SP/SP (16%, p=0.16) and the SP-monthly groups (0%, p=0.001). These SP regimens were effective in reducing placental malaria and LBW in this highly malarious area.

14 PROGRESSION OF PYRIMETHAMINE-RESISTANT *PLASMODIUM FALCIPARUM* IN MALIAN VILLAGES WHERE USE OF PYRIMETHAMINE-SULFADOXINE IS CONTROLLED VS. UNRESTRICTED. Plowe CV*, Djimde A, Wellems TE, and Doumbo O. Division of Geographic Medicine, University of Maryland School of Medicine, Baltimore, MD; Malaria Genetics Section, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and Malaria Research & Training Center, National School of Medicine & Pharmacy, Bamako, Mali.

The useful life of sulfadoxine-pyrimethamine (SP) as a first-line antimalarial replacement for chloroquine in subSaharan Africa is estimated to be as short as five years. Strategies to slow the development and spread of *Plasmodium falciparum* resistance to SP are urgently needed, as there is currently no suitable alternative drug that is affordable to countries in endemic areas. Previously we have reported an association between the prevalence of pyrimethamine-resistance conferring mutations in *P. falciparum* dihydrofolate reductase (DHFR) and unrestricted

use of SP in Mali, and suggested a model for slowing the spread of resistance based on a village with no resistance mutations where a community cooperative supports a physician who follows strict treatment protocols and a dispensary where appropriate antimalarial treatment is available at subsidized prices. Results will be presented of ongoing surveillance studies of the prevalence of the Asparagine-108 and other DHFR mutations in the study village compared with control villages where SP use is unrestricted. Mutation-specific PCR assays using parasite DNA extracted from finger-stick blood samples collected on filter paper strips will be used to determine the prevalence of the mutations, and the success of the strategy for deterring resistance will be assessed by comparing the rates of increase in the prevalence of resistance mutations at each site. Preliminary results will also be presented of PCR surveys for mutations in *P. falciparum* dihydropteroate synthase that have been implicated as possible determinants of sulfadoxine resistance.

15 LYMPHOCYTE SUBPOPULATIONS THAT FUNCTION IN THE PATHOGENESIS OF MURINE CEREBRAL MALARIA. Yanez D, Manning DD, van der Heyde HC, and Weidanz WP*. Department of Medical Microbiology & Immunology, University of Wisconsin, Madison, WI.

We examined whether selected lymphocyte subsets and cytokines were required in the pathogenesis of experimental murine cerebral malaria (CM) by using gene-targeted knockout and mAb suppressed mice. *Plasmodium berghei* ANKA (Pba) infection of A^{0-0} and Ii^{0-0} mice, which lack surface MHC class II expression and consequently have few CD4+ T cells in the periphery, induced CM. However, C57BL/6 and A^{0-0} mice severely depleted of CD4+ T cells by mAb treatment, in contrast to Ig-treated controls, did not exhibit CM; this finding confirms an earlier report that CD4+ T cells are required for CM to occur. Together, these data also indicate that the small numbers of residual CD4+ T cells in A^{0-0} mice are sufficient for CM development. The recently described CD3+,CD4+,NK1.1+(ab) T cells, known to be present in A^{0-0} mice, is not required for the development of CM because A^{0-0} and C57BL/6 mice depleted of NK1.1+ cells by mAb treatment still developed CM. Examination of the requirement in the pathogenesis of CM for several well characterized CD4+ T cell associated cytokines revealed that deficiency in TH1 cytokines, IFN-γ and IL-2,completely inhibited CM development, whereas the lack of Th2 cytokines, IL-4 and IL-10, did not. Our observation that B cell deficient mice, JHD and (m)MT, developed CM is consistent with our finding that TH2 cytokines are not required for CM. CD8+ T cell deficient mice, B^2M^{0-0} and anti-CD8 mAb-treated, did not develop disease, whereas control mice did.These data collectively demonstrate that both CD4+ and CD8+ T cells are crucial for the development of CM.

16 EFFECT OF NITRIC OXIDE ON NEURONAL NMDA CHANNELS HAS IMPLICATIONS FOR HUMAN CEREBRAL MALARIA. Rockett KA, Kwiatkowski DK, Bate CA, Premkumar LS, Gage PW, Awburn MM, and Clark IA*. Australian National University, Canberra, ACT, Australia; and Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

Malarial illness and pathology is now generally accepted to be caused TNF and other cytokines triggered by material released at schizogony. The partially purified released material, prepared by water lysis and solvent extraction of infected and control red cells, stimulated macrophages to make TNF, and also induced the mouse macrophage cell line, RAW 264.7, to produce inducible nitric oxide synthase and release nitric oxide. The material was active only in the presence of interferon-γ, which had only low activity itself. Activity was not caused by contaminant LPS. We have proposed that the altered mental states observed in human cerebral malaria may be caused by malaria-induced nitric oxide inhibiting N-methyl-D-aspartate (NMDA) channels in post-synaptic cells, in parallel with the action of general anaesthetics and ethanol. NMDA receptor-mediated current in hippocampal neurons, measured by a patch clamp technique, were altered when neurons were exposed to a buffer solution containing both NMDA and cells of mouse macrophage lineage, RAW 264.7, which had been stimulated to produce nitric oxide. Changes in the NMDA current by the stimulated cells was abolished in the presence of NG-methyl-L-arginine (L-NMMA) or haemoglobin, but was not affected when the medium contained NG-methyl-D-arginine (D-NMMA) or, L-NMMA plus excess arginine. These results suggest that nitric oxide produced by the stimulated cells is responsible for the change in NMDA currents we observed. These results are consistent with the idea that extra-neuronal nitric oxide induced by products of schizogony contributes to the seizures and unconsciousness seen in cerebral malaria.

17 DECREASED NITRATE EXCRETION IN TANZANIAN CHILDREAN WITH UNCOMPLICATED AND CEREBRAL MALARIA. Anstey NM*, Hassanali MY, Mwaikambo ED, Manyenga D, Mlalasi J, McDonald MI, and Granger DL. Duke-Muhimbili Clinical Research Lab, Muhimbili Medical Centre, Dar es Salaam, Tanzania; Department Paediatrics, Muhimbili Medical Centre, Dar es Salaam, Tanzania; and Division of Infectious Diseases & International Health, Duke University Medical Center, Durham, NC.

High levels of tumour necrosis factor (TNF) have been hypothesised to contribute to the pathogenesis of cerebral malaria (CM) via induction of nitric oxide (NO) synthesis. We thus compared urine nitrate excretion and plasma nitrate (markers of NO production) in four groups of children admitted to Muhimbili Medical Centre, April 94-Jan 95: diet controlled healthy controls (HC) [n=41], diet controlled asymptomatic parasitaemia (AP) [n=10], clinical malaria with no cerebral symptoms or signs (NCM) [n=53] and CM with Blantyre coma score ≤ 2 [n=87]. Nitrates were quantitated by bacterial nitrate reductase coupled with the Griess Reaction and spot urine nitrate:creatinine (N:C) ratios computed. Mean durations of fasting were: HC 13.0 hr, AP 12.9 hr, NCM 5.6 hr and CM 16.8hr. Urine N:C ratios were inversely correlated with disease severity, with levels lowest in CM: HC 0.47, AP 0.55, NCM 0.22, and CM 0. 14 (One way ANOVA: $p<0.0001$; CM and NCM differ from HC and AP, and CM differs from NCM, all at the 0.05 level). Preliminary plasma nitrate levels are consistent with the urine results. The excretion results do not appear to be explained by differences in dietary nitrate intake, chloroquine treatment or renal function among the study groups. NO synthesis appears suppressed in both NCM and CM. Further studies will correlate urine nitrates with plasma nitrates & WBC NO synthase (NOS) activity. Because nitrate excretion in malaria is so low (below that observed in the HC group) the suppression may involve the constitutive isoforms eNOS and nNOS as well as inducible NOS. If so, this could have deleterious effects on neurotransmission and cerebral perfusion in cerebral malaria.

18 CYTOKINE PRODUCTION BY ENDOTHELIAL CELL AFTER STIMULATION WITH *PLASMODIUM FALCIPARUM* BLOOD STAGE ANTIGENS. Xiao L*, Yang C, Saekhou AM, Udhayakumar V, and Lal AA. Division of Parasitic Disease, NCIC, CDC, PHS, U. S. Department of Health and Human Services, Atlanta, GA.

Previous studies on the involvement of endothelial cells (EC) in the pathogenesis of cerebral malaria have largely concentrated on the mechanisms of cytoadherence of parasitized erythrocytes (PRBC) to EC. In view of strong evidence indicating that cytokines may be involved in the pathogenesis of cerebral malaria, we have evaluated whether PRBC binding or parasite products such as soluble antigens have any direct effect on EC, especially interons of their cytokine production. Unstimulated SV40 transfected human dermal microvascular endothelial cells (HMEC-1) constitutively secreted IL-6, IL-8 and small amount of IL-1β, and no interleukin 1α (IL-1α). When HMEC-1 were stimulated with soluble malaria antigens or exposed to PRBC, the production of IL-6 and IL-1β significantly increased. The peak production of IL-6 occurred as early as 24 hours postactivation. The increase in IL-6 production paralleled the increased expression of mRNA for IL-6. IL-10, a regulatory cytokine down-regulated the malarial antigen stimulated cytokine production by HMEC-1. The increased production of IL-6 and IL-1β was also seen in human brain microvascular endothelial cells (HBEC-5I) and umbilical vein endothelialcells (HUVEC) after they were stimulated with soluble antigens. Based on these findings, we propose that in addition to cytoadherence of PRBC to EC, malarial antigens can also trigger activation of EC.

19 EXPRESSION-CLONING AND IMMUNOLOGIC ANALYSIS OF THE CD36-BINDING MALARIA PROTEIN, SEQUESTRIN: ADHERENCE CHARACTERISTICS AND MECHANISMS OF REVERSAL. Duffy PE*, and Ockenhouse CF. US Army Medical Research Unit- Kenya/Kenya Medical Research Institute, Kisumu, Kenya; Immunology, Walter Reed Army Institute of Research, Washington, DC.

Cytoadherence of *Plasmodium falciparum*-infected erythrocytes (irbc) to endothelial ligands mediates parasite survival as well as pathology, and is a target for therapeutic interventions. Radiolabelled CD36 identified a 2 kB gene fragment from a *P. falciparum* cDNA library constructed in lambda gt11;nucleotide sequencing suggests a deduced protein rich in degenerate repeat sequences, both tandem and non-tandem. Recognition of this protein by anti-idiotype antibodies against OKM8, a mAb to CD36 which blocks irbc binding, suggests the polypeptide is derived from sequestrin. Expression of overlapping sections of this protein with GST or trpE fusion partners specified a 127-residue polypeptide, called RO/1, containing the binding site; RO/1 also contains 9 Glu-Glu pairs within non-tandem degenerate repeats homologous to the mammalian annexin proteins. Immunization with RO/1 elicited parasitized erythrocyte to CD36-expressing cell lines or purified CD36. Antisera raised to RO/1 recognized a downstream fragment of the protein not involved in CD36 binding, called R3, which also contained Glu-Glu pairs within non-tandem annexin-like motifs. This"decoy" may be a parasite mechanism to divert humoral recognition from the biologically vital binding site within sequestrin toward a non-functional fragment. In fact, RO/1 is widely recognized in holoendemic western Kenya, but humoral recognition does not correspond to absence of infection or reduced level of parasitemia. Precise specification of the binding residues within RO/1 may be required to develop effective immunogens or passive peptide/ antibody therapy.

20 CYTOKINE SECRETION IN PLACENTAS OBTAINED FROM MALARIA-INFECTED WOMEN. Fried M*, and Duffy PE. US Army Medical Research Unit- Kenya/Kenya Medical Research Institute, Kisumu, Kenya.

During pregnancy, the placenta is a preferential site for sequestration of *Plasmodium falciparum*-infected erythrocytes, and can have a heavy parasite burden while the peripheral blood is free of parasites. Malaria infection during pregnancy can be particularly severe in primigravid women, and is associated with abortion, premature delivery, fetal growth retardation and fetal death. To understand the immunologic basis for clinical outcomes of malaria infection during pregnancy, we measured levels of TNF-α secreted in placentas obtained from primigravid, secundigravid, and multigravid women in western Kenya. Analysis was performed on 43 placental serum samples. High levels of TNF-α, detected in 30% of the women, were found in placentas with low parasitemia or with pigment suggesting past infection (determined in histologic sections of the tissue). In general, high placental parasitemia (more than 1% placental parasitemia) was observed exclusively in primigravid women. TNF-α was not detected or was at low concentration in placentas that were not infected with malaria or in cases of high parasitemia. No differences in placental secretion of TNF-α was apparent between primigravid,secundigravid, and multigravid women in cases where primigravid had low infection (less than 1% placental parasitemia). The results suggest that TNF-α correlates with the ability to control or completely clear malaria parasites from the placenta. The possible involvement of other cytokines in the control of placental malaria is under investigation.

21 CYTOKINE PROFILES IN RESPONSE TO MALARIA ACQUIRED ON THE PACIFIC COAST OF COLOMBIA. Duque S, Montenegro-James S*, Praba A, Hutchinson L, Herrera MA, Herrera S, and James MA. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA; Ochsner Medical Foundation, New Orleans, LA; and Instituto Nacional de Salud, Bogota, Colombia; Universidad del Valle, Cali, Colombia.

The analysis of a complete cytokine profile is needed to gain an accurate understanding of cell-mediated immune responses to malaria. Expression of 10 cytokine, ICAM-1 and iNOS mRNA transcripts was assessed in 12 presumptive malaria patients of Buenaventura, Colombia by RT-PCR. These individuals were all symptomatic; they were either infected with*P. falciparum* (n=4), *P. vivax* (n=5) or *P. malariae* (n=1), and/or had a past history of malaria. PBMCs were cultured for 24h after stimulation with PHA, Pf/Pv-infected RBC lysate antigens, peptide C3(Pf70 exoantigen), and a MAP construct from the PvCSP. PBMCs from parasitemic subjects showed an elevated expression of IL1β, IL12, IFNγ,TNF-α and iNOS (Th1-type response) to PHA; increased expression of the pyrogenic cytokines IL1β and IL6 was noted even in unstimulated cells. Expression was suppressed when cells were stimulated with Pf/Pv lysate antigens. Infected individuals responded to Pf70 peptide C3 with an elevated expression of IL1β, IL6, IL10, TNF-α, ICAM-1 (inducible by IL1b and TNF-α) and iNOS, typical of a pro-inflammatory response. Minimal expression of IL1β, IL6, TNF-α, ICAM-1 and iNOS was seen in Pv patients to the PvCS MAP. Nonparasitemic subjects with a history of malaria expressed IL1β, IL12 and TNF-α,β, with little or no expression of IL4,IL10, IFNγ, TGF- β, GM-CSF, ICAM-1 and iNOS. Quantitative RT-PCR isbeing carried out to determine relative differences in cytokine expression.

22 CYTOKINE EXPRESSION IN *AOTUS* MONKEYS AFTER IMMUNIZATION WITH SYNTHETIC AND RECOMBINANT *PLASMODIUM VIVAX* AND *P. FALCIPARUM* ANTIGENS. Montenegro-James S, Duque S, Herrera MA, Praba A, Hutchinson L, Herrera S, and James MA*. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA; Ochsner Medical Foundation, New Orleans, LA; and Instituto Nacional de Salud, Bogota, Colombia; Universidad del Valle, Cali, Colombia.

Cytokine responses were studied in *Aotus* monkeys previously immunized with candidate malaria antigens in pilot experiments carried out in Cali, Colombia. This is the first comprehensive study of its kind in this primate model as the expression of 10 cytokine, ICAM-1 and iNOSmRNA transcripts was assessed by RT-PCR. The first group of animals (n=5) was immunized with various MAP antigens [with $Al(OH)_3$] containing T/B epitopes of the Pv CS protein. Two other animals were immunized with Pf MSP-1 constructs plus Freund's adjuvant, one with the r190L protein linked to an 'universal' CS-T3 epitope (r190L-CST3) and the other with a mixture of 18 overlapping synthetic peptides, spanning the length of 190L. Eight and 33 months (MAPs and 190L monkeys,respectively) after immunization, PBMCs were isolated and cultured for 24h after stimulation with PHA and either MAPs, r190L or 190L peptide pools (3 pools of 6 peptides each). Monkeys immunized with PvCS MAPs (T,T+B) showed elevated IL1β, IL6, IL10, IL12, TNF-α,β, GM-CSF and iNOSin the absence of IL4, IFNγ, TGF-β and ICAM-1. Monkeys immunized with Pfr190L-CST3 or 190L peptides preferentially expressed IL1β, IL6, IL12,IFNγ, TNF-α,β and iNOS (little or no IL4, TGF-β, ICAM-1) indicating aTh1-type response typical of splenic cell-mediated activity. Cytokine expression may be affected by the immunogen (epitopes, adjuvants) and/or the MHC-restricted nature and kinetics of the host immune response.

23 IMMUNOREGULATORY ROLE OF *PLASMODIUM FALCIPARUM* SCHIZONT-INDUCED IL-12 PRODUCTION. Pichyangkul S*, Saengkrai P, Yongvanitchit K, and Heppner DG. Department of Immunology and Parasitology, U.S. Army Medical Component, AFRIMS, Bangkok, Thailand.

Blood stage malaria parasites have an unusual capacity to nonspecifically induce immune activation, resulting in the production of proinflammatory cytokines. We investigate *Plasmodium falciparum* schizont-induced production of IL-12 and its influence on IFNγ and TNFα production. IL-12 was produced when peripheral blood mononuclear cells (PBMC's) from malaria-naive individuals were exposed to schizont stage parasites. Two different peaks of IFN-γ production were observed when PBMC's were cultured with different ratios of parasitized red blood cells. High concentrations of parasites (schizont:PBMC = 1:1) induced early IFNγ production which peaked within 24 hours. In contrast, low concentrations of parasites (schizont:PBMC = 1:16) induced late IFNγ production which peaked at 4-5 days. Different cells and cytokines were involved under these two different experimental conditions. Antibody neutralization experiments indicated that IL-12 plays a major role in both early and late schizont-induced IFN-γ production, but IL-2 played a greater role for late schizont-induced IFN-γ production. Depletion of γδT cells, which were present in small numbers (2-10%) in PBMC's, abolished the schizont-induced early IFN-γ production, but had no effect on the late IFN-γ production. In addition to IL-12's role in schizont-induced IFNγ production, IL-12 also potentiated schizont-stimulated PBMC's production of TNFα. The present study describes IL-12 as an important immunoregulatory molecule for *P. falciparum*-induced IFNγ and TNFα production. Furthermore, it provides evidence of γδT cell involvement in the rapid release of IFNγ when PBMC's are exposed to high concentrations of schizonts.

24 ALTERED COURSE OF *PLASMODIUM YOELII* INFECTION IN CBA/J MICE WITH PRE-EXISTING *SCHISTOSOMA MANSONI* INFECTIONS. Bosshardt SC*, Louis-Wileman V, Freeman GL, Colley DG, and Lal AA. Vanderbilt University School of Medicine, Nashville, TN; and Immunology Branch, DPD/NCID/CDC, Atlanta, GA.

Patent *Schistosoma mansoni* infections in male CBA/J mice lead to a very strong Th2-type cytokine environment at 8 weeks postinfection that wanes as the infection progresses to chronicity. The progression of malaria in schistosome-infected mice was studied by inoculation of non-lethal (strain 17X) *Plasmodium yoelii* sporozoites into uninfected mice, or mice with existing *S. mansoni* infections of 3, 7, or 15 weeks duration. Parasitemia was first detectable in blood smears of mice inoculated with P. yoelii only (n=20) or mice with 3-week *S. mansoni* infections (n=18) at 5.8±1.0 days or 5.9±1.0, respectively. Mice with 7-week (n=13) or 15-week (n=18) schistosome infections had significantly delayed parasitemias ($p<0.001$) of 9.9±2.3 days or 8.7±1.6 days, respectively. Cytokine synthesis by spleen cells *in vitro* at 6 and 9 days after sporozoite inoculation showed a strong IFNγ response by +CD3-exposed cells from mice without pre-existing schistosome infections. Mice with schistosome infections of 7 weeks most effectively dampened the strong IFNγ response to sporozoite inoculation. These data indicate that pre-existing, patent *S. mansoni* infections in mice may alter the course of *Plasmodium* spp infections through hepatic inflammatory responses not specific for malaria parasites.

25 MUCOSAL IMMUNIZATION WITH RBC INFECTED WITH LETHAL *PLASMODIUM CHABAUDI ADAMI* PROTECTS AGAINST CHALLENGE WITH THE SAME STRAIN. Dimayuga FO*, Dimayuga ER, and Wei Y. Department of Biological Sciences, Ohio University, Athens, OH; and Edison Biotechnology Institute, Ohio University, Athens, OH.

Orally administered antigens have been shown to induce systemic tolerance but may also mediate protection against certain pathogens and autoimmune diseases. We wanted to determine whether this phenomenon holds true for murine malaria. We now report that intragastric immunization of BALB/c mice with intraerythrocytic parasites protected orally immunized animals but not naive mice against i.p. challenge with lethal *Plasmodium chabaudi adami*. To attempt to determine what lymphocyte subsets are expanded after intubation and participate in protective responses to challenge with lethal *P. chabaudi*, splenic and Peyer's patch T cells were analysed by flow cytometry before challenge, during ascending, peak, and descending parasitemia. The splenic and Peyers patch TCR + cells of the iRBC-fed group were comparable with the PBS-fed or normal RBC-fed (nRBC) groups during ascending parasitemia. At peak parasitemia, the iRBC-fed and nRBC-fed groups had 40% more splenic TCR + cells over the PBS-fed group but with no changes in numbers of this subset in the Peyer's patches. During ascending parasitemia, the iRBC-fed group had 2.5 times more splenic TCR + cells than the nRBC-fed group and 5 times more than the PBS-fed group. In contrast, the Peyer's patch TCR + cells of iRBC-fed animals were significantly decreased from levels seen in PBS- and nRBC-fed controls during ascending parasitemia. At peak parasitemia, the iRBC-fed group had 50% decrease of TCR + cells in the spleen compared to controls while in the Peyer's patches this subset were comparable in numbers among the iRBC- and PBS-fed groups.

26 REQUIREMENTS FOR THE EXPRESSION OF PRIMARY AND REINFECTION IMMUNITY TO MURINE MALARIA *PLASMODIUM YOELII*. Yadava A*, Denkers E, Ahlers J, Gorden J, Berzofsky JA, Miller LH, and Kumar S. Laboratory of Parasitic Diseases, National Institute of Allergy & Infectious Diseases & Metabolism Branch, National Cancer Institute, NIH Bethesda, MD.

In immunologically intact BALB/c mice *Plasmodium yoelii* 17XNL primarily infects reticulocytes and causes a self-resolving infection. Mice develop reinfection immunity following a single infection. Previously, using u suppressed mice, others have demonstrated that immunity to primary infection with *P. yoelii* is antibody mediated. We have found that nonlethal *P. yoelii* causes a lethal infection in B cell knock-out and in MHC class II knock-out mice, suggesting that both B cells and $CD4^+$ T cells are essential for immunity against primary infection. Also, *in vivo* depletion of $CD4^+$, but not $CD8^+$ T cells, during primary infection causes virulent infection and leads to death of mice. However, depletion of $CD4^+$ T cells has no effect on immunity in mice made immune by infection and self-cure. Following rechallenge, both immune non-depleted mice and immune CD4-depleted mice had similar antibody titer. How these immune CD4-depleted mice maintain their antibody titer is not known. One possibility is that the few remaining memory $CD4^+$ T cells are sufficient to boost antibody titer following parasite infection. These results may explain why HIV infected individuals in malaria endemic areas of Africa with very low $CD4^+$ T cell counts do not experience severe malaria. Alternatively, non-$CD4^+$effector cells in immune individuals may contribute to protective immunity against reinfection.

27 INDUCTION OF CTL RESPONSES AGAINST TWO DISTINCT EPITOPES ON THE *PLASMODIUM YOELII* CIRCUMSPOROZOITE PROTEIN BY IMMUNIZATION WITH PEPTIDES. Franke ED*, Sacci J, Corradin G, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Institute of Biochemistry, University of Lausanne, Epalinges sur Lausanne, Switzerland.

Adoptive transfer of CTL against a 9 amino acid peptide sequence (aa280-88; SYVPSAEQI) on the *Plasmodium yoelii* circumsporozoite protein (PyCSP) can completely prevent sporozoite (spz) induced malaria. Immunization of BALB/c mice with a peptide containing aa59-79 (YNRNIVNRLLGDALNGKPEEK) on the PyCSP induces T-cell proliferation and the activation of CD8+ T cells that eliminate hepatic stage parasites from culture. In the present study, BALB/c mice were immunized with Multiple Antigen Peptides (MAP) containing aa280-99 (SYVPSAEQILEFVKQISSL) or aa57-70 (KIYNRNIVNRLLGD). The MAP(280-99)p2p30 contains 4 branches of aa280-99 linked to the universal T helper epitope from tetanus toxin (p2p30) and a glycine-lysine core. The MAP(57-70) contains 4 branches of aa57-70 linked to a glycine-lysine core. Mice were immunized 3 times at 3 week intervals by subcutaneous injection at the base of the tail with either: (a) 40 μg of the MAP(57-70) + 16 μg of the MAP(280-99)p2p30 in Lipofectin, (b) 40 μg of MAP(57-70) in Lipofectin, or (c) 16 μg of MAP(280-99)p2p30 in Lipofectin. Splenocytes from mice immunized with either MAP alone lysed target P815 (H2d) cells sensitized with the autologous linear peptide; however, splenocytes from mice immunized with a combination of the 2 MAPs did not lyse targets sensitized with either peptide. The CTL response was CD8+ T-cell dependent and genetically restricted. T-cell proliferation and killing of hepatic stage parasites by splenocytes was induced by immunization with MAP(57-70). Partial protection (50-88%) against spz challenge was observed in mice immunized with either MAP alone, but not in the mice immunized with both MAPs.

28 CYTOTOXIC T CELL RESPONSES TO CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM FALCIPARUM* IN NATURALLY IMMUNE KENYA ADULTS REACTIVITY OF DIFFERENT POLYMORPHIC SEQUENCES. Udhayakumar V*, Ongecha JM, Shi YP, Orago AS, Duffy PE, Hawley WA, Nahlen B, Hoffman SL, Weiss W, and Lal AA. Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; CDC-KEMRI, VBCRC, Kissian, Kenya; Kenyatta University, Kenya; NMRI, Bethesda, MD; USAMRU, KEMRI, Kenya.

Genetic analysis of field isolates of *Plasmodium falciparum*has shown extensive polymorphism in the CD8+ CTL determinant of circumsporozoite (CS) protein. In this study, we determined the CTL responses to different CS variants using synthetic peptides corresponding to the genetic sequences of Kenyan field isolates. The naturally developed CTLs in Kenyan adults, were further expanded *in vitro* by stimulating with peptides (aa368-390) corresponding to 7G8 strain. While these CTLs were able to recognize autologous targets sensitized with six of the eight variant peptides analogous to Kenyan parasite isolates, they failed to recognize two of the variants (PL8 and PL11). These two sequences, PL8 and PL11, that failed to react with the 7G8 specific CTLs had two amino acid substitutions corresponding to position 372 (E to Q change) and 376 (E toA change in PL8 and E to I change in PL11). Interestingly, PL14, a variant with three amino acid substitution was consistently recognized by the 7G8 specific CTL. In a reverse experiment, we found a donor who repeatedly showed no reactivity to 7G8 sequence had CTL reactivity to PL8. The PL8 specific CTL showed partial reactivity to PL2, which has a single substitution at position 372 (E to Q),

and no reactivity to 7G8 sequence. Thus, we have found that individuals naturally exposed to malaria recognize different variants of the polymorphic CTL determinant.

29 PROBLEMS ASSOCIATED WITH IMMUNIZATION OF EUROPEAN BREED CATTLE WITH LIVE, ATTENUATED SMITHBURN ANTI-RIFT VALLEY FEVER VACCINE. Botros BA*, Moussa A, Taylor K, Soliman A, Salib A, Abdel Fattah M, and Arthur RR. US Naval Medical Research Unit No. 3, Cairo, Egypt; and General Organization for Veterinary Services, Ministry of Agriculture, Cairo, Egypt.

Following RVF recurrence in Egypt in 1993, native cattle and sheep were immunized with Smithburn live, attenuated RVF vaccine (SMVAC). In this report, 318 European breed cows and 115 native buffaloes were vaccinated with locally prepared SMVAC. One hundred cows and 20 B were pregnant. Between the 8th and 72nd day post-vaccination, 28 (28%) abortions occurred among pregnant cows; 19 of them within first 30 days. B did not abort (0/20). Sera collected at 80 days post-vaccination from all 28 aborting cows, 17 non-aborting cows, 5 non-aborting buffaloes and 32 unbred cows were tested by ELISA for anti-RVF virus IgG and IgM. All 28 aborting cows were strongly positive for IgG (geometric mean titer (GMT)=10,500). Five of 17 (29%) C that did not abort (GMT=459); 2 of the 5 (40%) buffaloes (GMT=1131) and 14 of 32 (44%) unbred C (GMT=4307) were positive. IgG GMT of aborting cows was higher than non-aborting cows ($p<0.001$), suggesting a severe reaction to the live virus SMVAC that resulted in abortions. IgG GMT of unbred cows was higher than pregnant non-aborting cows ($p<0.05$) indicating better immunologic response in unbred cows. Seven of 28 (25%) aborting cows vs 1 of 32 unbred cows were positive for IgM. Results demonstrate weak immunologic response to SMVAC; highest response (44%) was in unbred cows. Data indicate that vaccination of European cows with live, attenuated SMVAC may cause abortions, suggesting that a safer and more effective vaccine for cattle is needed.

30 SURVEILLANCE OF RIFT VALLEY FEVER IN EGYPT USING SENTINEL ANIMALS. Arthur R*, Taylor K, Soliman A, Caliamaio C, Ibrahim H, Berry J, Moussa A, and Botros B. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt; and General Organization for Veterinary Services, Ministry of Agriculture, Cairo, Egypt.

RVF recurred in Egypt in May 1993 after a 12-year period during which there was no evidence of virus transmission. Beginning in October 1993, sentinel groups totaling 1607 animals (1365 sheep, 154 goats and 88 cattle) were established at 26 locations in 5 governorates (Beheira (BH), Daqahliya (DQ), Kafr el-Sheikh (KS), North Sinai (NS) and Sharkiya (SH)), in the Nile River delta and the North Sinai. To identify individual study animals, metal tags with unique numbers were placed on the animals' ears. Base-line studies of anti-RVF IgG, as measured by ELISA, ensured that all animals were seronegative. Thereafter, animals were sampled at 2 month intervals and tested for seroconversion (SC). SC in sheep were first observed in SH (12/93-4/94). During the period of 6/94 - 12/94, SC occurred in DQ and KS. RVF virus was cultured from an abortus from a sentinel sheep in DQ in 8/94. No animals in the NS, bordering Israel and the Mediterranean basin, have seroconverted. Samples collections in BH and SH were terminated at the end of 1994 because animals were inappropriately vaccinated; collections are continuing in DQ, KS, and NS. No SC were observed in goats; results for bovine sera are pending. This prospective longitudinal study of RVF infections in animals provides useful data about the dissemination of RVF throughout Egypt during the current outbreak.

31 ENHANCED TRANSMISSION OF RIFT VALLEY FEVER VIRUS BY *ANOPHELES STEPHENSI* MOSQUITOES CO-INFECTED WITH MALARIA. Vaughan JA*, and Turell MJ. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Anophelines are susceptible to infection with Rift Valley fever virus. However, anophelines are inefficient laboratory vectors due to a salivary gland barrier. Dual infections of RVF virus and malaria may enable anophelines to transmit RVF virus because sporozoites disrupt the integrity of the salivary glands. To examine this, we used *Anopheles stephensi* and the rodent malaria, *Plasmodium berghei* as a model. We compared viral transmission rates for RVF virus-inoculated mosquitoes that had either previously fed on gametocytemic mice (=malaria-infected) or on uninfected mice (=control). Overall, 0/32 of the control-fed mosquitoes transmitted RVF virus by bite, whereas 4/25 of the mosquitoes containing salivary gland sporozoites transmitted virus. In addition, preliminary results indicate that enhanced RVF virus transmission also occurs in the co-indigenous vector system of *An. gambiae* infected with the human malaria, *P. falciparum*. Parasitic infections may enhance mosquito transmission of arboviruses.

32 A SPATIAL ANALYSIS OF THE DISTRIBUTION OF LACROSSE ENCEPHALITIS AND ITS ENVIRONMENTAL DETERMINANTS IN THE UPPER MIDWEST. Kitron U*, Kazmierczak JJ, Haramis LD, Aufmuth J, and Michael J. College of Veterinary Medicine, University of Illinois, Urbana, IL;

Communicable Disease Section, Wisconsin Division of Health, Madison, WI; and Vector Control Program, Illinois Department of Public Health, Springfield, IL.

Data on all human cases of LaCrosse encephalitis since 1976 in Illinois and since 1981 in Wisconsin were gathered, and georefenced to the maximum allowable degree of precision. The geographical and temporal patterns in distribution of cases were mapped using a GIS. Spatial measures of autocorrelation and local spatial statistics were used to quantify the degree of clustering among cases, and to identify "hot spots", and the buffer zone around them. Environmental data on demography, soil, vegetation and hydrology were derived from existing digital databases and from acquired satellite imagery. The distribution of cases was highly clustered around Peoria in Illinois, and in southwest Wisconsin. Local clusters occurred around several very small towns. Cases could be associated with changes in elevation, vegetation and land use patterns, as determined, in part, through remote sensing. This analytical approach to spatial data can be applied to surveillance and control programs of various arboviral diseases.

33 ARE NORTH AMERICAN BUNYAMWERA SEROGROUP VIRUSES THE ETIOLOGIC AGENTS OF CONGENITAL DEFECTS OF THE HUMAN CENTRAL NERVOUS SYSTEM? Calisher CH*, and Sever JL. Colorado State University, Fort Collins, CO; and NINCDS, NIH, Bethesda, MD.

1,000 human serum samples were selected from an archival collection of samples obtained between 1959 and 1964, at delivery or post partum, from women enrolled in a prospective study of congenital rubella syndrome. Samples from mothers of children with macrocephaly or microcephaly and from age- and site-matched controls (mothers of children without these defects) were tested for neutralizing antibody to bunyaviruses including Cache Valley(CV) and Tensaw (TEN) viruses. We determined a correlation between presence of antibody to CV or TEN virus in mothers and macrocephaly in their infants (Chi-square; $p=<0.05$). The presence of antibody to TEN virus (McNemar's Chi-square; $p=<0.05$) or antibody to CV or TEN virus (McNemar's Chi-square; $p=<0.02$) was related to the occurrence of macrocephaly in the infants of these mothers. Fourfold rises in titer were detected in paired samples, indicating recent infections with CV virus or with a closely related Bunyamwera serogroup virus. Six of the eight women in a subset with antibody to CV virus delivered macrocephalic infants, including the two with rises in antibody titer to CV virus. These and other analyses, to be presented, provide the first evidence that Bunyamwera serogroup viruses in North American are associated with congenital defects in humans.

34 EPIDEMIOLOGY OF OROPOUCHE FEVER IN THE AMAZAON REGION OF PERU. Hayes C*, Phillips I, Wi SJ, Callahan J, Griebenow W, Hyams K, and Watts D. Naval Medical Research Institute Detachment, Lima, Peru; and Naval Medical Research Institute, Bethesda MD.

Oropouche (ORO) virus is a member of the Simbu serogroup in the genus *Bunyavirus*. Acute fever caused by this virus has emerged as an increasing public health problem in Central and South America over the past 20 years. ORO virus was first implicated as a cause of acute fever in Peru in 1990 based on IgM serological results obtained during the first confirmed dengue epidemic in the city of Iquitos. Acute fever surveillance was established in Iquitos after this epidemic to determine if dengue was endemic, and during this surveillance, several isolates of ORO virus were obtained. To determine the extent of ORO virus transmission and associated risk factors in the population of this area, a cross sectional study was conducted in 1992 in an urban and a rural community and 3 riverine villages. An antibody negative cohort identified during the cross sectional study also was rebled after one year to obtain incidence rates. ORO antibody prevalence was associated with location, age, and occupation in the cross sectional study by multivariate analysis. The highest rate (46%) was in the smallest riverine village and the lowest rate (18%) was in the rural community. In the cohort study, only the rural site showed a substantial seroconverion rate (28%). This study suggests that ORO has been endemic in the study population for many years, but that transmission rates can vary considerably among sites from year to year.

35 MOSQUITO SALIVA POTENTIATES LACROSSE VIRUS VIRULENCE. Edwards JF*, Higgs S, and Beaty BJ. AIDL, Colorado State University, Ft. Collins, CO.

LaCrosse virus (LAC) virulence is age dependent; mice older than 30 days are resistant to subcutaneous (SC) infection, but younger mice become infected and die of encephalitis. However, 6 week old ICR mice died six to ten days after being fed upon by *Aedes triseriatus* mosquitoes transovarially infected with LAC. Disease was dose dependent; mortality occured sooner and viremias were higher titered and of longer duration as the number of feeding mosquitoes was increased. Mice inoculated SC (9/9) with LAC infected mosquito salivary gland homogenates died, as did mice (8/9) inoculated SC with LAC in areas of intense mosquito feeding. One mouse (1/18) inoculated SC with LAC alone or with salivary gland suspensions derived from noninfected mosquitoes died. This study demonstrates for the first time that mosquito saliva secretions can potentiate viral infections of

mammals. This suggests that evaluation of an arboviral pathogen should include studies in which the virus is delivered naturally by the arthropod vector.

36 QUALITATIVE ANALYSIS OF LAC S SEGMENT TRANSCRIPTION IN *AEDES TRISERIATUS* MIDGUTS BY TRANSCRIPT SPECIFIC RT-PCR. Wasieloski LP*, Blair CD, and Beaty BJ. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Arthropod Borne and Infectious Diseases Laboratory, Department of Microbiology, Colorado State University, Ft Collins CO.

Virogenesis in mosquitoes has traditionally been studied using virus isolation and/or antigen detection, but these techniques do not analyze viral transcription and replication in vectors. Alternatively, Nucleic acid hybridization has been utilized mainly for detection of virus in vectors. Furthermore, hybridization assays are relatively insensitive. One assay, polymerase chain reaction (PCR) is equisitely sensitive for the detection of analyte even in minute quantities. Here, reverse transcription coupled PCR was adapted for: the detection of viral analyte from mosquito samples, the amplification of specific S segment transcripts, and the qualitative analysis of LAC S segment transcription and replication in individual mosquito midguts. RNAs were assayed from midguts removed at days 1, 3, 6, 9, 14, 21, and 28 postinfection with a LAC blood meal. LAC genome was detected in more of the samples than either mRNA or viral complementary RNA, and it was detected in almost a uniform number of LAC-infected samples at each time point from days 3 to 28 (n=22). LAC S segment mRNA was the second most detected transcript. It was detected in relatively equal number of samples at days 3 to 21 (n=29). Viral complementary RNA was detected in the fewest number of mosquitoes, and the number of positive samples declined from days 14 to 28 (n=12). These data demonstrate that regulation of LAC transcription and replication does occur in mosquitoes. Persistent LAC infection of *Ae. triseriatus* was correlated with lower rates of detectable genome replication during the course of midgut infection.

37 AMPLIFICATION OF LA CROSSE VIRUSES FROM AUTOPSIED CNS TISSUES AND CHARACTERIZATION OF THE VIRUSES BY SSCP ANALYSIS. Beaty BJ*, Chandler LJ, Dobie D, Vanlandingham DL, Sweeney WP, and Black WC. Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

La Crosse (LAC) virus small (S) segment RNA sequences were detected in CNS tissues obtained at autopsy from two La Crosse encephalitis patients. The tissues had been stored frozen (-70°C) for up to 35 years. Primers complementary to sequences of the LAC S RNA segment were used for reverse transcription and PCR amplification of the virus. Single strand conformational polymorphism (SSCP) analysis was used to compare genetically the two virus sequences with prototype LAC virus, which was originally isolated from CNS tissues of one of the patients. The RT-PCR products from the viruses were denatured, rapidly chilled to promote intrastrand reassociation, separated electrophoretically on a nondenaturing gel at room temperature, and silver stained. The resultant SSCP patterns were very similar for the two sequences amplified directly from human tissues, but these differed significantly from the passaged prototype LAC virus. SSCP analysis has also been used to differentiate rapidly the S RNA segments of three California serogroup bunyaviruses. The S RNA segments of LAC, snowshoe hare (SSH), and Tahyna (TAH) viruses were reverse transcribed and PCR amplified. The cDNAs were then subjected to SSCP analysis; and the resultant patterns were specific for the respective viruses. This molecular technique offers great potential for virus typing and taxonomic studies.

38 DETECTING BUNYAVIRUSES OF THE CALIFORNIA-BUNYAMWERA COMPLEX BY PCR. Kuno G*, Mitchell CJ, Chang GJ, and Smith GC. Division Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO.

Many Bunyaviruses of the California-Bunyamwera Complex are well recognized human pathogens. Development of a reliable PCR technique for those viruses is critical for improving clinical diagnosis and virologic surveillance in the field. We evaluated the characteristics of many primers using 37 members of the Genus Bunyavirus representing 13 antigenic groups of the world. A pair of the Complex-specific primers could detect 3 antigenic groups (California, Bunyamwera, and Bwamba). Among 2 pairs of primers for LAC, one was virus-specific while the other was LAC and TEN reactive; and for SSH, one was virus-specific while the other was SSH and South River reactive. Two pairs were CE-specific. One pair was reactive to JC and INK. Efficacy of the PCR protocol for LAC was evaluated in a blind test using 24 pools (20 positive and 4 negative) of mosquitoes and a diluent (negative control). Specificity was 100% with all primers. Discrepant negative results were obtained in 2,2, and 3 pools containing one mosquito harvested within 6.5 hr after inoculation, with the Complex-specific, LAC/TEN-reactive, and LAC-specific primers, respectively. Overall, LAC virus could be detected with all primer pairs as early as 3.5 hr after inoculation in a pool of 100 mosquitoes containing one positive individual, and the lowest infectious titer detectable in the pools was 0.9 log PFU/ml.

39 RISK FACTORS FOR ACUTE MORBIDITY IN BANCROFTIAN FILARIASIS. Kazura J*, Bockarie M, Alexander N, Dagoro H, Bockarie F, Hyun P, Perry R, and Alpers M. Case Western Reserve University, Cleveland, OH; and Papua New Guinea Institute of Medical Research, Maprik, Madang, and Goroka.

Adenolymphangitis (ADL) of the extremities and acute epididymitis/funiculitis (EPD) are among the most debilitating disease manifestations of *Wuchereria bancrofti* infection. The epidemiologic and demographic risk factors for these acute and transient inflammatory responses are not known. As part of a prospective study of the impact of mass chemotherapy on transmission and disease due to *W. bancrofti* infection, we established a weekly morbidity survey system which allowed us to monitor the relationship between ADL/EPD and age, sex, chronic pathology, and infection status. ADL/EPD was defined as a self-limited episode of fever and swelling of the specific anatomic area lasting >1 and < weeks. A population of 2256 persons in East Sepik Province, Papua New Guinea was monitored for a 14 month period prior to administration of chemotherapy. ADL of the lower extremities occurred >1 time in 10% of the population between the ages of 10-19 years, increased to ~22% in 20-29 year olds, with no change in older age categories. There were no differences according to sex. EPD was first noted in 20-29 year old males (10% of the population) and remained at this level in older age categories. ADL of the lower extremities and EPD were more frequent among persons with pre-existing Grade 11-111 lymphedema ($p<0.001$ vs disease-free group) or hydroceles ($p<0.001$), respectively. Mf+ status predisposed to EPD (relative risk 3.7 vs mf- status) but mf status did not correlate with ADL. Persons with the most frequent episodes of ADL or EPD (> 3 episodes) were residents of areas with the highest transmission intensities. The implications of these findings for strategies to control lymphatic filariasis will be discussed.

40 FIVE HUMAN FILARIASIS IN EQUATORIAL AFRICA: FIRST DESCRIPTIONS OF MULTIFILARIAL ENDEMIC AREAS IN GABON. Richard-Lenoble D*, Kombila M, Chandenier J, Eyang Obame E, Thérizol Ferly M, and Duong TH. Department of Parasitology and Tropical Medicine University School of Medicine Libreville Gabon and Tours France.

Gabon as a muggy equatorial African country, covered by 80% of high forest more or less degraded and divided by numerous large and calm waterways interrupted by waterfalls or fast flowing rivulets. Vegetation, climate, and hydrography favors large vector development for human filariasis (*Simulium* sp., *Chrysops* sp., and culicoides). The University Department of Parasitology and Tropical Diseases has systematically studies human filariasis through field survey all around Gabonese districts from 1978 to 1990. Skin biopsy and peripheral blood specimens were collected on more than 8000 identified and clinically analysed patients. Except *Wuchereria bancrofti* and *Dracunculiasis medinensis* two main filariases are endemic (*Loa loa* and *Onchocerca volvulus*) associated with 3 *Mansonella*: *M. perstans* and *M. streptocerca* the most frequent and *M. rodhaini* recently discovered in man (44 cases). The results of field surveys Fougamou, Makokou, Lastourveille, Lebamba, Doussala, Tchibanga, Wolen N'tem, Ogooue Boue ...) allow precise prevalence and epidemiological mapping for each filariasis and evaluation on the multifilarial condition and best approach on mass treatment. *M. perstans* is the most frequent with prevalence of microfilaria carriers often more than 90%. Loaisis is important all over the forest area with never more than 35% microfilaria carriers but with many amicrofilaremic and clinically loiasis patients. Onchocerciasis unknown in 1980 is in fact present in 4 large areas around Makokou, Fougamou, Lebamba, and Lastourveille, and with high clinical implication in many villages (in Moukoro village 146 patients examonates, 83% with *O. volvulus* microfilaria and 6% epileptic seizures. Mectizan Program is in course. For *M. streptocerca* prevalence is high in many areas (34% near Fougamou, 19% for all the country) and *M. rodhaini* discovered in man for the first time in Gabon concerned less than 50 patients at this time. Concerning the Mectizan Program for onchocerciasis, multifilarial evaluation is important to evaluate secondary effects risk; for instance around the Fougamou area in South West Gabon 1070 patients were examined: 7% associated with 4 different filariases, 25% with 3, 30% with 2, and 13% with none. One elderly woman was a carrier of five different microfilaria.

41 EFFECT OF REPEATED IVERMECTIN TREATMENTS ON OCULAR ONCHOCERCIASIS: EVALUATION AFTER SIX TO EIGHT DOSINGS. Chippaux JP*, Boussinesq M, Lafleur C, Fobi G, Auduge A, Banos MT, Ngosso A, Ernould JC, and Prod'hon J. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon; Hopital Central, Yaounde, Cameroon; Hopital General, Douala Cameroon; Ministère franais de la Cooperation, Paris, France; and Ophtalmo sans frontieres, France.

Large scale ivermectin treatments were carried out annually between 1987 and 1995 in North Cameroon in an area hyperendemic for onchocerciasis. Detailed ophthalmologic examinations were performed prior to each treatment round between 1987 and 1992, and then in 1995. The effect of ivermectin on ocular lesions was evaluated by two ways. First, longitudinal data were analysed for 1309 male patients recruited between 1987 and 1989 (3 cohorts) and aged 15-45 at the time of inclusion. Second, analysis was carried out on cross sections of patients: pre-treatment data

obtained in 1987-1989 on males 15-20 were compared with the results obtained in 1995 on patients belonging to the same age group, examined for the first time but supposed to have received regular ivermectin treatments since the outset of the study. The lesions were defined by their clinical appearance and topography, using slit lamp and ophtalmoscope. The indicators used were prevalence of ocular onchocerciasis lesions and scores resulting from the combination of indicators useful for statistical comparisons. In cohorts, prevalence of punctuate keratitis (PK) decreased by 60% and prevalence of microfilariae in anterior chamber (MFAC) by 90%. The prevalence of retinal lesions increased. In young men under 20, incidence of PK and MFAC decreased drastically, e.g. down to 0% for MFAC. However, the prevalence of retinal lesions did not show significant difference between 1988 and 1995. The prevalence of optic atrophy declined but did not disappeared. Improvement of lesions of anterior segment of the eye was strongly confirmed but impact of ivermectin on posterior segment remained disappointing.

42 ONCHOCERCIASIS IN ECUADOR: EVOLUTION OF CHORIRETINOPATH AFTER AMOCARZINE TREAMTENT. Cooper PJ*, Proano R, Beltran C, Anselmi M, and Guderian RH. Department of Pathology, Cambridge University, Cambridge, United Kingdom; Onchocerciasis Control Progamme, Hospital Vozandes, Quito, Ecuador.

Changes in chorioretinopathy were examined in 4 separate cohorts treated with the macrofilaricide amocarzine and followed subsequently for 2 to 5 years. A total of 294 individuals living in an area of Ecudaor hyperendemic for onchocerciasis were followed-up completely. Amocarzine therapy did not prevent the natural evolution of chorioretinal disease. The prevalence of chorioretinopathy increased in all cohorts (overall prevalence increased from 14.1% to 33.6%). The cumulative incidence (CI) of cases with new or extending chorioretinal lesions was greater with increasing periods of follow-up (range of CIs of new lesions 13-55%; range of CI of extending lesions 16-90%). The risk of progression of existing chorioretinal lesions was greater than the risk of developing new lesions in all cohorts. An association was seen between the cumulative microfilarial loads in the skin and the development of new chorioretinal lesions (multiple log regression $p<0.05$). No relationship was noted between cumulative microfilarial loads and the progression of existing disease. The data suggest that ocular microfilariae are necesary for the induction of chorioretinopathy in previously unaffected eyes; the extension of existing disease may also be related to the presence of ocular microfilariae although other mechanisms (particularly immunological) must also be considered.

43 EFFECT OF REPEATED IVERMECTIN TREATMENTS ON THE INCIDENCE OF ONCHOCERCIASIS: A 7-8 YEARS FOLLOW-UP IN NORTHERN CAMEROON. Boussinesq M, Prod'hon J, and Chippaux JP*. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon.

Large scale annual ivermectin treatments have been carried out since 1987 in northern Cameroon where onchocerciasis is hyperendemic. The impact of these distributions on the transmission of onchocerciasis was demonstrated on a first occasion in 1992 by comparing the prevalence and intensity of infection recorded in 1987 (before the first distribution) and 1992 in children who never received the drug but were members of the treated communities. In 1995, skin snips were again performed on untreated 5-7 year old children from Ngoumi and Babidan, two villages which were first treated in 1987 and 1988, respectively. In Ngoumi, the prevalence of microfilariae (PMF) in the 47 children examined was 4.3% (in comparison with 52.3% in 1987, and 23.3% in 1992 in children of the same age group), and the mean microfilarial density (MFD) was 0.1 microfilariae per skin snip (mf/ss) (versus 3.1 mf/ss in 1987 and 0.7 mf/ss in 1992). In Babidan, 25 children were examined in 1995; the PMF and the MFD were 32.0% and 2.45 mf/ss, respectively whereas the corresponding pretreatment values in 1988 were 72.5% and 12.1 mf/ss. The differences observed between the two villages are probably due to the facts that (1) the initial microfilarial loads were higher in Babidan than in Ngoumi, (2) Babidan is located few km from Chad, where ivermectin treatment started recently, whereas Ngoumi is situated at the middle of the treated area, and (3) distribution in Babidan started one year after the first treatment in Ngoumi. Repeated large scale ivermectin treatments may bring about a dramatic reduction in the transmission of onchocerciasis. However, satisfactory drug coverage is essential to obtain such results.

44 IVERMECTIN BASED COMMUNITY CONTROL PROGRAM FOR LYMPHATIC FILARIASIS. Streit TG*, LeConte FA, Addiss DG, Lammie PJ, and Eberhard ML. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; and Hopital Ste. Croix, Leogane, Haiti.

High dose administration of ivermectin shows promise for use in community based control of lymphatic filariasis based on the suppression of *Wuchereria bancrofti* microfilaremias for periods up to two years. A pilot treatment program was initiated in a Haitian community endemic for filariasis. Studies to follow clinical, entomologic, and parasitologic dynamics related to this infection were initiated four months prior to delivery of drug. Nocturnal 20 cmm thick blood smears from community residents indicated that 25% were microfilaremic. Banked sera collected

at one year and at three months prior to treatment were assayed for filarial antigen to confirm the infection rate and determine occult infection rates. In pretreatment surveys, vectors were collected in gravid traps spaced throughout the center of the community. Of 3,353 *Culex quinquefasciatus* females collected, 64 (1.7%) contained *W. bancrofti* larvae. Pretreatment education regarding the disease and treatment was conducted in collaboration with local health workers and was well received, contributing to greater than 90% coverage of 1,400 eligible residents at the time of drug delivery. Pregnant women and children <5 years of age were excluded. Over a 10 day period, 400 mcg/kg ivermectin was administered orally to each resident in a single dose; side effects were recorded and palliative care offered. Posttreatment infection figures for mosquitoes and a follow-up KAP survey in the community will be presented.

45 IMMUNODIAGNOSIS FOR SURVEILLANCE OF ONCHOCERCIASIS : EVALUATION OF AN ENZYME-IMMUNOASSAY USING A "COCKTAIL" OF RECOMBINANT ANTIGENS ("TRI-COCKTAIL"). Weiss N*, Toe L, Bradley J, Oettli A, Back C, and Boatin B. Swiss Tropical Institute, Basel, Switzerland; Onchocerciasis Control Programme, Ouagadougou, Burkina Faso; and Imperial College, London, U.K.

The WHO Expert Committe on onchocerciasis recommended that immunodiagnostic tests should be developed capable of detecting early infections, especially new infections in area under control. Within the TDR Programmeof WHO, various recombinant *Onchocerca* antigens were evaluated by multi-center trials. With three highly specific antigens, Ov-7 (cloned by S. Lustigman, New York), Ov-11 (J. Bradley, London) and Ov-16 (E. Lobos, Basel) were collected in the OCP area from children aged 5 to 15 years, born during vector control, and tested for IgG antibodies in a centrallaboratory in Bouaké, Ivory Coast. By evaluating 103 sera of helminthiasis patients and 573 sera and blood spots from Burkina (Jan. 94), the cut-off values for a positive result was calculated. The seroprevalence rates for 6 villages within the OCP area ranged from 2.4%-11.5%. In a second survey (N=529, Dec. 94) the range was 0 to 11.1 % The village with the highest seroprevalence (11.5% and 11.1%) had an ATP of 48 in 1992. Two mf-positive children were detected in early 1994. The seroprevalence rate in a non-controlled area of Ghana was 21.7% (N=244), in Guinea (1 year after a first dose of Ivermectin) 16.2% (N=253). Only 3 out of 200 individuals from a nonendemic area (Niger) were classified sero-positive. A seroprevalence rate > 10% seems to be strong indicator for transmission.

46 EARLY IMMUNODIAGNOSIS OF *ONCHOCERCA VOLVULUS* INFECTIONS IN TRANSMIGRANTS TO AN ENDEMIC AREA IN CAMEROON. Schneider D*, Chippaux JP, Boussinesq M, Vogt I, and Weiss N. ORSTOM, Centre Pasteur, Yaoundé, Cameroon; and Swiss Tropical Institute, Basel, Switzerland.

In 1991, migrants from North Cameroon settled in two new villages, Mafaré (vM, pop.>5y: 301) and Fragong (vF, pop. 146) in the Vina River Valley, an area of high endemicity for onchocerciasis. Baseline investigations (census, clinical examinations, two skin snips, blood sample) were done in 1992 (vM) and 1993 (vF). Cohorts of microfilariae (mf)-negative individuals were followed annually until 1995. Individuals who became mf-positive were treated with Ivermectin and excluded from further follow-up. Mf rates were 5.9% (vM) and 17.3% (vF), 1 and 2 years after settlement. The seropositivity rates, using a "tricocktail" ELISA for IgG antibodies against Ov-7,-II,-16, were 14.3% (vM) and 7.3% (vF). The annual incidence rates of new (mfpos) cases were 15%, 15%, and 14% (vM in 93-95) and 29% and 23% (vF in 94-95) leading to a cumulative total of 75 (vM) and 48 (vF) cases. The baseline serology in vM detected 23/75 (31%) cases and 9/129 "non-cases" (RR=4.40, Mantel-Haenszel $P< 10^{-5}$)resulting in predictive values of 0.72 and 0.70 for positive or negative test results respectively. In contrast, only 13/75 (17%) cases were detected by skin biopsies in 1992. For unknown reasons the test performance was less satisfactory in vF; only 7/48 (15%) cases and 0/40 "non-cases" were detected in comparison to 19/48 (40%) by skin biopsies. By testing for IgE antibodies a somewhat higher sensitivity (23%) could be achieved.

47 SERODIAGNOSIS OF EARLY ONCHOCERCIASIS IN CHILDREN: IMPLICATIONS FOR MONITORING TRANSMISSION. Ogunrinade AF*, Chandrashekar R, and Weil GJ. University of Ibadan, Nigeria; Washington University School of Medicine, St. Louis, MO.

Improved methods are needed for monitoring onchocerciasis transmission. Skin snips and sera were collected from 208 children (mean age 9.7 S.D. 1.4 years) in a mesoendemic savannah area in Nigeria at two time points 9 months apart to assess the value of a recombinant antigen (OC3.6/9.3) based antibody assay for detecting early infections. 20 of 22 children with positive skin snips had positive antibody tests. 4 of 5 children who converted skin snips over the 9 month interval had antibodies to OC3.6/9.3 at time 0. Skin snip conversion rates were 17% (4/23) for children with positive antibody tests and negative skin snips at time 0 vs. 1% (2/185) for those with negative antibody tests ($P<0.001$). 19% of children with negative antibody tests at time 0 were seropositive 9 mo later, while only 3% of all children became skin snip positive in this interval. These preliminary results show that antibody serology is sensitive for detection of early *Onchocerca volvulus* infections in children. In addition, they suggest a strategy of following annual antibody seroconversion rates in standardized samples of children (sentinel populations) as an

efficient means of monitoring changes in onchocerciasis transmission over time. Additional (later) follow-up data from this study will be presented at the meeting.

48 TRANSMISSION OF *ONCHOCERCA VOLVULUS* STRAINS BY *SIMULIUM DAMNOSUM S.L.* SIBLING SPECIES IN WEST AFRICA. Toe L, Tang J, Back C, and Unnasch TR*. IOnchocerciasis Control Programme, Bouake, Cote d'Ivoire; and Division of Geographic Medicine, University of Alabama at Birmingham, Brimingham, AL.

Experimental transmission studies have sugested that different members of the *Simulium damnosum s.l.* species complex permit development of the forest and savanna strains of *Onchocerca volvulus* with different efficiencies, leading to the hypothesis that distinct parasite-vector transmission complexes exist. The recent development of PCR based methods to identify the strain of *O. volvulus* and the different sibling species of *S. damnosum s.l.* has offered the opportunity to evaluate the importance of these putative transmission complexes under natural conditions. Infected flies were collected from a number of river basins throughout the Onchocercaisis Control Programme where previous data had demonstrated that the different sibling species of *S. damnosum s.l.* and the two strains of *O. volvulus* were sympatric. The flies were classified by morphological criteria and by directed heteroduplex analysis, and the infective larvae carried by these flies were classified by O-150 PCR. Approximately equal numbers of forest strain larvae were found in both the forest and savanna dwelling *S. damnosum s.l.* sibling species. Similarily, transmission of the savanna strain of *O. volvulus* was not restricted to the savanna dwelling black fly species. These results suggest that distinct vector-parasite transmission complexes do not exist in the OCP control area, a finding that has significant implications for the strategic planning of control efforts in the post-OCP era.

49 MONITORING BY PCR OF *WUCHERERIA BANCROFTI* INFECTIONS IN WILD POPULATIONS OF *AEDES POLYNESIENSIS* IN FRENCH POLYNESIA. Nicolas L*, Luquiaud P, Lardeux F, and Mercer DR. Institut Territorial de Recherches Medicales Louis Malarde, Papeete, Tahiti, French Polynesia.

The detection of the lymphatic filariasis parasite *Wuchereria bancrofti* in mosquitoes requires the individual dissection and microscopic observation of hundreds of mosquitoes. We are trying to evaluate entomological indices on pools of vectors using molecular probes. A PCR assay amplifying a repeat sequence from W. bancrofti, the "SspI DNA repeat", was previously established. The assay now allows the specific detection of even a single infective L3 larva in a pool of 100 *Aedes polynesiensis* mosquitoes. Following mass chemotherapy of the human population, a field trial was carried out in French Polynesia to compare dissection and PCR methods formonitoring the infection of mosquito population. At 4 time periods, ca. 2,000 *A. polynesiensis* were captured and dissected. Infection rates ranged from 0.4 to 1.35%. In parallel, 50 pools of 5 mosquitoes were processed for PCR assay. As the assay is not yet quantitative, a pool of mosquito which is PCR positive indicates that it contains at least one infected mosquito. With the dissection data, we have simulated a random sampling of 50 pools of 5 dissected mosquitoes to determine the mean number of "dissection positive pools". The number of positive pools were always higher by PCR than by dissection. This indicates that dissection may overlook some mosquitoes infected by small stages of W. bancrofti, difficult to separate from the mosquito tissues by dissection.

50 THE CYTOLYTIC ARMAMENT IN GRANULES OF *ENTAMOEBA HISTOLYTICA*. Jacobs T, Berninghausen O, Andrä J, and Leippe M*. Department of Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, F.R. Germany

One of the major pathogenic functions and probably the most prominent feature of *E. histolytica* is the extraordinary cytolytic capacity of the parasite. We are interested in the molecular mechanisms of the amoebic killing reaction, particularly in the potency of amoebapores and in the structure/function relationships of this unique family of pore forming peptides. The biological activities of three isoforms of amoebapore purified from cytoplasmic granules were compared using various targets; it was established that all of them cause ion channel formation in membranes, exhibit antibacterial activity, and are cytolytic toward eukaryotic cells. Furthermore, in a search for additional aggressive components contained inside the granular vesicles of amoebae two phospholipases, a major antibacterial protein with lysozyme-like properties, and a novel cysteine proteinase that tends to associate with membranes were detected among a variety of proteins. The aforementioned factors have been purified, biochemically characterized, and the primary structures of most of them elucidated on the protein and DNA level. They constitute an armament that may prevent growth of phagocytozed bacteria inside the amebic digestive vacuoles but may also enable pathogenic amebae to kill nucleated cells extracellularly with remarkable efficiency, in particular when some of these factors are acting in synergy.

51 REPETITIVE ELEMENTS FROM THE *ENTAMOEBA HISTOLYTICA* RIBOSOMAL DNA EPISOME CONFER STABILITY TO TRANSFECTED DNA. Dhar SK*, Vines RR, Mann BJ, Bhattacharya S, Bhattacharya A, and Petri, Jr. WA. University of Virginia, Charlottesville, VA, USA; and School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India.

Ribosomal RNA genes of *Entamoeba histolytica* strain HMd IMSS are located on a circular extrachromosomal 24.5 kB DNA molecule present in approximately 200 copies/trophozoite. The lack of detectable chromosomal rDNA genes in *E. histolytica* is consistent with autonomous replication and segregation of the rDNA episome during cell division; therefore the rDNA episome should contain all the sequences required in cis for replication and segregation. Replication and segregation of the rDNA episome are likely to be novel and important processes to study in *Entamoeba*, as trophozoite cell division occurs without dissolution of the nuclear membrane or formation of condensed metaphase chromosomes. We and others have demonstrated that transfected plasmids containing short (< 3 kB) amebic flanking sequences from actin and lectin genes autonomously replicate, indicating that they contain sequences sufficient to direct initiation of replication. However in the absence of selection these plasmids are unstable, suggesting that they lack sequences required for segregation of replicated plasmid between daughter trophozoites. We have cloned restriction fragments encompassing the rDNA episome into the circular transfection vector BΔ1R8.D3. Ligation of the HMd restriction fragment, a region of the rDNA episome containing BΔ1R8.D3 repeats, but not the HMe or C6HdIII fragments, stabilized luciferase expression from the transfection vector in the absence of selection. Resolution of the mechanism by which the HMd fragment stabilizes the vector DNA awaits localization of the replication origin on the transfected plasmid.

52 PHYSICAL MAPPING AND THE STUDY OF EXPRESSION OF GENE FAMILIES ENCODING N-ACETYLGALACTOSAMINE ADHERENCE LECTIN OF *ENTAMOEBA HISTOLYTICA*. Mann BJ*, Ramakrishnan G, Ragland BD, Purdy JA, and Petri, Jr. WA. University of Virginia, Charlottesville, VA.

Adherence of *Entamoeba histolytica* to host cells is mediated by the N-acetylgalactosamine (GalNAC) specific lectin, a heterodimeric protein consisting of a 170 kDa heavy subunit and 31/35 kDa light subunits. Three genes (*hgl*) encoding the heavy subunit and two genes (*lgl*) for the light subunit have been reported previously. We have used a clamped homogeneous electric field gel system to characterize the genes encoding the lectin subunits. We have shown that the heavy subunit is encoded by a family of five *hgl* genes and have physically mapped them to restriction fragments. The *lgl* genes are present at six to seven loci in the genome. We have obtained partial sequences of two new *hgl* genes, and also of a new *lgl* gene. We have used reverse transcriptase and polymerase chain reaction techniques to analyze mRNA from trophozoites grown in culture and in gerbil liver abscesses. cDNA sequences from three out of the five *hgl* genes and three different *lgl* genes were detected in both growth conditions with no obvious differential expression. Only a small number of sequences corresponding to *hgl5* were detected and no sequences for *hgl3* were found. Determining the complexity and functions of the individual members of the GalNAc lectin gene families will be important for understanding the role of this adherence protein in pathogenesis.

53 INDUCTION OF IL-8 RELEASE FROM HUMAN CELLS BY *ENTAMOEBA HISTOLYTICA* TROPHOZOITES IS MEDIATED BY PREFORMED IL-1A AND THE GALACTOSE-INHIBITABLE LECTIN. Eckmann L, Kagnoff MF, and Reed SL*. Departments of Medicine and Pathology, University of California, San Diego School of Medicine, San Diego, CA.

In animal models, neutrophils are detected in early amebic lesions, however the signals controlling the acute inflammatory response are poorly understood. We have used human epithelial and stromal cell lines co-cultured with amebic trophozoites as an *in vitro* model system to identify the profinflammatory signals in response to infection by *Entamoeba histolytica*. The release of a number of chemoattractant and proinflammatory cytokines, including IL-8, GROa, GM-CSF, IL-1a, and IL-6, was stimulated by co-culture with amebic trophozoites. IL-8 specific mRNA was increased 10- to 1000-fold in infected cells, resulting in release of bioactive IL-8. IL-8 secretion could be reproduced by the addition of lysates of human cells and was linked to release of preformed IL-1a. IL-8 release required viable trophozoites and could be blocked in HT29 cells by galactose. IL-8 secretion by HT29 cells could also be increased with the calcium elevating agents, thapsigargin and ionomycin, suggesting that the galactose inhibitable lectin may mediate increased IL-8 secretion via an increase in intracellular calcium levels. These studies define novel mechanisms by which cell lysis following infection with *E. histolytica* trophozoites may upregulate the secretion of proinflammatory cytokines by viable neighboring cells.

54 MOLECULAR TRANSFER FROM *ENTAMOEBA HISTOLYTICA* TO HUMAN ENTEROCYTES IN COCULTURE. Leroy A*, De Bruyne G, Bailey G, Mareel M, and Nelis H. Laboratory of Pharmaceutical

Microbiology, University of Ghent, Ghent, Belgium; Laboratory of Experimental Cancerology, University Hospital, Ghent, Belgium; and Morehouse School of Medicine, Atlanta, GA.

To investigate early interactions of *Entamoeba histolytica* with the colon mucosa, we cocultured trophozoites with human enterocytes. After coculture the monoclonal antibody (MAb) CD6, raised against an ameba surface antigen, recognized not only trophozoites but also the lateral surface of trophozoite-associated enterocytes. Binding of the CD6 antigen to enterocytes was first observed after 5 minutes of coculture; trophozoite-mediated target cell lysis was first detected by propidium iodide staining after 15 minutes. The typical display of the CD6 antigen on the enterocytes was found neither at 4°C, nor with prefixed trophozoites, nor with trophozoite-conditioned media, nor with amebic sonicates or detergent lysates, nor when a filter prevented direct cellular contact. We presume that the galactose-N-acetylgalactosamine (Gal/GalNAc)-specific lectin is transferred to the enterocyte surface, because the CD6 antibody immunoprecipitated from trophozoite lysates the same bands as the MAb 7F-4 directed against the cystein-rich domain of the 170-kDa subunit of this lectin. This presumption is not contradicted by the fact that trophozoite-associated enterocyte immunostaining cannot be produced by the 7F-4 antibody, since CD6 and 7F-4 recognize non-overlapping domains of the lectin. Coculture in presence of antibody showed that CD6 does not cover the enterocyte-binding domain of the transferred lectin. The enterocytic acceptor site is sensitive to trypsin/EDTA. We concluded that the transfer of the intact amebic Gal/GalNAc-specific lectin or a portion of it to the lateral surface of enterocytes initiates invasion of trophozoites.

55 LYMPHOCYTE CYTOKINE RELEASE PATTERNS IN GERBILS WITH AMOEBIC LIVER ABSCESS. Campbell JD*, and Chadee K. Institute of Parasitology, Macdonald Campus of McGill University, Montreal, Quebec, Canada.

Amoebic liver abscess (ALA) formation is accompanied by a transient state of immunosupression, however, immunized animals are resistant to reinfection with *Entamoeba histolytica*. Several studies have demonstrated that macrophage functions are downregulated in animals with ALA and that macrophage activation is neccessary for immunity. In contrast, T and B cell functions in amoebiasis are less well characterized. In gerbils with ALA, we examined the proliferative responses of spleen and hepatic lymph node cells to the B cell mitogen lipopolysaccharide (LPS), the T cell mitogen concanavalin A (Con A) and amoebic antigen (AA) in the presence or absence of sera (10%) from homologous infected gerbils. We also quantified Interleukin-2 (IL-2), IL-4 and Tumour Necrosis Factor (TNF) production in response to Con A and AA by these cells using bioassays. Cell proliferative responses to Con A, LPS and AA were suppressed at day 20 compared to days 5 and 10 but increased from days 20 to 60 post infection. While sera from infected animals augmented proliferative responses, sera from animals at day 20 post infection suppressed Con A induced proliferation in cells from infected and uninfected control gerbils. IL-4 release was highest at day 5 and 10, declined at day 20 and remained low (<0.5 U/ml) at day 30 and 60. In comparison, IL-2 production, while also suppressed at day 20, increased significantly thereafter. TNF activity remained low from days 5 to 60 (< 1 U/ml). Gerbils which were drug-cured at day 20 and subsequently challenged were resistant to reinfection. Cells from gerbils isolated 5 and 10 days post challenge and from drug-cured but not challenged gerbils showed strong proliferative responses to mitogens and AA and increased IL-2 and TNF, but low IL-4 production. Thus, early development of ALA in gerbils is associated with IL-2 and IL-4 production (ThO-like response), while control and resistance to reinfection is associated with enhanced levels of IL-2 and TNF, but low IL-4 secretion (Th1-like response).

56 HUMAN COLONIC MUCIN HETEROGENEITY FOR DIFFERENTIAL INHIBITION OF *ENTAMOEBA HISTOLYTICA* ADHERENCE TO TARGET CELLS. Goettke MU*, and Chadee K. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

Secretory mucins in the gastrointestinal tract are the host's first line of defense against invasion by *Entamoeba histolytica* and other pathogens. Binding to target cells via the 260kD amoebic adherence lectin is an absolute prerequisite for pathogenesis. High affinity binding of the lectin by the Gal/GalNAc residues of colonic mucins is a host defense mechanism preventing amoebic adherence to mucosal cells. Genetic heterogeneity of secretory mucins has been demonstrated recently with MUC2 and MUC3 being the predominant genes in the colon. Mucins undergo extensive posttranslational modi- fications resulting in very large molecules (>1000 kD) with up to 80% sugar content. They are difficult to purify and to date have not been separated into subclasses reflecting the genetic polymorphism. In this study we have isolated distinct secretory mucins, characterized them immunogenically and biochemically and measured their functionality as inhibitors of amoebic adherence to chinese hamster ovary (CHO) cells. Using the culture medium of the mucin producing human colonic adenocarcinoma cell line LS174T, high M_r mucins were purified sequentially through Sepharose-4B and FPLC Superose-6 chromatography and then separated according to charge using FPLC Mono-Q, a strong anion exchanger. Two protein peak clusters eluted consistently between 250-430M NaCl and between 600-730M NaCl. Functional activity as expressed as adherence inhibition to CHO-cells varied from no inhibition to 53% inhibition of adherence in the peaks isolated from Mono-Q and was

up to five-fold higher than in Superose-6 mucins. The isolated peaks exhibited marked differences in antigenicity as determined by Western blot using antibodies against CsCl purified heterogeneous mucins. These results suggest that different subclasses of mucins confer different functions in health and disease.

57 DEVELOPMENT OF A SCID-HU-INT MODEL FOR INVASIVE INTESTINAL AMEBIASIS. Seydel K*, and Stanley, Jr. SL. Department of Medicine Washington University School of Medicine, St. Louis, MO.

The major morbidity associated with *Entamoeba histolytica* infection is intestinal disease, yet the study of the intestinal aspects of this disease has been hampered by a lack of a suitable small animal model to study the disease. In an attempt to remedy this, we have utilized an intestinal isograft system to accurately mimic human disease. Human fetal intestinal tissue of gestational age 95 to 120 days was implanted in the subscapular space of immunocompromised SCID mice. The intestinal isografts become vascularized and after eight weeks show a histologically normal mucosa. Subsequent infection of the isografts with *E. histolytica* trophozoites results in mucosal invasion and damage mimicing human disease. Thus we have developed a system where the interactions between human mucosa and ameba can be studied in a physiologic setting. Further studies characterizing intestinal responses to infection and amebic requirements for pathogenesis are currently underway.

58 RAPID DIAGNOSIS OF AMEBIASIS BY ANTIGEN DETECTION. Haque R*, Hahn P, Neville LM, Shamsuzzaman SM, and Petri, Jr. WA. International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh; TechLab, Blacksburg, VA; Dhaka Medical College, Dhaka, Bangladesh; and University of Virginia, Charlottesville, VA.

Current diagnosis of amebiasis is unsatisfactory in several aspects. Microscopic examination of stool specimens for amebic cysts and trophozoites is insensitive and unable to distinguish pathogenic *Entamoeba histolytica* from nonpathogenic *E. dispar*. Identification of the parasite in aspirated pus from liver abscess even in the most experienced hands is only 20% sensitive. Here we report diagnosis of invasive amebiasis via the detection of the amebic Gal/GalNAc lectin in sera, liver abscess pus, and stool, and the results of a field trial of the first commercial antigen detection kits (TechLab *Entamoeba* and *E. histolytica* kits) designed to rapidly detect and differentiate *E. histolytica* from *E. dispar* in stool using monoclonal antibodies specific for the *E. histolytica* lectin. Acute sera from 39 patients with amebic liver abscess and 31 sera from patients with other illness were tested for Gal/GalNAc lectin by a mAb-based ELISA; lectin was detected in 32/39 patients with amebic liver abscess and 3/31 controls for a sensitivity of 82% and a specificity of 90%. The Gal/GalNAc lectin was also detected in aspirated pus from 100% (7/7) of patients with amebic liver abscess. Stool specimens from 202 patients with diarrhea were examined for *E. histolytica* and *E. dispar* by microscopy, culture, and antigen detection. Compared to culture, microscopic identification of *E. histolytica/dispar* complex was 60% sensitive and 79% specific, while the screening antigen detection test for *E. histolytica/dispar* complex was 80% sensitive and 99% specific. Differentiation of *E. dispar* from *E. histolytica* with the *E. histolytica*-specific test was 95% sensitive and 93% specific compared to zymodeme analysis. We conclude that the antigen detection tests for *E. histolytica/ dispar* complex are more sensitive and specific than microscopy, and offer a rapid non-invasive means for the diagnosis of invasive amebiasis.

59 FIRST RECOVERY OF THE LAB-MADE *ENTAMOEBA HISTOLYTICA* ZYMODEME XX IN HUMANS. Gatti S*, Cevini C, Bruno A, Novati S, Marchi L, AND Scaglia M. Lab. Clinical Parasitology, Institute of Infectectios Diseases, University-IRCCS S, Matteo, Pavia, Italy; and Sospiro Hospital, Sospiro, Cremona, Italy.

Isoenzyme analysis by starch-gel electrophoresis has proved a useful method for the biochemical identification of *Entamoeba* spp., and has been used to differentiate pathogenic *Entamoeba histolytica* and non-pathogenic *E. dispar* stocks. Till now 24 "zymodemes" (zym) have been identified: of 21 isolated from human beings, 9 have pathogenic patterns and 12 non-pathogenic. Three are lab-made pathogenic zym (XI α-, XX, XXI), experimentally obtained by mixing in culture pathogenic zym previously recognized in man. During a parasitological screening in an Institution for mentally retarded patients, we isolated several *Entamoeba* spp. The cloned stocks were studied by starch-gel electrophoresis, in order to distinguish pathogenic zym of *E. histolytica* from non-pathogenic ones of *E. dispar*. The electrophoretic runs of the isolates from 2 subjects showed isoenzyme banding patterns consistent with those of zym XX reference strain (double fast bands in HK, double banding in β and δ position in GPI and PGM respectively). Repeated experiments with recloned cultures of the same strains gave the same results. This first identification of zym XX in human amoebic isolates demonstrates that: a) the process of "genetic exchange", experimentally obtained *in vitro* and in mice by mixing two pathogenic zym as described previously, may take place also in humans; b) the severely mentally ill subjects, institutionalized for several years in close communities, because of peculiar life style, may acquire *E. histolytica* stocks with different pathogenic zym and then may act as a "culture system" where the amoebic strains can give rise to a "hybrid progeny".

61 HEPATITIS E VIRUS EXCRETION AND SEROLOGIC RESPONSE FROM AN EPIDEMIC OF HEPATITIS IN PAKISTAN. Zhang HY, Iqbal M, Bryan JP*, Tsarev S, Longer CF, Rafiqui AR, Caudill JD, Duncan JF, Ahmed A, Miele TA, Malik IA, and Purcell RA. Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC; Pakistan United States Laboratory for Seroepidemiology, Rawalpindi, Pakistan; Department of Preventive Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD; and Hepatitis Viruses Section, National Institutes of Allergy and Infectious Diseases, Bethesda, MD.

Hepatitis E virus (HEV) is an important cause of sporadic and epidemic hepatitis in Southwest Asia. An outbreak of hepatitis which occurred among men in an academic community in Abbottabad, Pakistan, was traced to contamination of a water supply. Of 109 men hospitalized with a clinical diagnosis of hepatitis, 104 (95%) were found to have serologic evidence of acute hepatitis E. Both IgM and IgG anti-HEV were present in 92% of cases on admission. Among 44 men from whom three serum specimens were obtained over 4 months, IgG anti-HEV geometric mean titers (GMT) decreased from 1534 on admission to 651 at 4 months. IgM anti-HEV was detected in 40 (91%) of 44 men at a GMT of 525 during acute disease but was observed in only 8 (18%) four months later. Affinity capture PCR (AC/PCR) detected HEV in serially-collected feces from 18 of 19 men. The intensity of viral excretion was greatest during the first week of symptoms (present in 79% of patients) but persisted intermittently for up to 4 weeks after the onset of jaundice. PCR studies of single fecal specimens from 41 patients detected HEV in 13 (32%). This study identifies hepatitis E as the etiologic agent of this outbreak, elucidates the pattern of anti-HEV in patients, and demonstrates the pattern of HEV excretion in feces as measured by AC/PCR.

62 GNATHOSTOMIASIS CONTRACTED IN EAST AFRICA. Wolfe MS*. Traveler's Medical Service of Washington, Clinical Professor of Medicine, George Washington University Medical School, Washington, DC.

In September, 1991 a group of 5 American tourists visited the Selous Game Reserve in southeast Tanzania. All ate sushi from a catfish caught in the Rufiji River. A few months after return home, 3 individuals began having intermittent migratory painless subcutaneous nodules. These involved the chest and upper arm on a 63 year old male and his 58 year old wife, and the eyelid and face of an unrelated 25 year old male. When first seen in October 1994, *Gnathostoma* serologies (ELISA and Western blot) performed in Bangkok were positive on all 3 symptomatic individuals. Albendazole was adminsitered in a dose of 400 mg bid for 28 days. On day 17 of treatment, the 58 year old female developed a one cm linear tract with a small vesicle at one end, one her her neck. A *Gnathostoma* sp. larva measuring 2.7 mm long and 600 micrometers wide was removed from the vesicle. This is the first report of human gnathostomiaisis from east Africa. Further studies of the larva will be made to attempt to determine if this is a previously known or a new *Gnathostoma* sp. To date, there has been no return of symptoms in the treated individuals.

63 EPIDEMIC VISCERAL LEISHMANIASIS IN SOUTHERN SUDAN: PATIENT CHARACTERISTICS AND TREATMENT OUTCOMES. Seaman J, Mercer AJ, Sondorp HE, and Herwaldt BL*. Medecins Sans Frontieres-Holland, Amsterdam; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Civil-war affected southern Sudan is experiencing a massive epidemic of visceral leishmaniasis. During the period 1989-94, Medicins sans Frontieres-Holland treated approximately 19,000 patients. To determine the proportions of patients who had various treatment outcomes and to identify patient characteristics associated with the outcomes,

we analyzed data for a cohort of 3,076 patients treated with sodium stibogluconate during the period August 1990-July 1991. The patients had a median age of 15 years and were markedly anemic (median hemoglobin value [HB], 7.7 g/dL) and malnourished (median body mass index [BMI] for adults aged >=18 years, 15); most (91.0%) had been sick <5 months. Most patients (2,544 [82.7%]) were apparently cured. Only 437 (14.2%) are known to have had an adverse outcome: 344 (11.2%) died, and 93 relapsed (3.5% of those discharged alive). The main patient characteristics associated with death were young and older age (aged <5 or >=45 years), long duration of illness (>=5 months), severe anemia or low BMI on admission, and vomiting during the treatment course. In multivariate analysis of data for a subgroup of 1,194 adults, the estimated relative risks for death were significantly elevated at 2.3, 3.6, 4.6, and 12.2 for those with a long duration of illness, aged ≥45 years, with a HB<6, or with a BMI<12, respectively. Despite the patients' severe debility and the difficult circumstances under which they were treated, most fared remarkably well.

64 *OESOPHAGOSTOMUM BIFURCUM* IN MAN: TRANSMISSION IN NORTHERN TOGO AND GHANA. Polderman AM*, Eberhard ML, Pit D, Blotkamp J, and Baeta S. Department of Parasitology, University of Leiden, The Netherlands; Division of Parasitic Diseases, Centres for Disease Control & Prevention, Atlanta, GA; Regional Hospital of Dapaong, Ministry of Health, Togo & Department of Parasitology, Leiden, The Netherlands; Department of Parasitology, University of Leiden, The Netherlands; and Department of Gynaecology, University of Lome, Togo.

Human infections with *Oesophagostomum bifurcum* are very common in parts of northern Togo and Ghana. Children as young as 10 months have been found infected and the prevalence of children of 3-4 years old is over 40% in some villages. Although most infections remain without symptoms the clinical expression may be impressive and the morbidity is significant. The mode of transmission remains ill understood. Association with hook- worm infections might suggest cutaneous transmission but attempts to percutaneously infect one of us failed. Similarity with transmission of other species, too, suggests oral trans- mission. However, it is difficult to visualize how man consu- mes large numbers of infective L3 larvae. Reinfection studies after treatment at different times of the year show that transmission is limited to the rainy season. The biological function of the infective larvae's capacity to survive months of aestivation during the dry period, needs to be elucidated.

65 CHARACTERIZATION OF THE INFLAMMATORY RESPONSE IN THE HUMAN BRAIN INFECTED BY THE CYSTICERCUS OF THE FLATWORM *TAENIA SOLIUM*. Restrepo BI*, Llaguno P, Sandoval MA, Enciso JA, and Teale JM. Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, TX; and Division Investigaciones en Enfermedades Infecciosas y Parasitarias, Hospital Pediatria CMN-IMSS Mexico D.F.

Cysticercosis is the most common parasitic infection of the human central nervous system (CNS) worldwide. Its etiological agent is the cysticercus of the porcine tapeworm, *Taenia solium*, which develops preferentially in the brain of a human that ingests a *Taenia* egg. While alive, the cyst only induces a mild inflammatory response and the patient will usually have no clinical symptoms. However, symptoms are common upon parasite death, when an inflammatory response is elicited. Therefore, characterization of the immune response in the brain is pivotal for understanding the pathogenesis of cysticercosis. We are defining by immunohistochemical analysis the inflammatory cells and cytokines produced adjacent to the parasite in the human brain. Two patients with a cysticercus in leptomeninges exhibited significant inflammation with abundant T and B lymphocytes, and granulocytes. In contrast, a patient with the parasite in the temporal lobe had a mild inflammatory response, with scanty T cells, and discrete regions with either IgM-producing cells and/or NK cells. In this third patient IL-12 was abundant. IL-6, γ-IFN and NK cells were present in three IL-12 positive regions. IL-4 producing cells were only present in an IgM-rich area. Although other interpretations are possible, these data suggest that the type of inflammation induced by the cysticercus is influenced by its location in the brain. This may be explained by the closer vicinity of the leptomeninges to primary blood vessels, when compared to brain parenchyma. Proximity to blood vessels may facilitate the initial access of activated T leukocytes to the brain, and the subsequent entrance of other inflammatory cells.

66 LYMPHOCYTE RESPONSE TO TETANUS IMMUNIZATION DURING EXTENDED USE OF CHLOROQUINE OR PRIMAQUINE CHEMOPROPHYLAXIS. Fryauff DJ*, Church P, Mouzin E, Widjaja H, Sutamihardja MA, Ratiwayanto S, Hadiputranto H, Saraswati A, Subianto B, Tjitra E, Wignall FS, and Hoffman SL. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; Provincial Health Service, Irian Jaya, Indonesia; National Institute of Health Research, Jakarta, Indonesia; Provincial Health Service, Irian Jaya, Indonesia; and U.S. Naval Medical Research Institute, Bethesda, MD.

Immune suppression is a potential side effect of antimalarial therapy and prophylaxis. Therapeutic levels of chloroquine and primaquine *in vitro* can suppress lymphocyte proliferation and chloroquine prophylaxis has been shown to depress antibody response to rabies and typhoid vaccination. The effect of extended use of primaquine on immune response and vaccination success are unknown. Cellular immune response to tetanus was evaluated in Javanese men who were randomized to either weekly chloroquine (300 mg base/wk), daily primaquine (0.5 mg base/kg), or a daily placebo. After 12 months of supervised prophylaxis a tetanus-diphtheria inoculation (Td adsorbed for adult use USP, 0.5 ml IM) was administered to men in each group (92 men total) and respective chemoprophylaxis continued 1 month longer. In vitro lymphocyte responses to PPD, PHA, whole tetanus toxoid, and 3 synthetic subunits (P2, P30, P2P30) of tetanus exotoxin were evaluated at baseline, 1, 3, and 7 months post-Td immunization. Non-immunized, non-prophylaxed men served as control. After 12 months of prophylaxis there was no evidence of reduced lymphocyte response in either primaquine or chloroquine groups to any protein/mitogen concentration tested. At 1 month post-Td all 3 immunized groups responded comparably to whole tetanus toxoid but the proportions of subjects responding and the indices of lymphocyte proliferation to whole tetanus toxoid and subunit preparations were greatest in the primaquine group. At 3 and 7 months post-Td, lymphocyte responses to tetanus preparations had declined in all immunized groups but the primaquine group still registered highest responses to whole tetanus toxoid. These findings lend positive support for the safety and acceptability of primaquine for use as a chemoprophylactic agent.

67 HUMAN COENUROSIS IN NORTH AMERICA: REPORT OF TWO CASES AND REVIEW. Ing MB*, Schantz PM, and Turner JA. Division of Infectious Diseases, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA; and Epidemiology Branch, Division of Parasitic Diseases, NCID, Centers for Disease Control, Atlanta, GA.

Coenurosis is a zoonotic disease of humans caused by the cystic larval stages of *Taenia (Multiceps)* species developing in the central nervous system (CNS), eye, subcutaneous or intramuscular tissues. Canids are the definitive hosts. Hares, rabbits, and squirrels serve as intermediate hosts. Our cases represent CNS and intramuscular involvement. Case 1 is a 2 year old Native American girl from northern Wisconsin who developed neurologic signs and symptoms and intracranial hypertension. Imaging studies showed cystic masses involving the base of the brain and lumbar spinal cord. Coenurosis was diagnosed following a craniotomy. Case 2 is a 39 year old Hispanic male from Los Angeles, California who developed a mass in the left infrascapular area. Systemic symptoms occurred. The diagnosis of coenurosis was made preoperatively from examination of aspirate from the lesion. Both patients received praziquantel, and their responses will be discussed. Case 1 lived in an area where dogs ranged free, and larval stages were common in hares and rabbits. Case 2 had a pet dog, and had briefly visited a ranch near Juarez, Mexico prior to the onset of his illness. Previous case reports of human coenurosis in North America will be reviewed, and the current information on the prevalence of *Taenia (Multiceps) serialis* in definitive and intermediate hosts in this geographic area will be discussed.

68 MYIASIS: AN EMERGING OR ENDEMIC PROBLEM IN KUWAIT? Hira PR*, Farooq R, Al-Ali F, Hajj B, Al-Muzairai IA, and Hall MJ. Department of Microbiology, Faculty of Medicine, Kuwait University, Safat,Kuwait; Laboratory Departments, Adan and Farwaniya Hospitals, Kuwait City, Kuwait; Ophthalmology Department, Ibn Sina and Mubarak Hospitals, Kuwait City, Kuwait; and Natural History Museum, London, England.

Ophthalmomyiasis due to the sheep nasal botfly *Oestrus ovis*, has been identified and continues to be of clinical significance in Kuwait. We report cases of intestinal and urinary myiasis. Examination of the stool of a child who presented with diarrhea showed the presence of a 3rd instar larva of *O. ovis* which usually infests the nasal passages of sheep and goats. How the specimen came to be in the child's gastrointestinal tract is unknown: ingestion is a possibility. The stool of a 6 year old child who complained of stomach upset showed organisms, approximately 4-8 mm in length, identified as the 3rd instar larvae of *Megaselia* (probably *ik*), a filth-inhabiting phorid fly. A soldier complained of passing worms in urine; a 28-year old female reported noticing worms in her urine. In both specimens, the organisms were identified as 3rd instar larvae of *Pyschoda* sp. The patients were counselled and reassured and remain well to date. In these unique and other myiasis cases counselling and reassurance resolves the associated anxiety and confusion. This is the first report of intestinal and urinary myiasis from Kuwait, an area with a paucity of data on myiasis. Lack of expertise and reference centers are major contributing factors in that many cases may pass unnoticed and unreported. Intensive coordinated research is strongly suggested to determine whether myiasis is an endemic or newly emerging disease.

69 STRATEGIC CONTROL OF CYSTICERCOSIS THROUGH SEROLOGIC MONITORING OF SENTINELS AND TREATMENT OF INFECTED PIGS WITH ALBENDAZOLE AND OXFENDAZOLE. Gonzales AE, Garcia HH, Gilman RH, Gavidia C, Pilcher JB, and Tsang VC*. Universidad Nacional Mayor de San Marcos, Lima, Peru;

Universidad Peruana Cayetano Heredia, Lima, Peru; Division of Parasitic Diseases, NCID, Centers for Disease Control & Prevention, Atlanta, GA; and School of International Health, The Johns Hopkins University, Baltimore, MD.

We have shown previously that serodiagnosis of sentinel piglets is an effective and practical means to detect *Taenia solium* eggs in the environment. Uninfected native (n=28) and sentinel pigs (n=12) from non-endemic areas were shown to acquire new infections very soon after first exposure to *T. solium* eggs. There is great economic incentive for farmers to allow access to their pigs for testing and treatment, because cysts-free pork is likely to fetch much higher prices on legitimate markets than a cyst infested product having to be sold in street markets. Furthermore, virtually all pigs are replaced through slaughter very year, thus eliminating the concern for persistence of antibody. Serologic monitoring of sentinel pigs is, therefore, practical and effective for indirect assessment of human risk. Effective treatment of infected pigs is the next logical step for controlling this disease. We will show that multiple doses of albendazole are required for cure in pigs (n=17). Severe side effects and incomplete resorption of cysts were observed with this drug. A single dose of oxfendazole, on the other hand, is 100% effective for cure, free from side effects, and was able to clear cysts from the tissue of treated animals (n=16). This drug is also inexpensive (U.S.$1.20 per animal). Pilot control programs based on diagnosis of infected humans, improved pig husbandry, treatment of infected animals, and monitoring of sentinels are underway in several Peruvian villages.

70 ROLE OF FINE NEEDLE ASPIRATION BIOPSY IN DIFFERENTIAL DIAGNOSIS AND TREATMENT OF LIV ER ECHINOCOCCOSIS. Stefaniak J*, Lemke A, Paul M, and Pawlowski ZS. Clinic of Parasitic and Tropical Diseases and Institute of Radiology, University of Medical Sciences, Poznan, Poland.

Some cases of liver echinococcosis are difficult to be clinically diagnosed. The presence of the protoscolices, hooks or parasite antigen can be demonstrated in the cyst fluid obtained by fine needle aspiration biopsy (FNAB). Out of 285 cases with liver space occupying lesions 121 patents were qualified for FNAB. Echinococcosis has been diagnosed in 24 cases either by finding *Echinoccus granulosus* protoscolices or hooks (16 cases) or by finding *E. granulosus* antigen 5 (8 cases). Among the lesions, which requires differential diagnosis with echinococcosis there were 9 cases of neoplasm, 3 cases of bacterial abscess, 4 cases of angioma, 67 cases of simple cyst and 14 cases of polycystic disease. FNAB was performed under 3-days albendazole (ALB) cover, using a needle 22 gauge, being introduced transhepatically under US guidance. There were neither direct adverse reactions nor late complications related to FNAB. In all the cases of confirmed echinococcosis ALB treatment has been extended to one month at the daily dose of 600 or 800 mg. The treated cases were followedup for at least 18 months and all of the cases has demonstrated the signs of cyst damage (diminished size and calcifications). The other imaging signs of cyst damage (increased hyperechogenicity, endocyst detachment) were statistically invalid criteria of a cyst damage. There was no justification to extend ALB treatment up to 3 months, a usually recommended. In conclusion: FNAB was a safe diagnostic procedure, which in addition allowed to shorten the time of ALB treatment to one month only.

71 GEOGRAPHIC INFORMATION SYSTEMS (GIS) DURING THE CEASE FIRE INITIATIVE IN SOUTHERN SUDAN: PROBLEMS WITH THE AVAILABLE DATA BASES. Richards F*, Roberts J, Doyle R, Ruiz-Tiben E, and Pietrantonio F. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; The Carter Center, Atlanta, GA; and Somalia/Sudan Mission, Medicins sans Frontieres-Belgium, Nairobi, Kenya.

Former US president Jimmy Carter negotiated a cease-fire that on March 28, 1995 temporarily halted the 12-year old civil war in southern Sudan. The cease fire afforded the guinea worm eradication operations, Mectizan® community treatments and childhood immunization (EPI) activities. Large scale paper maps showing locations of small villages, roads, and rivers in the region were urgently needed by field teams for epidemiological assessment and control-eradication activities. Using Atlas*GIS® software, we were quickly able to produce 24X32 inch color maps using a large format plotter. Latitude- longitude "point" data for human settlements (n=7193) and river names (n=132) in south Sudan were overlayed on a commercially available (1: 1,000,000 scale, 22 megabyte vector-based) map of south Sudan (Digital Chart of the World-DCW). However, when the printouts were field-tested around the village of Mvolo in Western Equatoria province, < 20% of villages listed in the "point" data base could be confirmed in the field, and the two rivers (Woko and Bahr el Naam) that converge near the village were not found on the DCW. This study underscores that the principal limitation to rapid deployment of GIS-generated products is the quality of available data sets.

72 GENETIC ANALYSIS OF ROUNDWORM BURDEN IN A NEPALESE POPULATION. Williams-Blangero S*, Blangero J, Upreti RP, Adhikari BN, Upadhayay RP, Jha B, Rai SK, Subedi J, and Robinson ER. Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; Tribhuvan

University Institute of Medicine, Kathmandu, Nepal; and Department of Sociology/Anthropology, Miami University, Miami, OH.

Little is known about the role of human genetic factors in susceptibility to helminthic infection. Detection of genetic effects requires the collection of data in families and some mechanism for discriminating between the effects of shared environmental exposure and genes. To assess the relative contribution of genetic factors in roundworm (*Ascaris lumbricoides*) burden, we performed a household-based study among the Jirel population of eastern Nepal. Quantitative egg counts were obtained from 400 individuals who were members of 32 pedigrees residing in 90 households. The observed prevalence of roundworm infection was 27%. Because of the study design, we were able to examine statistical genetic models that allowed for the effects of genes and shared household environment. Statistical genetic analysis, using a robust variance component method, revealed a significant genetic component to worm burden ($p<0.001$). Genetic factors account for 34% of the variation in egg counts, while shared environmental exposure cannot account for observed correlations among family members. This is the first study on helminthic burden in humans with adequate power to disentangle the effects of genetics from shared environment. Our data suggests that exposure may be relatively homogeneous in this population and that genes are a primary determinant of egg count covariation between individuals.

73 *ASCARIS LUMBRICOIDES* ASSOCIATED WITH LOW HEIGHT FOR AGE Z-SCORES IN INFANTS AND YOUNG CHILDREN FROM A RURAL AREA IN SUB-SAHARAN AFRICA. Rockhold P*, and Ash L. Department of Epidemiology, UCLA, Los Angeles, CA.

A community based cross-sectional study was done to identify intervention strategies to improve the nutritional status in a rural area of Malawi in Africa. The study examined the association between characteristics of the household environment of early childhood and the nutritional status of children measured by height for age Z-score (HAZ) and weight for height Z-score (WHZ). A random sample of 171 children between the ages of 6 month and 4 years was included in the study. Nearly 65 percent of the children were stunted, 2.8 percent wasted and 39 percent underweight. Laboratory data on 130 of the chidren found 95 percent to be anemic and 50 percent had malaria. Of the 112 children with stool examinations, 18 percent had *Ascaris lumbricoides*. Multivariate regression based on two initially similar mathematical models for HAZ and WHZ, found HAZ to be significantly lower in boys and negatively associated with child's age and *Ascaris*. WHZ was negatively associated with the number of children under five in the household and positively associated with current household wealth and the child's hemoglobin. Most studies have found *Ascaris* to be negatively associated with WHZ. The here found, association between HAZ and *Ascaris*, seems biological plausible, but lacks sufficient documentation.There is a need for community based cohort studies to explore the association of *Ascaris* infection with HAZ further.

74 COMPARISON OF THE CAPACITY OF ROBINS AND STARLINGS AS RESERVOIRS OF EEE VIRUS. Komar N*, Turell MJ, Pollack RJ, Monath TP, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

To compare the importance of various birds as reservoir hosts of eastern equine encephalitis (EEE) virus, we conducted a combined experimental and computer modelling study. American robin and European starling were candidate reservoirs because they roosted abundantly in wetland sites near dense infestations of the enzootic vector, *Culiseta melanura*. Although both birds were competent hosts for the virus, starlings were more infectious to mosquitoes and remained infectious twice as long as did robins. Robins survived infection, but starlings generally died. Reservoir capacity of these birds was compared by means of a deterministic, differential equations model, derived using Stella II software. Reservoir capacity was defined as the number of secondary infections in vectors generated from one infected reservoir host. Components of the model included densities of susceptible, infected, infectious and immune reservoir hosts, as well as non-reservoir hosts; densities of noninfected biting, noninfected gravid, infected gravid and infectious biting vectors, and the following host factors: intrinsic incubation period, duration of infectivity, mean daily infectious dose, mortality, roost fidelity and forage ratio. To estimate reservoir capacity, mortality and competence data for robins and starlings were combined with preliminary estimates of robin and starling forage ratios of 0.4 and 0.1, respectively. Whereas starlings were 3.2 times more competent than robins, robins infected 8.4 times more vector mosquitoes than did starlings. Communal roosts of robins, therefore, may serve as important foci of EEE virus transmission, and may be targetted for virus surveillance and anti-mosquito interventions. Interestingly, mortality in reservoir hosts appears to increase reservoir capacity by eliminating immune hosts that otherwise would be zooprophylactic for virus transmission.

75 MULTIPLE EMERGENCES OF EPIDEMIC/EPIZOOTIC VENEZUELAN EQUINE ENCEPHALITIS VIRUSES FROM ENZOOTIC PROGENITORS. Weaver SC*, Oberste MS, Smith JF, Tesh RB, Shope RE, and Rico-Hesse

R. Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX; US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Yale Arbovirus Research Unit, Yale School of Medicine, New Haven, CT.

We used phylogenetic methods to study emergence of epidemic/epizootic Venezuelan equine encephalitis (VEE) viruses. Because previous work indicated that antigenic variety IAB and IC epidemic/epizootic viruses evolve from variety ID enzootic progenitors, we focused on these serotypes. The N-terminal half of the E2 envelope glycoprotein gene, totaling 856 nucleotides, was amplified by reverse transcription/polymerase chain reaction (RT-PCR) and sequenced. Nucleotide sequences were aligned and analyzed using parsimony and distance-matrix algorithms to generate phylogenetic trees. Homologous sequences from other alphaviruses, as well as variety IE VEE viruses, were included as an outgroup. The IAB, IC and ID sequences comprised a paraphyletic group that also included Everglades virus, subtype II. Within this large group, four minor monophyletic groups representing distinct VEE genotypes were delineated by the trees: (1) variety ID enzootic viruses isolated in coastal Ecuador and southwestern Colombia; (2) Everglades virus from Florida; (3) ID viruses isolated in Panama and Iquitos, Peru and (4) a large group of IAB, IC and ID viruses primarily from Colombia, Venezuela and Peru. Relationships within the trees indicated that epidemic/epizootic VEE viruses emerged 5 or more times from enzootic ID progenitors in Venezuela and/or Colombia, while genotypes circulating in Panama, Ecuador and Florida have not initiated major outbreaks.

76 ATTENUATED VENEZUELAN EQUINE ENCEPHALITIS VIRUS VACCINE VECTORS EXPRESS IMMUNOGENS OF HETEROLOGOUS PATHOGENS *IN VIVO* AND INDUCE MUCOSAL IMMUNITY. Davis NL, Brown KW, Charles PC, Caley IJ, Swanstrom RI, Smith JF, and Johnston RE*. Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC; and Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

A molecularly cloned mutant of Venezuelan equine encephalitis virus (VEE) with two strongly attenuating mutations was inoculated subcutaneously (sc.) into mice. The mutant replicated in the draining lymph node without producing any clinical signs of disease and induced protective immunity against sc. or intranasal (in.) challenge with virulent VEE. Effective mucosal immunity was demonstrated by the induction of VEE-specific IgA and complete protection of the nasal tissues of in. challenged mice from VEE infection. This attenuated cDNA clone was altered to contain a second subgenomic RNA promoter and a complete influenza HA gene immediately downstream of the structural gene region. Antigenically authentic influenza HA protein was expressed from the second promoter as determined by immunoprecipitation with anti-influenza antibody. CD-1 mice were inoculated sc with 2×10^4 pfu of the HA vector, vector without insert, or diluent with no ill effects. Expression of HA mRNA was detected in the draining lymph node of HA vector-inoculated mice by *in situ* hybridization, consistent with the organ tropism of the original VEE mutant. Sera from HA vector-inoculated mice had measurable anti-influenza neutralizing activity and anti-HA IgG by ELISA. Significant increases in these titers were observed after administration of a booster immunization, and influenza-specific IgA was detected in vaginal washes 11 to 15 days after the boost. After in. influenza challenge, 100% morbidity and 50% mortality were observed in control animals, whereas morbidity/mortality were 17% and 8% following primary immunization with the HA vector and 0% and 0% following the boost. Significantly reduced replication of influenza challenge virus in the lung and in the nasal mucosal itself was consistent with the observed protection and the presence of protective mucosal immunity. Currently, this vector is being used to express high levels of either HIV-1 MA/CA (gag) or HIV-1 gp160 (env) which are reactive with patient sera and with specific monoclonal antibodies on Western blots. These experiments demonstrate the feasibility of using vectors based on attenuated VEE cDNA clones to induce protective systemic and mucosal immunity against heterologous pathogens.

77 DEVELOPMENT OF RNA REPLICON AND HELPER SYSTEMS FROM ATTENUATED STRAINS OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS. Pushko P*, Parker M, Ludwig G, Davis N, Johnston R, and Smith J. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC.

We have developed VEE RNA replicon and helper systems for high level expression of foreign genes in animal cells and as vaccine delivery systems. These systems were derived from full-length cDNA clones of VEE with multiple attenuating mutations which have been extensively evaluated during the development of new live-attenuated VEE vaccines. In the replicon systems, these mutations will assure attenuation in the event that full-length genomes are regenerated by recombination between replicon and helper RNAs. Initially, several truncated VEE helper transcripts were produced which contained different combinations of attenuating mutations in the structural genes and different deletions within the nonstructural genes. These helper constructs were evaluated for their relative efficiency of replicon packaging in cotransfection experiments. To evaluate the levels of expression achieved in cell cultures, and the ability of packaged replicons to induce appropriate B and T cell responses in mice,

individual recombinant replicons were constructed in which the VEE structural genes were replaced with relevant reporter genes encoding influenza HA, Lassa N, or Lassa GPc proteins. In studies with the Lassa N replicon, we have observed efficient packaging with a helper transcript containing attenuating mutations at the E2- 209 and E1-272 codons, and a deletion encompassing 94% of the nonstructural genes. With this helper, approximately 10^8 single-cycle infectious units per ml were produced. The resulting particles induced high level expression of Lassa N protein after infection of BHK cells, and inoculation of Balb/c mice induced a prompt antibody response to the Lassa N protein. The potential advantages of this system for foreign gene expression and vaccine delivery will be discussed.

78 VENEZUELAN EQUINE ENCEPHALITIS VIRAL INFECTION AMONG PERUVIAN MILITARY TROOPS IN THE AMAZON RIVER BASIN OF PERU. Watts DM*, Callahan J, Cropp CB, Rossi C, Oberste MS, Karabatos N, Nelson W, Roehrig JT, Lavera V, Wooster MT, Smith JF, and Gubler DL. U.S. Naval Medical Research Institute Detachment, Lima, Peru; U.S. Army Medical Research Institue of Infectious Diseases, Frederick, MD; Division of Vectroborne Infectious Diseases, CDC, Ft. Collins, CO; and Peruvian Military, Iquitos, Peru; and U.S. Naval Medical Research Institute, Bethesda, MD.

An outbreak of a febrile illness characterized by headache, ocular pain, myalgia and arthralgia occurred among Peruvian military personnel during May and June, 1994 in Amazon jungle of Northern Peru. On 14-16 June, 1994 clinical data and blood samples were obtained from 8 individuals with febrile illness and 26 others who had recovered from a febrile illness. A second blood samples was obtained from 11 of the troops 107 days later. Virus isolation was attempted in newborn mouse and Vero cell culture assays. Sera were tested for dengue (DEN), Oropouche (ORO) and Venezuelan equine encephalitis (VEE) IgM and/or IgG antibodies by ELISA. Viral isolates were identified by indirect immunofluorescence employing alphavirus and flavivirus polyclonal and VEE monoclonal antibodies. VEE viruses similar to the Colombian and Venezuelan subtypes 1C and 1D were isolated from 2 of 8 persons with febrile illness. Acute sera of both were negative for VEE antibody, but a second serum obtained from one of these patients had a greater than four-fold increase IgG titer. One other patient seroconverted and IgG antibody was detected in sera of 2 others. Among the 26 individuals who had recovered from their illness, 4 were positive for either VEE IgM and/or IgG antibodies. Evidence of previous DEN and ORO infections was demonstrated in some of the troops. These data suggest that VEE virus may be an important cause of illness among military personnel in Northern Peru.

79 ARBOVIRUSES ASSOCIATED WITH HUMAN INFECTION IN THE PERUVIAN AMAZON RIVER BASIN. Wooster MT, Watts DM*, Rossi C, Oberste MS, Callahan J, Smith JF, Hayes CG. U.S. Naval Medical Research Institute Detachment, Lima, Peru; U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD; and U.S. Naval Medical Research Institute, Bethesda, MD.

Studies were conducted during 1993 and 1994 to determine the etiology of febrile illnesses in the city of Iquitos, Peru, and in surrounding rural and jungle villages of the Amazon Jungle. All patients enrolled had a clinical diagnosis of a viral associated, acute febrile illness of less than 3 days duration. Clinical data and blood were obtained from each patient. Sera were tested for virus by the newborn mouse and cell culture assays. Viral isolates were identified by indirect immunofluorescence employing hyperimmune sera and/or monoclonal antibody to selected alphaviruses and flaviviruses. Sera were tested by ELISA for IgM and IgG antibodies to several alphaviruses, DEN and Bunyamwera serogroups. A total of 221 patients were enrolled with fever, headache, myalgia, arthralgia, ocular pain, and chills. VEE virus was isolated from at least 8 cases. Sequence analysis showed the isolates to be related to the 1D subtype. Additional 4 cases of VEE were diagnosed based on seroconversions, 3 others had IgM antibody in both the acute and convalescent sera, and IgM was detected in 4 patients for whom only acute sera were available. DEN was shown to cause 24 cases and ORO 8 cases. All sera were negative to antibody to EEE, Punta Toro, and Bunyamwera serogroup viruses.

80 IDENTIFICATION AND SUBTYPING OF HUMAN ISOLATES OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN PERU BY SEQUENCING AND PHYLOGENETIC ANALYSIS. Oberste MS*, Weaver SC, Watts DM, and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX; U.S. and Naval Medical Research Institute Detachment, Lima, Peru.

Venezuelan equine encephalitis (VEE) virus was isolated from patients during two 1994 outbreaks of acute undifferentiated febrile illness in the Peruvian Amazon basin. Two viral isolates were recovered from Peruvian soldiers at a jungle outpost near Pantoja, in northern Peru, and at least eight isolates were made from military and civilians in Iquitos, an urban center in northeastern Peru. The viruses were initially identified as VEE by ELISA and immunofluorescence using polyclonal and monoclonal antibodies specific for alphaviruses and flaviviruses

known to be endemic to the Amazon region of Peru. To determine the subtype(s) of these VEE isolates, we used RT-PCR to amplify 517 nt of the nS4 gene and 858 nt of the E2 glycoprotein gene. The PCR products were directly sequenced and the sequences were compared to those of other VEE strains, including representatives of the IAB, IC, ID, IE, II, IIIA, IIIB, and IIIC subtypes. VEE subtypes ID and IIIC were recovered from sentinel hamsters and mosquitos near Iquitos in the early 1970s and VEE IAB was previously isolated in coastal Peru during equine epizootics. The two Pantoja isolates were most closely related to the subtype IC and ID viruses previously isolated in the nearby Colombian Amazon basin and in Iquitos. All of the new Iquitos isolates were similar to one another and most closely related to strain 3880, a 1961 ID isolate from Panama, and distinct from previous Iquitos and Colombian isolates. This marks the first identification of 3880-like VEE virus outside of Panama. The recovery of 3880-like virus in Iquitos may represent the first detection in Iquitos of a newly imported VEE lineage. It is also possible that 3880-like viruses have been present in Iquitos previously, along with IIIC and Colombian-type ID viruses, but have remained undetected due to ecological restrictions or lack of surveillance.

81 SUSCEPTIBILITY OF CENTRAL AND SOUTH AMERICAN MOSQUITOES FOR EPIZOOTIC AND ENZOOTIC STRAINS OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS. Turell MJ*, Coleman RE, Dohm DJ, and Barth J. Applied Research Division, U. S. Army Medical Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

Mosquitoes collected in Peru, Panama, and Belize were evaluated for their susceptibility to epizootic (IAB, IC, and ID) and enzootic (IE) strains of Venezuelan equine encephalitis (VEE) virus. *Culex (Melanoconion) taeniopus* was nearly refractory to the IAB strain (0/41 with a disseminated infection), but was highly susceptible to the IE strain (100% infected and 9/17 with a disseminated infection). In contrast, *Culex (Melanoconion) ocossa* was equally susceptible to both IAB (91%) and IE (84%) strains of VEE virus. Susceptibilities of other *Culex (Melanoconion)* spp. will also be presented. Although *Psorophora columbiae* was susceptible to infection with the IAB and IE strains (infection rates >55%), this species did not transmit either strain of VEE virus by bite (0/12 disseminated, refeeding mosquitoes).

82 PROTECTION OF MICE AGAINST AEROSOL CHALLENGE WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS BY ALPHAVIRUS SPECIFIC MONOCLONAL ANTIBODIES. Pratt WD, and Ludwig GV*. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

The Venezuelan equine encephalitis complex viruses (VEE) have a longhistory of enzootic and epidemic activity throughout the Americas. Development of anti-VEE monoclonal antibodies (MAbs) has been important in defining glycoprotein epitopes which are important for induction of protective immune responses following infection. It is not known, however, if protection from aerosol infection can be afforded via animmune response against the same epitopes. This study tested the ability of a panel of anti-alphavirus MAbs to protect against parenteral and aerosol challenge with virulent VEE. For this study, 10^6 antibody secreting cells from each hybridoma tested were inoculated subcutaneously (SC) into 20 Balb/c mice. Mice were held 7 days when groups of 10 mice were challenged SC with 10^4 PFUs of VEE, Trinidad donkey strain. Matchedgroups were challenged with 10^4 PFUs delivered via the aerosol route. For most antibodies tested, protection was similar regardless of the route of challenge. However, three MAbs were found to protect better against a parenteral than an aerosol challenge. In cases where two different MAbs directed against the same epitope were available, IgG2a antibodies protected better than any other isotype regardless of the route of challenge. Taken together, these data suggest that both the type and specificity of an antibody response against VEE is important for protection against aerosol infection.

83 EFFECT OF GAMMA IRRADIATION ON VENEZUELAN EQUINE ENCEPHALITIS VIRUS ENVELOPE PROTEIN EPITOPES. Lind CM, Kondig JP*, Shoemaker MO, Smith JF, and Ludwig GV. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

Venezuelan equine encephalitis virus (VEE) is a New-World alphavirus,responsible for epidemic disease in the Americas in recent decades. A new inactivated VEE vaccine in PLGA microspheres is currently under development. Use of this vaccine will require that virus be completely inactivated yet maintain adequate immunogenicity. Two forms of virus inactivation, formalin treatment and gamma irradiation, were tested in these studies. Groups of mice were immunized with microencapsulated or saline suspended virus which had been exposed to ^{60}Cobalt and/or treatedwith formalin. Mice receiving microencapsulated preparations were incompletely protected from challenge unless the virus had been pretreatedwith formalin, suggesting that formalin stabilizes virus epitopes during microencapsulation. Mice immunized with irradiated virus failed to mount a pre-boost neutralizing response, suggesting that such treatment negatively affected VEE neutralization epitopes. To test this hypothesis, VEE virus, diluted in four different solutions, was gamma irradiated and tested by ELISA against a

panel of anti-VEE monoclonal antibodies. Results showed that gamma irradiation negatively affected all VEE epitopes tested but that such an effect could be reduced as a function of the solution in which virus was irradiated. Taken together these data suggest that gamma inactivation must be carefully optimized during VEE vaccine development.

84 IMMUNOMAGNETIC SEPARATION AS A SIMPLIFIED PROCEDURE FOR PROCESSING VENEZUELAN EQUINE ENCEPHALITIS VIRUS SAMPLES FOR IDENTIFICATION BY RT-PCR. Knauert FK*, Parrish BA, Ibrahim SI, Johnson DE, Craw PD, and Lofts RS. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

We developed an immunomagnetic separation procedure as a simplified, alternative method for preparing Venezuelan equine encephalitis (VEE) virus samples for identification by using the reverse transcription - polymerase chain reaction (RT-PCR) procedure. Magnetic bead - immune capture complexes (MB-ICC) with anti-VEE monoclonal antibody attached through an anti-mouse IgG antibody intermediate were used as a solid support for adsorbing viral particles from the sample. Sample contaminants were removed in subsequent washes, and the virus was concentrated by resuspending the collected magnetic beads into a 10-fold reduced volume. Viral RNA was released from the captured particles by proteinase K digestion and amplified by using the RT-PCR process. We identified conditions for preparing the MB-ICC and performing the proteinase K digestion release step that resulted in the lowest detection limits with minimum false positive results. The process was four to eight times more sensitive than the guanidine isothiocyanate (GITC) extraction procedure, our laboratory standard. It eliminated the use of hazardous material commonly used to extract and purify RNA, and lends itself to automation which will minimize labor intense steps in current procedures. This simplified procedure has the potential of making the RT-PCR procedure more suitable for routine use in a diagnostic laboratory.

85 BIOCHEMICAL CHARACTERIZATION OF AN ANTIGEN LOCALIZED TO DISCOID BODIES IN THE PARASITIC TREMATODE *SCHISTOSOMA MANSONI*. Hoffmann KF*, Lewis SA, and Strand M. The Johns Hopkins University, School of Medicine, Baltimore, MD.

We report the biochemical characterization of an antigen localized to the discoid bodies in the parasitic trematode, *Schistosoma mansoni*. This antigen was originally identified by its reactivity with a monoclonal antibody (mAb), designated mAb709. By immunofluorescent microscopy, the antigen was exclusively localized to the tegument and by immunogold electron microscopy the staining was shown to be specifically associated with discoid bodies. Immunofluorescent studies also indicated that the expression of the antigen recognized by mAb 709 was developmentally regulated: the antigen was not detected in cercaria, but was present in 3 hour, lung stage, and adult worms. Mab 709 immunoprecipitated two bands of approximately 6- and 9kDa from adult male worms metabolically labeled with ^{35}S-methionine. Phosphorylation studies have shown that neither of these two bands are phosphorylated in adult male worms. However, both bands contain a glycosylphosphatidyl inositol (GPI) anchor. This was determined by immunoprecipitation of adult worm proteins metabolically labeled with either ^{3}H-myristate or ^{35}S-methionine followed by Triton X-114 phase separation. Both the localization and developmental stages of expression suggest that this antigen may be a useful immunoprophylactic candidate. As a first step in addressing this question, a passive transfer experiment was performed in C57/bl 6 mice using mAb 709. A 69% reduction in worm burden was observed in mice receiving mAb 709 as compared to mice receiving only PBS. This experiment is currently being repeated. Molecular characterization of the target antigen for mAb 709 is ongoing.

86 ASPARAGINYL ENDOPEPTIDASE ACTIVITY IN ADULT *SCHISTOSOMA MANSONI*. Dalton JP, and Brindley PJ*. School of Biological Sciences, Dublin City University, Dublin, Ireland; and Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

Previous immunoblot analysis has shown that *Schistosoma mansoni* proteins Sm31 and Sm32, while minor components in tissue extracts, are highly immunogenic in infected humans. Both Sm31 and Sm32 have independently been ascribed roles as hemoglobinases but whether either enzyme is actually involved in digesting hemoglobin (Hb) is controversial. We (and others) have recently shown by sequence comparisons that Sm32 is an asparaginyl endopeptidase, a member of a novel family of cysteine proteinases of which legumains from leguminous plants are the best characterized. By synthesizing and employing fluorogenic peptide substrates for the specific detection of asparaginyl endopeptidases, we have characterized this type of activity in extracts of adult S. mansoni. The schistosome activity is similar to that of the legumain of seeds from the jack bean *Canavalia ensiformis*, but differs in its pH and temperature optimum for sensitivity. In contrast to the legumains, the schistosome asparaginyl endopeptidase activity was not activated by the reducing agent dithiothreitol. Rather than being involved directly in the cleavage of Hb, Sm32 may function in the posttranslational modification processes that regulate the activity of other molecules, including proteinases, as suggested for legumains.

87 PUTATIVE MOLECULAR IDENTIFICATION OF ANDROGEN RECEPTOR GENE(S) IN *SCHISTOSOMA MANSONI*. Fantappie MF*, Sluder AE, Colley DG, and Secor WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; and Department of Zoology, University of Georgia, Athens, GA.

Based on preliminary investigations, it appears that host testosterone can affect the cercarial-to-adult development of *Schistosoma mansoni*, and that exogenous testosterone has a direct negative effect on the parasite, rather than a potentiating effect on the anti-worm protective mechanisms of the host. If schistosomes are directly affected by physiologic levels of testosterone, they should express receptors for this hormone. Human androgen receptor (hAR) cDNA was used as a probe in Southern and Northern blots, as well as to screen an adult worm cDNA library. Digestion of schistosome genomic DNA with a cadre of restriction enzymes revealed a single banding pattern upon hAR probing, suggesting the occurence of a single copy gene. Northern blots with total RNA from schistosomula, adult male, and adult female worms showed identical banding patterns to male mouse total RNA for schistosomula and adult female worms, but no there was no apparent hybridization of the hAR to the adult male worm RNA. Screening of an adult worm cDNA library revealed two distinct populations of clones which were bound by the hAR. Cloning and sequencingof these inserts is ongoing. These data are consistent with a hypothesis that we have identified the gene for a homologue of mammalian androgen receptors in the genome of *S. mansoni*, and that this gene is transcribedin immature and female worms, but not by adult male worms.

88 CLONING AND CHARACTERIZATION OF A CALPONIN HOMOLOGUE FROM *SCHISTOSOMA MANSONI*. Karim AM*, Shalaby KA, and LoVerde PT. Department of Biochemistry, Ain Shams University, Cairo, Egypt; and Department of Microbiology, State University of New York, Buffalo, NY.

Calponin is a vertebrate smooth muscle protein thought to function as a homologue for troponin in regulating contractile activity. We recently identified a protein of 357 amino acids in *Schistosoma mansoni* which shares 36% identity to chicken calponin. Comparison between the schistosome sequence and that of the chicken reveals similar tandem repeats at the carboxy terminus. Sequences within the repeats are highly conserved across species. The schistosome protein was identified by immunoscreening a cercarial lambda gt11 cDNA library using antibodies from patients with chronic *S. mansoni* infection. A cDNA insert encoding the 296 carboxy terminal amino acids was cloned into a pGEX vector and expressed in *E. coli*. The purified protein was used to immunize rabbits. Antibodies identified bands of ~40 kDa in Western blots of 3˜hour schistosomula and adult worm extracts. By hybridization a cDNA clone encoding the entire sequence of the schistosome calponin homologue was isolated. This will be used in studies on the interaction with other muscle proteins and in assessing the molecule's vaccine potential.

89 EXPRESSION AND CHARACTERIZATION OF GLUTATHIONE PEROXIDASE ACTIVITY IN *SCHISTOSOMA MANSONI*. Mei H, Thakur AN, and LoVerde PT*. Department of Microbiology, State University of New York, Buffalo, NY.

Antioxidants may play an important role in immune evasion by schistosome parasites. In the present study glutathione peroxidase (GPX) activity was measured in different fractions of worm extracts from several developmental stages of *Schistosoma mansoni*. The enzyme activity was shown to be developmentally regulated with higher specific activities in the tegument-enriched NP-40 extract of adult worms (the stage least susceptible to immune killing) compared to the larval stages (which are most susceptible to immune elimination). In all extracts tested, the activity against cumene hydroperoxide, even when glutathione S-transferase (GST) activity was removed, was higher than that for hydrogen peroxide. The expression of GPX cDNA in pGEX-2T in bacteria produced a 50˜kD fusion protein and a 32˜kD truncated protein. The latter presumably due to termination at the internal TGA codon that codes for selenylcysteine. GPX activity was detected in the recombinantly produced GPX but not with Sj26-GST from the vector. Mutating the TGA codon to TGT produced a full-length product, GPXm (19˜kD). Anti-GPXm monoclonal antibodies recognized a 19˜kD molecule in adult worm extracts which upon removal by immunoprecipitation resulted in the loss of over 90% of the GPX activity, suggesting a single form of GPX exists in the schistosome. In immunofluorescence studies GPX was localized to the tegument and gut epithelium of adult worms and barely detectable in 3h schistosomula.

90 TREMATODES DIFFER IN THEIR SUSCEPTIBILITY TO FREE RADICAL KILLING *IN VITRO*: COMPARISON OF LARVAE OF *SCHISTOSOMA MANSONI* AND *FASCIOLA HEPATICA*. Piedrafita DM*, Brindley PJ, Dalton JP, Spithill TW, Sandeman M, Wood P, and Parsons JC. Victorian Institute of Animal Science, Victoria, Australia; Queensland Institute of Medical Research, Queensland, Australia; Dublin City

University, Dublin, Ireland; La Trobe University, Victoria, Australia; and CSIRO Division of Animal Health, Victoria, Australia.

Our previous experiments have suggested that newly excysted juvenile *Fasciola hepatica* (NEJ) are resistant to free radical killing *in vitro*. In contrast, schistosomula of the related trematode, *Schistosoma mansoni*, appear to be highly susceptible to killing by either chemically or cell-generated reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). Therefore, in order to confirm the relative resistance of NEJ to the actions of free radicals, we compared the susceptibility of NEJ and schistosomula to killing by ROI and RNI. When exposed to high levels of chemically-generated ROI, >90% of schistosomula were killed; in comparison, only 21% of NEJ were killed. When NEJ were incubated for 72 hours with LPS-stimulated peritoneal lavage cells (PLCs) of naive rats, the level of parasite mortality was 5%; however, 79% of schistosomula were killed when incubated with stimulated cells from the same rat. Co-culture of NEJ, schistosomula and LPS-stimulated PLCs, resulted in killing of 7% of NEJ and 87% of schistosomula. No increase in parasite mortality was observed following extended culture of NEJ with stimulated PLCs for 5 days. The schistosomulocidal activity of rat PLCs was inhibited by 0.5 mM L-NMMA and 50 mM $FeSO_4$ and suggests killing was mediated by RNI. From this study we conclude that, in comparison to schistosomula of *S. mansoni*, NEJ of *F. hepatica* are highly resistant to free radical killing by reactive oxygen/nitrogen intermediates *in vitro*. Potential mechanisms of resistance to free radical killing in NEJ, such as a glycocalyx and the level of free radical scavenger enzymes, are currently under investigation.

91 EXPRESSION OF LEWIS X BY SCHISTOSOMES AND AUTOIMMUNITY TO THIS DETERMINANT IN SCHISTOSOMIASIS. Nyame AK*, Pilcher JB, Tsang VC, and Cummings RD. Oklahoma University Health Sciences Center, Department Biochemistry & Molecular Biology, Oklahoma City, OK; and Centers for Disease Control and Prevention, Division of Parasitic Disisease Immunology Branch, Atlanta, GA.

We reported recently that *Schistosoma mansoni* synthesize glycoproteins containing Lewis x (Le^x) antigen (Galβ1,4[Fucα1,3]GlcNAcβ-R). We now report that Le^x occurs also on *Schistosoma japonicum* and *S. hematobium*. Sera from *S. mansoni* infected humans, primates and rodents contain both IgM and IgG antibodies to Le^x. Surprisingly, these sera can mediate specific complement-dependent cytolysis of human leukocytes which bear surface Le^x determinants. The major cytolytic activity toward leukocytes in the sera from infected animals is due to the anti-Le^x antibodies. These results demonstrate that schistosomes synthesize Le^x and that *S. mansoni* infection is accompanied by an autoimmune disorder based on reactivity to Le^x.

92 IL-12 BOOSTS CELL-MEDIATED AND HUMORAL IMMUNE RESPONSES IN MICE VACCINATED WITH ATTENUATED CERCARIAE OF *SCHISTOSOMA MANSONI*. Wynn TA*, Reynolds A, Fouad S, James S, Hieny S, Jankovic D, Strand M, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, MD.

The production of Th1-type cytokines is associated with strong cell-mediated immunity while Th2-type cytokines are typically involved in the generation of humoral immune responses. In mice vaccinated a single time (1X) with attenuated cercariae of *Schistosoma mansoni*, the immunity induced is higly dependent on IFN-γ. In contrast, mice vaccinated multiple times (3X) have decreased IFN-γ expression and develop a dominant Th2-type cytokine response. Additionally, the immunity induced by 3X immunization can be passively transferred to naive recipients with serum. Previously, we demonstrated the ability of IL-12, a potent IFN-γ-inducing cytokine, to enhance (1X) schistosome immunity when administered during the period of immunization. In the present study, we asked whether administering IL-12 during each vaccination of 3X-immunized mice could maintain the dominant Th1-type responses seen previously in 1X/IL-12-vaccinated mice and whether serum from these animals could transfer protection. 3X-vaccinated mice demonstrated an average 77% reduction in parasite burden while 3X/IL-12 treated mice displayed a significantly greater 94% ($p<.005$) reduction in challenge infection. Spleen and lymph node cells from 3X mice produced predominantly Th2-type cytokines while the 3X/IL-12 immunized animals secreted high levels of IFN-γ upon re-stimulation *in vitro* with parasite antigen (SWAP). Serum obtained from both groups at 2 to 3 weeks post-challenge contained high titers of SWAP-specific IgG_1 antibodies. More importantly, 3X/IL-12 serum, when transferred to naive mice on day 3 post-challenge, reduced worm burdens by over 60% while 3X serum transferred significantly ($p<.05$) less protection (47%). Together, these data demonstrate that IL-12 can be used as an adjuvant to boost IFN-γ, cell-mediated immunity while maintaining or even enhancing humoral immune responses.

93 IMMUNITY IN HUMAN URINARY SCHISTOSOMIASIS: ASSOCIATION OF CELLULAR RESPONSES WITH AGE AND INTENSITY OF INFECTION. King CL*, Malhotra I, Koech D, Wamachi A, Kioko J, Mungi P, and Ouma JH. Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH; and Kenyan Institute of Medical Research and Division Vector Borne Diseases, Nairobi, Kenya.

Previous studies have shown an age-dependent resistance to infection both before and after curative chemotherapy in humans schistosomiasis. To examine the relationship between cell-mediated responses and acquired immunity, peripheral blood lymphocytes (PBMC) were collected from 301 individuals both before and 6 months after chemotherapy in a population endemic for *Schistosoma haematobium* in Kenya. Lymphocyte cultures were stimulated with parasite (PAg) and non-parasite antigens, subsequently enriched for CD4+ cells, and examined for cytokine production. Positively selected CD4+ cells released significant quantities of cytokines, especially IL-4 and IL-10. PAg-, but not PPD-driven, IFN-γ production inversely correlated with IL-4 and IL-10 release by both PBMC and CD4+ cells suggesting cross-regulation of their production. Among individuals 8-14 yrs, PAg-driven IL-4 and IL-5 production were 4- and 3.5- fold higher respectively with heavy infection (>1000 ova/10ml urine) compared with lightly infected subjects (1-10 ova/10ml urine; $p<0.04$). IFN-γ levels were statistically equivalent between the 2 groups (mean=514±385 vs 189±70 pg/ml, $p=0.3$). Older individuals (>20 years; putatively resistant) compared to children (8-11 years; susceptible), matched for intensity of infection, tended to have diminished Ag-driven IL-4, IL-5 and IFN-γ production. The relationship between cellular immunity and re-infection are currently being examined. These data suggest that generation of a Th2 pattern of cellular immunity may not always correlate with acquired immunity although the confounding effects of age and exposure need to be fully examined.

94 INTERLEUKIN-10 REGULATES *IN VIVO* T-CELL REACTIVITY IN HUMAN URINARY SCHISTOSOMIASIS. Malhotra IJ*, Medhat A, Nafeh M, Shata A, Helmy A, Khoudary J, and King CL. Divison of Tropical Medicine, Case Western Reserve University; Departments of Medicine and Microbiology, Assiut Egypt.

IL-10 contributes to parasite Ag (PAg) specific anergy observed in individuals with chronic helminth infections. This hypothesis derives from findings that endogenously produced IL-10 inhibits PAg-induced IFN-γ release and T cell proliferation I in vitro I by peripheral blood lymphocytes (PBL) from helminth infected individuals. However, these observations are based on *in vitro* cultures conditions and may not reflect an immunomodulatory role for IL-10 *in vivo*. To address this question, both spontaneous and PAg-driven IL-10 and IFN-γ production were examined by PBMC, CD4+ cells and monocytes before and 2 weeks after praziquantel therapy in 22 Egyptians infected with *Schistosoma haematobium*. Spontaneous as well as PAg-driven IL-10 release increased by 2.2-, 7- and 2- fold from PBL, CD4+ cells and monocytes respectively after chemotherapy relative to pretreatment levels ($p<0.01$). PAg-driven IL-4 also increased by 3.5-fold after treatment ($p<0.01$). In contrast, net PAg-driven IFN-γ production decreased by 8.5-fold post-treatment. No significant difference in PAg-driven IL-5 or PPD-driven IFN-γ production was observed with therapy. These data indicate that Ag challenge in vivo induced by parasite death with chemotherapy generated enhanced endogenous IL-10 and IL-4 production and diminished IFN-γ release and support the concept that IL-10 has an immunomodulatory role *in vivo* in patients with helminth infections.

95 MODELLING OF MULTIVALENT DNA VACCINES WHICH PROTECT AGAINST MALARIA IN THE *PLASMODIUM YOELII* SYSTEM. Doolan DL*, Hedstrom RC, Charoenvit Y, Rogers WO, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD.

PyHEP17 is localized on the parasitophorous vacuole membrane of *Plasmodium yoelii*-infected hepatocytes and erythrocytes. After determining that the genomic sequence of PyHEP17 is characterized by intergenic noncoding regions, oligo d(T) primed first-strand cDNA was prepared from total parasite RNA in order to obtain the PyHEP17 cDNA. Reverse transcription polymerase chain reaction amplification was performed using oligonucleotide primers predicted from the genomic DNA sequence. The derived sequence was consistent with that of a PyHEP17 cDNA clone subsequently isolated from a *P. yoelii* cDNA library. Amplified cDNA fragments were cloned into a mammalian expression vector. *In vitro* expression of the antigen in this construct was confirmed by IFAT on transfected COS cells. Immunization of mice with plasmid DNA encoding partial PyHEP17 induced genetically restricted, CD8+ T cell dependent protective immunity to malaria (71%, H-2^a; 54%, H-2^k; 26%, H-2^d; 17%, H-2^q, 40%, outbred mice). Furthermore, immunization with a combination of plasmid DNAs encoding PyHEP17 and the *P. yoelii* circumsporozoite protein circumvented the genetic restriction of protective immunity to the individual antigens. We have identified the gene encoding the *P. falciparum* homolog of PyHEP17, by structural and amino acid sequence identity and *in vitro* reactivity. Since genes encoding other pre-erythrocytic stage *P. falciparum* antigens such as the circumsporozoite protein and sporozoite surface protein 2 are available, our data provide the foundation for studies of multivalent pre-erythrocytic malaria DNA vaccines in humans.

96 PROTECTIVE IMMUNITY AFTER IMMUNIZATION WITH A PyCSP DNA VACCINE: OPTIMIZATION OF DOSAGE REGIMEN AND CHARACTERIZATION OF GENETIC RESTRICTION. Sedegah M*, Hedstrom RC, Margalith M, Hobart P, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Vical Incorporated, San Diego, CA.

We have reported on the efficacy of a *Plasmodium yoelii* circumsporozoite (PyCSP) DNA vaccine in BALB/c mice as a model for the development of a malaria vaccine for human use. Immunization with 3 intramuscular (IM) doses of 200 μg of the PyCSP vaccine induced high levels of antibodies and CTL, and protection of up to 85% of BALB/c (H2d) mice by a CD8+ T cell dependent immune mechanism. We now report that: 1) 3 doses of 10 μg PyCSP DNA gives comparable protection; 2) as little as 3 doses of 0.63 μg PyCSP DNA provides modest protection; 3) one dose of 100 μg PyCSP DNA protected 55% of recipients; 4) divided administration in 2 sites is preferable to administration of all of the DNA in one site (92% vs 50% protection); 5) administration at 6 week intervals apparently provides better protection than administration at 3 week intervals; and 6) IM administration is much more effective than intradermal immunization (82% vs 14% protection). Unfortunately protection induced by this vaccine is genetically restricted; A/J (H-2a), C57BL/6 (H-2b), and B10. BR (H-2k) are poorly or not protected at all. Work is now in progress to construct multi-gene DNA vaccines that will protect recipients of all MHC backgrounds.

97 DNA VACCINES AGAINST MALARIA BASED ON THE SSP2 ANTIGEN. Hedstrom RC*, Sedegah M, Wang H, Kaur M, Hobart P, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Vical Incorporated, San Diego, CA.

We recently reported that *Plasmodium yoelii* CSP DNA vaccines induced specific antibodies and nearly complete CD8+ CTL-dependent protection against sporozoite challenge in BALB/c mice. Preliminary studies have now shown that a full-length *P. yoelii* SSP2 (PySSP2) DNA vaccine protected approximately 50% of A/J mice. In an effort to improve upon the results with PySSP2, we constructed two DNA vaccine plasmids both of which contained a truncated PySSP2 gene lacking the 5' terminal region of the gene thought to encode the native signal peptide: one contained an in-frame fusion with a mammalian (tPA) signal peptide while the other one remained signal minus. The ability of both plasmids to express PySSP2 *in vitro* was demonstrated by immunoblot analysis of the cell pellet and culture supernatant material from transiently transfected COS-7 cells. An immunoreactive band of an expected molecular mass was detected with anti-PySSP2 Mab (NYS4) in both fractions from cells transfected with the tPA-PySSP2 plasmid but only in the cell pellet material of the COS-7 cell cultures transfected with the signal minus plasmid. The ability of these plasmids to induce protective immunity is being evaluated in multiple strains of mice. So far, both BALB/c and A/J mice have produced antibodies after a single immunization with the tPA-PySSP2 DNA vaccine as determined by IFAT; the immunoreactive pattern on sporozoites was similar in appearance to that obtained with NYS4. Mice immunized with the signal minus construct have not yet produced measurable antibodies to sporozoites. Initial protection studies and the results of the ongoing immunogenicity and protection studies will be presented.

98 PROTECTIVE EFFICACY AGAINST SPOROZOITE CHALLENGE WITH A COMBINATION SPOROZOITE AND ERYTHROCYTIC STAGE MALARIA VACCINE. Charoenvit Y*, Wang R, Daly TM, Long CA, Corradin GP, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Department of Microbiology and Immunology, Hahnemann University, Philadelphia, PA; and Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland.

Recent reports indicated that immunization of mice with GST-PYC1, a recombinant protein based on the *Plasmodium yoelii* MSP-1 protects against challenge with infected erythrocytes, and immunization of mice with MAP4 (QGPGAP)4 P2P30, a multiple antigen peptide based on the *P. yoelii* CS protein protects against sporozoite challenge. The current studies were designed to determine if: (1) GST-PYC2 (a modified GST PYC1) protects against sporozoite challenge, and (2) if immunization with GST-PYC2 plus MAP4 (QGPGAP)4 P2P30 would provide additive or synergistic protection. Groups of 10 mice were immunized with individual or combined vaccine emulsified in TiterMax adjuvant and challenged with *P. yoelii* sporozoites. One of 10 mice immunized with GST-PYC2 was completely protected against sporozoite challenge. The remaining nine mice had significantly lower levels of parasitemia on the last day of follow up (day 14) than did control mice ($p<0.01$). Immunization with the combined vaccine reduced by 50% the level of parasite specific antibodies as compared to that induced by the individual components. Nonetheless, 50% of the mice immunized with the combined vaccine, 40% of the mice immunized with MAP4 (QGPGAP)4 P2P30 vaccine, and 10% of the mice immunized with the GST-PYC2 vaccine were fully protected. This modest increase in protection in the combination group was not statistically significant, but may have been a reflection of additive activity of antibodies against each immunogen. These studies provide a foundation and direction for work designed to optimize the construction, delivery, and assessment of multi-component malaria vaccines.

99 OPTIMIZATION OF IMMUNE RESPONSES TO A *PLASMODIUM* DNA VACCINE IN *AOTUS* MONKEYS. Gramzinski RA*, Maris DC, Obaldia N, Rossan R, Sedegah M, Wang B, Hobart P, Margalith M, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and ProMed/Gorgas Memorial Laboratory, Panama City, Panama; and Vical Incorporated, San Diego, CA.

We have demonstrated that intramuscular (IM) immunization with a plasmid DNA vaccine encoding the *Plasmodium yoelii* CSP protein (PyCSP) induces high antibody levels and cytotoxic T- cells against PyCSP, and induces a CD8+ T-cell dependent protection in 85% of BALB/c mice. In preparation for development of DNA vaccines designed to produce protective antibodies against *P. falciparum* erythrocytic stage antigens, we conducted studies to optimize antibody responses in *Aotus* monkeys after immunization with the PyCSP DNA vaccine. Groups of 3 monkeys were immunized at 3 week intervals with 125, 500, or 2000 μg PyCSP DNA. IM alone (tibialis anterior), IM after pre-treatment with 0.25% bupivacaine, and intradermal (ID) routes were compared. Only monkeys immunized by the ID route produced antibodies against sporozoites. Antibody titers, obtained by indirect fluorescent antibody test (IFAT) against air-dried sporozoites and by ELISA using a PyCSP recombinant protein, peaked at 3 weeks after the third ID dose and were reduced 50% by 7 weeks after the third dose. There was a trend toward a dose response with peak geometric mean IFAT titers of 806, 508, and 403 for the 2000, 500, and 125 μg doses respectively. In a second experiment antibody responses to a 500 μg dose at multiple sites (2 or 6) were found superior to responses generated by a single site administration.These data clearly demostrate that ID immunization generates significantly better antibody responses to the PyCSP DNA vaccine than IM immunization, and support the use of the ID route for the initial studies of the efficacy of DNA vaccines in inducing protective antibodies against *P. falciparum* erythrocytic stage antigens.

100 MALARIA VACCINE TRIALS AGAINST PREERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM* IN CHIMPANZEES: 1) EFFECT ON PARASITEMIA ELICITED BY FOUR VACCINE CANDIDATES. Millet P*, Luty A, Dubreuil G, LeRoy E, Tartar A, Eling WM, Georges AJ, and Druilhe P. Centre International de Recherche Médicale de Franceville (CIRMF), Franceville, Gabon; Institut Pasteur, Roux, Paris, France; and University Hospital Nijmegen, Department of Parasitology, Nijmegen, The Netherlands.

We report here the effect on blood stage development of the vaccination of 5 non-splenectomized chimpanzees with 4 *Plasmodium falciparum* liver stage proteins: Liver Stage Antigen 1 (LSA-1) and 3 (LSA-3), Sporozoite Threonine and Asparagin Rich Protein (STARP), and Sporozoite And Liver Stage Antigen (SALSA). LSA-1 is specific to liver stages while LSA-3, SALSA, and STARP are also expressed by the sporozoite. To activate multiple immune mechanisms using the same antigen, 3 types of immunization per chimpanzee were used: immunization by lipopeptides only, by peptides associated with the adjuvent Moutaulde ISA 31 (SEPIC, Paris France), and by recombinant proteins associated with the some adjuvant. One chimpanzee per vaccine candidate was used; a control animal was immunized with adjuvant only. After the course of vaccination each chimpanzee received a dose containing 120,000 sporozoites intravenously at day 0. The control animal developed a parasitemia of 270 parasites/μl at day 7. Parasitemia decreased rapidly to 15 parasites/μl the next day, and no parasites were detectable at day 10. The 2 chimpanzes vaccinated with LSA-1 and SALSA developed identical parasitemias (270 parasites/μl at day 7). Interestingly, the chimpanzee vaccinated with STARP developed a 3 fold greater parasitemia at day 7. In marked contrast, the chimpanzee vaccinated with products from the protein LSA-3 developed a 3 times lower parasitemia than the non-protected animals at day 7 (80 parasites/μl). According to the time of maturation of liver stage of *P. falciparum* (minimum of 6 days), parasitemia observed 7 days after sporozoite inoculation is directly linked with merozoites from the hepatic stage, and not from blood-stage multiplication. Thus, the decreased parasitemia observed for the LSA-3 vaccinated animal could be linked with a protective immune mechanism. Although this experiment does not allow to prove vaccine efficacy, interest of such research using limited a number of Apes will be discussed with reference to these observation.

101 COMPARTMENTALIZED T LYMPHOCYTE RESPONSES IN *MACACA MULATTA* TO THE MULTIANTIGEN MALARIA VACCINE CANDIDATE NYVAC-PF7. Stewart A*, Tongtawe P, Ngampochjana M, Lanar D, Tine JA, Krzych U, Ballou WR, and Heppner DG. Department of Immunology and Parasitology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Virogenetics Corporation, Troy, NY; and Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

Obstacles to effective vaccine-induced immunity against falciparum malaria include antigenic variability in parasite strains and genetic restriction of the host response to particular antigens. NYVAC-Pf7, a highly attenuated vaccinia virus strain into which the genes for seven different *Plasmodium falciparum* antigens from multiple life-cycle

stages, (CSP, SSP2, LSA-1, MSA-1, AMA-1, SERA, and Pfs25) have been inserted, has been constructed in an attempt to overcome these obstacles. Because of the viral nature of the vector, intracellular expression of *P. falciparum* genes is expected, with subsequent presentation of antigenic epitopes in the context of Class I surface molecules of NYVAC-Pf7 infected cells. Evidence from murine malaria models suggests that cytotoxic T lymphocyte (CTL) responses are a prominent part of the development of immunity to malaria, and NYVAC-based recombinants have conferred CD8+ CTL mediated protection in an experimental murine sporozoite challenge model. Our study is designed to examine the CD8+ CTL-inducing potential of NYVAC-Pf7 in a primate model, where samples can be obtained from the important splenic and hepatic compartments. NYVAC-Pf7 is safe and well tolerated when inoculated into rhesus monkeys, and the ensuing development of antibody responses to all stages of the parasite has been described. Currently, fourteen monkeys are receiving either 108 PFU of NYVAC-Pf7, the unmodified NYVAC vector, or sham inoculations on a 0, 1 and 4 month schedule. Lymphocytes are being harvested before, during, and after the vaccination schedule from sequential splenic and hepatic biopsies as well as from the blood. Immunophenotyping of these lymphocytes with flow cytometry has revealed a profound compartmentalization of the baseline and postinocuation distribution of T cell subsets within the spleen, liver, and peripheral blood of rhesus monkeys.

102 VACCINATION WITH LIVE NYVAC-PF7 CAN PRIME THE IMMUNE SYSTEM TO DEVELOP AN AMNESTIC RESPONSE TO A BOOST WITH ADJUVENTED PROTEIN. Lanar DE*, Tine JA, Wellde BT, Kaslow DC, Sadoff JC, Paoletti E, and Ballou WR. Department Immunology, WRAIR, Washington DC; Virogenetics Corp, Troy, NY; and NIAID, NIH, Bethesda, MD.

NYVAC-Pf7 is a multistage, multiantigen live vaccinia virus based vaccine containing the *Plasmodium falciparum* CSP, SSP2, LSA1(repeatless), AMA1, MSA1, SERA and Pfs25 genes under control of vaccinia viral promoters. The malaria proteins are produced by the virus once it infects a cell. In safety and immunogenecity tests rabbits and Rhesus monkeys received three doses of $1x10^7$ or $1x10^8$ pfu. Antibody (Ab)titers were low to moderate for most antigens. However, vaccinia virus based vaccines normally induce better cellular immunity, particularly CTL responses, than humoral responses. Therefore, 6 to 12 months after the last viral vaccine dose the animals were given injections of selected adjuvented protein vaccines derived from some of the proteins in the NYVAC-Pf7 array in order to study the ability of the live NYVAC-Pf7 to induce a priming B-cell immune response that could be boosted with a formulated complementary polypeptide vaccine. Pfs25 conjugated to alum induced an animistic response resulting in a >10 fold increases in Ab titers over those seen with NYVAC-PF7 alone. These sera had the ability to greatly reduce or completely block transmission of malaria to mosquitos when mixed with blood containing gametocytes in a standard transmission blocking assay. Animals that had received NYVAC parental virus or saline in the primary vaccination regime did not show any boosting of antigen specific antibody responses. Adjuvanted polypeptides from CSP and MSA1 are also being test for their ability to induce high titer boosted responses. The results of these studies may show the efficacy of a regime of priming with a NYVAC based vaccine and boosting with a complementary adjuvented polypeptide to stimulate immunologic memory.

103 INDUCTION OF MULTI-SPECIES IMMUNITY TO RODENT MALARIA USING MULTPLE EPITOPE TREES. Reed RC*, Lousis-Wileman V, Fang HS, Jue D, Wohlhueter R, Hunter RL, Lal AA. Department of Pathology, Emory University; and Biotechnology Core Facility Branch and Immunology Branch, Division of Parasitic Diseases, CDC, Atlanta, GA.

We prepared multiple epitope trees (TREEs) with B-epitopes from *Plasmodium berghei*, (PPPPNPND) 2, and*Plasmodium yoelii*, (QGPGAP) 3QG, circumsporozoite protein (CS). TREE molecules were synthesized containing one species of B-epitope (single-species) or two different species of B-epitopes (multi-species), both containing block copolymer (P1005) and detoxified LPS (RaLPS). Results demonstrated that single-species TREEs in adjuvant alone induced the highest level of protection (21/22) in mice immunized with either*Plasmodium bergheior Plasmodium yoelii*REEs. Three immunizations with both single-species TREEs, together in adjuvant, injected on the same day at the same site, on the same day at different sites, and on different days at different sites, induced protective antiboldies against *P. bergheibut notP. yoelii*parasite challenge. Multi-species TREEs induced immune responses to both B-epitopes. Therefore, it can be concluded that single and multi-species TREEs are capable of inducing strong and protective immune responses to their respective species of malaria.

104 IMMUNOGENICITY OF DI-EPITOPE TREES, SYNTHETIC PEPTIDE VACCINES CONTAINING REPEATS OF CIRCUMSPOROZOITE PROTEIN OF TWO DIFFERENT PLASMODIAL SPECIES. Lal A*, Saekhou AM, Fang S, Jue D, Wohlhueter R, and Udhayakumar V. Division of Parasitic Diseases, Scientific Resourse Program, Biotechnology Core Facility, NCID, CDC, Atlanta, GA.

Unlike multiple antigen systems, which typically carry the same peptide sequences in different arms, CDC-TREES are designed to carry different peptide sequences in alternate arms. As an experimental vaccine, we developed three CDC-TREES with four arms. Two arms in all the three TREES contained the antigenic repeats from the circumsporozoite (CS) protein of *P. vivax*-like parasite as T-helper epitope. The alternate arms contained epitopes from CS protein repeat sequences of either *P. vivax*type-1 (PL359), *P. vivax* type-2 (PL360), or *P. falciparum*(PL361). Mice of four genetic background (H-2a, H-2b, H-2d, and H-2k) were immunized with these TREES in Freund's adjuvant. Except H-2d mice, all other strains of mice developed high titers of IgG antibodies specific to *P. vivax*-like CS repeat peptides. H-2b mice, which is a non-responder to *P. vivax*-type 1 CS repeats, developed antibodies to this repeat following immunization with PL359 TREES. Similarly, H-2a and H2-k mice which are non-responders to NANP repeats, elicited NANP specific antibodies following immunizationwith PL361. PL360 induced moderate levels of antibodies to *P. vivax* type-2 repeat in H-2a mice. Thus, it is evident that: 1) synthetic peptides in TREE configuration are highly immunogenic; 2) helper epitopes from one arm can provide cognate help to B cell epitopes in another arm; and 3) genetic restriction can be overcome in some strains.

105 CD4+ T CELL DEPENDENT STERILE PROTECTION AGAINST MALARIA AFTER VACCINATION WITH PySSP2 PEPTIDE. Wang R*, Charoenvit Y, Corradin GP, De La Vega P, Franke ED, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland.

Plasmodium falciparum sporozoite surface protein 2 (SSP2), also known as TRAP, is included in experimental malaria vaccines because *P. yoelii* SSP2 is the target of protective $CD8^+$ CTL in BALB/c mice. To determine if, in addition to CTL, antibodies against PySSP2 protect mice, we immunized 3 strains of inbred mice with a synthetic branched chain polymer including the PySSP2-derived B cell epitope (NPNEPS)4 and 2 T helper epitopes from tetanus toxin. Vaccine-induced antibodies from the 3 strains of mice had minimal anti-parasite activity, yet A/J mice were completely protected against sporozoite challenge. Transfer of T lymphocyte-enriched immune splenocytes protected naive A/J mice, *in vivo* depletion of $CD4^+$ T cells eliminated vaccine-induced protection, and *in vivo* treatment with anti-IFN-γ reversed vaccine-induced activity against infected hepatocytes. This is the first demonstration in any infectious disease of a synthetic or recombinant vaccine inducing $CD4^+$ T cell-dependent, genetically restricted sterile protective immunity.

106 DIFFERENTIAL GENETIC REGULATION OF PROTECTIVE IMMUNE RESPONSES INDUCED BY RECOMBINANT GST-$MSPI_{19}$ OF *PLASMODIUM YOELII* IN CONGENIC MICE. Tian JH*, Miller LH, Berzofsky JA, and Kumar S. Laboratory of Parasitic Diseases, National Institute of Allergy & infectious Diseases; and Molecular Vaccine Section, NIH, National Cancer Institute, Bethsda, MD.

The 19 kDa carboxy-terminus of the merozoite surface protein of *Plasmodium yoelii* ($PyMSP1_{19}$) has been shown to protect mice against challenge with lethal parasite. We wanted to determine the mechanism of $MSP1_{19}$ induced protective immunty and to see if this response is widely recongnized by different MHC haplotypes. We expressed $PyMSP1_{19}$ fused to glutathione-S-transferase (GST) of *Schistosoma japonicum* in *E. coli*. Five congenic strains of mice on the B10 background were immunized with recombinant fusion protein in Freund's complete adjuvant and challenged with lethal *P. yoelii*. Following challenge infection, all the control mice developed high parasitemia and died between days 10 to 20. Varying degrees of protection were induced among the 5 immunized groups. C57BL/10 mice showed complete protection with no detectable parasitemia. In contrast, B10.BR mice failed to develop any protection and all the mice died after reaching high parasitemia. Moderate to low level protection was observed in B10.A(4R), B10.D2 and B10.A strains of mice. The difference between the $H\text{-}2^kb$/ recombinant strain B10.A(4R) and $C57BL/10(H\text{-}2^b)$ maps one protective effect to a gene in the left part of H-2, whereas that between B10.A(4R) and $B10.BR(H\text{-}2^k)$ maps another gene to the right end of H-2. Thus, two distinct mechanisms may be involved in protection. Prechallenge sera from all immunized groups of congenic mice had similar total antibody level and there was no correlation between total antibody titers and protective immunity. However, following parasite challenge, sera from C57BL/10 mice had a 10 fold increase in total IgG and 2-4 fold increase in IgG1 titers. On the other hand, among B10.D2 and B10.A(4R) mice total IgG titers remained unchanged but there was a 10 fold increase in IgG2a titers. These results suggest that prechallenge antibody titers do not reflect protective immunity, which may be associated with differential isotype switch following parasite infection.

107 VACCINATION WITH P30P2-$MSP1_{19}$ BUT NOT $MSP1_{19}$ OR EVE-$MSP1_{19}$ ELICITS PROTECTIVE IMMUNITY IN *AOTUS NANCYMAI* CHALLENGED WITH FVO *PLASMODIUM FALCIPARUM*. Kumar S*, Wellde B, Miller LH, Ballou R, Hall T, and Kaslow DC. Malaria Vaccine Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD; and Division of Immunology, WRAIR, Washington, DC.

The 195-kDa precursor of the merozoite surface protein, MSP1, is proteolytically processed, ultimately leaving only the 19-kDa ($MSP1_{19}$) carboxy-terminus lipid anchored to the parasite cell membrane. Evidence from murine and primate malaria model systems indicate a protective immune response is elicited to $MSP1_{19}$. Our preliminary mouse studies of immunogenicity of His_6-tagged, yeast-produced *Plasmodium falciparum* $MSP1_{19}$ ($yMSP1_{19}$) suggested that by itself it was not highly immunogenic. We, therefore, designed a His_6-tagged fusion protein ($yP30P2\text{-}MSP1_{19}$), consisting of the P30 and P2 universal helper T cell epitopes of tetanus toxoid fused to the amino terminus of $MSP1_{19}$. Ni-NTA affinity purified fusion protein consisted of a heterogenous degraded product. The major products were: 1) intact $MSP1_{19}$ having an additional three amino acids (Glu-Val-Glu) at the N-terminus ($yEVE\text{-}MSP1_{19}$); and 2) $MSP1_{19}$ missing the N-terminal amino acid (Asn). Previously, we reported that *Aotus nancymai* vaccinated with $P30P2\text{-}MSP1_{19}$ were protected from lethal challenge of virulent, *Aotus* -adapted FVO strain of *P. falciparum*. The mechanism of immunity was unclear. To determine if $yMSP1_{19}$ or if $yEVE\text{-}MSP1_{19}$, in the absence of minor contaminants of $yP30P2\text{-}MSP1_{19}$ (e.g., intact $yP30P2\text{-}MSP1_{19}$), could induce a protective immune response (and thus elucidate the immunogen mediating immunity), *Aotus nancymai* vaccinated with $yP30P2\text{-}MSP1_{19}$, $yEVE\text{-}MSP1_{19}$ or $yMSP1_{19}$ were challenged with FVO parasites. Only monkeys vaccinated with $P30P2\text{-}MSP1_{19}$ were protected. Thus, the biologically active components(s) of the vaccine could be a conformational isomer of one of the aforementioned major products, a mixture that consists of the two major products, or, more likely, a minor component of the protein mixture (e.g., intact $yP30P2\text{-}MSP1_{19}$). Studies are in progress to produce highly purified, intact $yP30P2\text{-}MSP1_{19}$ (e.g., in protease-deficient yeast and in other yeast species), an alternative form of $yP30P2\text{-}MSP1_{19}$ (e.g., with the helper T cell epitopes at the carboxy terminus), and other fusion proteins (e.g., with other universal helper T cell epitopes) in the hopes of producing a homogenous product that elicits a protective immune response.

108 *BRUGIA MALAYI* MICROFILARIAE UTILIZE A B-7-LIKE PATHWAY TO PROVIDE COSTIMULATION FOR HUMAN T LYMPHOCYTES. Weller PF*, Liu LX, and Kim J. Beth Israel Hospital, Harvard Medical School, Boston, MA.

In human and experimental lymphatic filarial infections, parasite-specific suppression of immune responses is most pronounced in hosts with microfilaremia. We have evaluated mechanisms by which *Brugia malayi* microfilariae (Mf) modulate the activation responses of normal human T lymphocytes. Mf, isolated from infected jirds, were fully depleted of jird peritoneal macrophages, as confirmed by anti-CD14 flow cytometry. Lymphocytes, isolated from normal donors with Hypaque-Ficoll followed by depletion of adherent mono- cytes, were cultured 24-48 hrs without stimulation or with Con A or immobilized anti- CD3 mAb stimulation, both without Mf and with Mf present at 1:1/16 and 1:1/4 lymphocyte:Mf ratios. Mf exerted no direct stimulatory effect on co-cultured T cells. In contrast, if cocultured T cells were activated either by Con A or cross-linking CD3, the addition of Mf to stimulated lymphocytes yielded significantly greater lymphocyte responses, including enhanced IL-2 and transferrin receptor expression and augmented release of IL-2 and IL-4. These actions of Mf were compatible with being mediated via a costimulatory pathway. When a CTLA4-Ig soluble fusion protein was included during Mf- lymphocyte cocultures, Mf induced costimulation was dose-dependently suppressed by CTLA4-Ig. Since CTLA4-Ig blocks B7-type co-stimultory molecules, Mf costimulation is mediated by a B7-like mechanism. Since differential engagement of costimulatory pathways can lead to varying responses ranging from anergy to activation, the capacity of Mf to act via a B7-like pathway for T lymphocyte costimulation may account for some of the altered lymphocyte responses observed in microfilaremic patients.

109 DIFFERENTIAL REGULATION OF ANTIGEN-SPECIFIC IGG4 AND IGE BY THE RECOMBINANT FILARIAL PROTEINS OV27 AND OVD5B. Garraud O, Perler FB, Bradley JE, and Nutman TB*. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; New England Biolabs Inc., Beverly, MA; and Imperial College of Science Technology and Medicine, London, England.

Immune responses to filarial infections are associated with the production of antigen (Ag)-specific IgG4 and IgE antibodies (Abs) and type-2 cytokines. To assess the role Ags themselves play in inducing Ag-specific B cells to switch to γ-4 (IgG4) and epsilon (IgE), purified B cells obtained from *O. volvulus* and *L. loa* infected individuals were cultured with either of two recombinant filarial proteins, Ov27 and OvD5B, after stimulation with anti-CD40 monoclonal Ab (mAb) and interleukin (IL)-4 and the IgG4/IgE production was compared to the production in peripheral blood mononuclear cell (PBMC) cultures from the same patients (n=24). Ag-specific IgG4/IgE Abs were detected in 14/24 PBMC- and in 24/24 B cell-cultures, with a higher level of Ab detected in the B cell cultures ($p<0.001$). The OvD5B/Ov27 induction of specific IgG4/IgE by B cells (n=7) required both Ag and IL-4, as B-cells were incapable of producing IgG4/IgE specific Abs in the absence of either Ag or IL-4. Ov27- and OvD5B-specific IgG4/IgE Abs were differentially regulated by additional cytokines (IL-13, TNF-a) or by neutralizing anti-cytokine Abs (anti-IL2, anti-IL-10), or by monoclonal Abs to B-cell differentiation Ags (anti-CD21, anti-CD23). Most notably, Ov27-

induced IgE production was unaffected by these reagents, while OvD5B-induced IgE was down-regulated by anti-IL-10, anti-IL-2, and anti-CD21 Ab ($p<0.05$). These data suggest not only that Ag-specific IgG4/IgE Abs can be polyclonally expanded *in vitro*, but also that certain epitopes on filarial proteins differentially regulate the overall Th2-mediated IgG4/IgE responses.

110 LARVAL AND ADULT FILARIAL ANTIGENS STIMULATE DIFFERENT CYTOKINE RESPONSES IN "ENDEMIC NORMAL" AND MICROFILAREMIC INDIVIDUALS FROM A HAITIAN POPULATION. Dimock KA*, Lammie PJ, and Eberhard ML. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Antifilarial immune responses of endemic normal, i.e. microfilaria- and antigen-free, individuals are characterized by a Th1-like response to adult filarial antigens. PBMC proliferative responses, IL-2 production, IFN-γ mRNA and antifilarial IgG2 levels are all significantly higher in this group (EN) than in microfilaremic (Mf+) individuals. In contrast, responses to adult antigens seem to have a Th2-like character in Mf+ individuals, who exhibit significantly higher levels of antifilarial IgG4. Initial findings suggest that responses to larval antigens may not conform to this pattern. Although PBMC proliferative responses to larval antigens (L3) are higher for EN persons, IL-2 production to L3 is significantly higher for Mf+ individuals. RT-PCR data indicate that L3-induced IFN-γ mRNA production is also significantly higher in Mf+ individuals. The highest L3-induced IL-4 message levels were obtained from Mf+ individuals, however there were no significant differences between groups in the production of IL-4 mRNA or in production of IL-10. Preliminary RT-PCR data indicate that adult antigens stimulate more IL-5 mRNA production from Mf+ individuals than from EN persons, however larval antigens did not stimulate signigicant IL-5 production from either group. These data suggest that Mf+ individuals may mount an anti-larval response which is predominantly Th1-like in character.

111 CHANGES IN Vß RECEPTOR USAGE ARE LINKED TO *IN UTERO* EXPOSURE TO *WUCHERERIA BANCROFTI* INFECTION. Steel C*, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and Division of Control of Tropical Diseases (CTD), World Health Organization, Geneva, Switzerland.

Previously, 11 adolescents (from the *Wuchereria bancrofti*-endemic island of Mauke, Cook Islands) born 17-19 years earlier to mothers who were microfilaremic (Mf+) were found to have a marked cellular hyporesponsiveness to microfilarial antigen (Ag) in comparison to 10 adolescents born of uninfected (Mf-) mothers. To examine the mechanisms underlying this hyporesponsiveness, analysis of 24 families of Vß T cell receptors (TCR) was carried out using a Vß-specific strategy of semiquantitative RT-PCR on RNA from the PBMCs of these 21 adolescents. Vß usage in unstimulated cells (media alone) revealed that children born of Mf- mothers had a higher percentage of Vß1 (3.0% vs 1.2%; $p<.02$) and Vß19 (1.6% vs 0.6%; $p<.05$) than children born of Mf+ mothers. Children born of Mf- mothers also had a higher percentage of Vß3 usage (4.3% vs 2.3%; $p<.02$) in response to adult filarial Ag than children of Mf+ mothers. When the expression of individual Vß receptors in response to parasite Ag was evaluated in comparison to the corresponding expression in media alone, however, children born of Mf+ mothers showed a dramatic increase in the expression of Vß19 compared to children born of Mf- mothers (who showed either no change or a decrease) to adult (geometric mean % change = 328% vs 80%; $p<.05$), microfilarial (232% vs 22%; $p<.01$), and L3 antigen (387% vs 44%; $p<.03$). These data suggest that the Vß19 TCR may play a critical role in the response to parasite Ag; future studies will focus on the clonality of Vß19 expression and on the T cell subsets expressing this receptor in response to Ag.

112 *IN UTERO* EXPOSURE TO *ONCHOCERCA VOLVULUS* PREDISPOSES TO HIGHER LEVELS OF INFECTION AND ALTERS SUBSEQUENT CELLULAR IMMUNE RESPONSES. Elson LH*, Days A, Calvopina M, Parredes W, Araujo E, Guderian RH, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD; Investigaciones Clinicas, Hospital Vozandes, Quito, Ecuador.

Black individuals living in an area hyperendemic for onchocerciasis in northwestern Ecuador have been monitored for *Onchocerca volvulus* infection over the past 16 years. To determine if *in utero* exposure to *O. volvulus* biases a child's subsequent immune responses, children between the ages of 9 and 16 years, for whom the mother's infection status was known within two years of the time of birth, were chosen for study. Although all of the children had been treated for onchocerciasis 1-3 years prior to the present study (and only 3 were infected at the time of the study), prior to treatment, children of infected mothers (n=17) had significantly higher levels of skin microfilariae than children of uninfected mothers (n=13; $p=0.021$). Analysis of the serum levels of *O. volvulus*-specific IgG, IgG subclasses and IgE showed no significant differences between the two groups of children. Using L3 and mf antigen from *B. malayi* and adult antigen from *O. volvulus*, analysis of the cellular immune responses revealed that peripheral blood mononuclear cells of children of infected mothers had lower proliferative responses

to L3 antigen (p=0.047). These same children produced more IL-5 and IL-2 to adult antigen (p=0.045 and 0.039), more IL-5 to L3 antigen (p=0.016), and more IL-4 to mf antigen (p=0.028) although they produced less IFN-γ and IL-2 to non-parasite antigens (p=0.054 and 0.007) than children of uninfected mothers. Thus, it appears that *in utero* exposure to *O. volvulus* has a long term effect on the child's subsequent cellular immune response that may render the child more susceptible to *O. volvulus* infection postnatally.

113 T-CELL MIGRATION THROUGH ENDOTHELIAL CELL MONOLAYERS IS INCREASED IN INDIVIDUALS WITH SYMPTOMATIC BANCROFTIAN FILARIASIS. Plier DA*, Maia e Silva MC, Maciel A, de Almeida AB, and Freedman DO. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil.

The migration of circulating T-memory cells through vascular endothelium at the site of the inflammatory reaction in human *Wuchereria bancrofti* infection has been shown to result in a CD8+ infiltrate in infected tissues. Using cultured endothelial cell monolayers, *in vitro* adhesion and migration of purified T-cells from 11 Brazilian individuals with clinical filarial disease (Dis+) were compared to T-cells from 11 asymptomatic microfilaremic individuals (Dis-). Fresh T-cells from Dis+ individuals migrated through monolayers significantly more than T-cells from Dis- individuals (% migration = 1.0 Dis+ vs 0.6 Dis-; p=0.04). Dis+ T cells had greater adhesion to the monolayers when compared to the Dis- T-cells (% adhesion = 9.3 Dis+ vs 5.4 Dis-; n.s.). 48hr filarial antigen (BmA) stimulated T-cells from Dis+ individuals migrated significantly more than those stimulated with media alone (% migration = 2.9 BmA vs 1.2 media; p=0.01) but no significant upregulation was seen with Dis- T-cells. When 48hr BmA-stimulated T-cells and endothelial cells were pre-incubated with anti-VLA-4 and anti-VCAM-1, respectively, 66.5% inhibition of migration and 39.2% inhibition of adhesion were achieved. Our findings further demonstrate that inflammatory pathological manifestations of *W. bancrofti* infection may result from the enhanced ability of T-cells to migrate through endothelium. A potential therapeutic role for blockade of the VCAM: VLA4 pathway is suggested.

114 ROLE OF NITRIC OXIDE (NO) IN HOST DEFENCE AGAINST THE HUMAN FILARIAL PARASITE, *BRUGIA MALAYI*. Rajan TV*, Keefer L, Yates J, Schultz LD, and Porte P. Department of Pathology, University of Connecticut Health Center, CT; National Cancer Institute, Frederick, MD; Department of Biology, Oakland University, Rochester, MI; and Jackson Laboratory, Bar Harbor, ME.

In order to determine whether the small diffusible radical gas, nitric oxide (NO), plays a role in host defense against filarial parasites, we used two complementary approaches. In one, we sought to determine whether the immunocompetent murine host, BALB/cByJ can be made permissive to *Brugia malayi* by chronic treatment with aminoguanidine (AG), a known inhibitor of nitric oxide synthase (NOS). The data from two independent experiments indicate that chronic administration of AG makes the mice partially susceptible to *B. malayi*, with an adult worm burden significantly higher than in control, untreated mice. In the other, complementary model, we have chronically administered the drug DEA/NO, which generates nitric oxide in aqueous buffer with a half-life of two minutes, to permissive scid mice challenged with *B. malayi* L3 larvae. The data from this experiment indicate scid hosts can be protected against the establishment of *B. malayi* infection by such treatment. Together, these data indicate that NO may be an important, but not sole, host defense against filarial infection.

Experiment 1		Experiment 2	
Group	Mean Worms ± SD	Group	Mean Worms ± SD
No Ttment	2.9 ± 3.7	No Ttment	0.9 ± 1.4
Ag Tted	9.0 ± 7.6	Ag Tted	13.0 ± 4.7

115 CHARACTERIZATION OF MTA/SAH NUCLEOSIDASE AND MTR KINASE FROM ENTERIC BACTERIA: POTENTIAL TARGETS FOR CHEMOTHERAPEUTIC INTERVENTION. Cornell KA*, and Riscoe MK. Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR; and Medical Research Service, Veterans' Affairs Medical Center, Portland, OR.

In contrast to higher eukaryotes, many lower eukaryotes and bacterial species metabolize 5'-methylthioadenosine (MTA) and S-adenosylhomocysteine (SAH) via a single bifunctional enzyme, MTA/SAH nucleosidase. 5'-methylthioribose (MTR) produced by the degradation of MTA is further metabolized via MTR kinase to MTR-1-phosphate and hence recycled back to methionine. These two enzymes represent attractive chemotherapeutic targets because of their role in salvage of adenine and methionine, and in maintaining low levels of MTA and

SAH. Elevated MTA and SAH levels are known to inhibit polyamine biosynthesis, transmethylation reactions, and cell proliferation. Ideally, substrate analogs could be developed to selectively inhibit or subvert the nucleosidase and/or kinase, yielding useful agents for the treatment of a variety of infectious diseases. To study of these enzymes as chemotherapeutic targets, they were purified to homogeneity from bacterial lysates by exploiting a novel affinity chromatography resin. Amino terminal sequence data from the purified protein led to the cloning and sequencing of the MTA/SAH nucleosidase gene from *Escherichia coli*. Sequencing of the MTR kinase gene is in progress. The MTA/SAH nucleosidase gene was further subcloned into the pGEX expression system and kinetic analysis of the resulting fusion protein yielded similar enzymatic activities to native protein. In summary, we describe the purification to homogeneity and amino terminal sequencing of two microbial enzymes involved in the methionine salvage pathway, and report for the first time the cloning, sequencing, and expression of a gene encoding MTA/SAH nucleosidase.

116 CYTOKINE GENE EXPRESSION IN BLOOD, LYMPH NODES, AND SPLEEN OF JIRDS DURING A PRIMARY INFECTION OF *BRUGIA PAHANGI*. Mai Z*, Horohov DW, and Klei TR. Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton, Rouge, LA.

A shift in the dominant cytokine response has been hypothesized to be associated with the spectrum of clinical conditions seen in lymphatic filariasis patients. Jirds infected with *Brugia pahangi* rapidly, 14 days post-infection (DPI), develop an antigen specific lymphoproliferative response in renal lymph nodes (RLN) and a corresponding granulomatous inflammatory response both of which are subsequently downregulated. Examination of cytokine responses during this infection has begun using quantitative competitive RT-PCR ELISA to measure cytokine gene expression. For this purpose full length cDNAs of jird IL-2, IL-4, IL-5, IL-10, IFN-γ and HPRT were isolated and characterized using a protocol involving cross-species PCR, inverse PCR or RACE, and conventional PCR. A mutant competitor was constructed for each cytokine gene. Total RNA was isolated from the axillary LN, RLN, spleen, and peripheral blood at 14, 28, 56, and 150 DPI. Increased levels of IL-2, IL-5 were seen during the initial stages of the infection (14 to 28 DPI). An increase in IL-4 first detected 28 DPI in RLN continued during the infection. Increased levels of IL-10 were first seen in the spleen 56 DPI and in all tissues at 150 DPI. This shift to a dominant Th2 like response is associated with the hyporesponsive, susceptible, microfilaremic state seen in this model. The limited early detection of IL-5 corresponds to the increase and subsequent decrease seen in eosinophilia.

117 AN ORAL *SALMONELLA* VACCINE IN MICE INDUCED A TH1-LIKE RESPONSE TO *ONCHOCERCA VOLVULUS* GST BUT NOT TO THE OV103 SURFACE-ASSOCIATED PROTEIN. Catmull J*, Wilson ME, Kirchhoff LV, and Donelson JE. Howard Hughes Medical Institute, Departments of Biochemistry, Microbiology and Internal Medicine, University of Iowa; and Department of Veterans Affairs Medical Center, Iowa City, IA.

Two antigens of *Onchocerca volvulus* were examined for their ability to induce a cellular immune response in mice when expressed by *Salmonella typhimurium*: Ov103, a surface-associated antigen; and glutathione S-transferase (GST), a detoxification enzyme of which a functional homologue in schistosomes was shown to provide protection against schistosomiasis. Mice were immunized orally with recombinant *S. typhimurium* (*Aro*-A) expressing these two antigens. After infection, maximal numbers of *Salmonella* were recovered from the Peyer's patches and spleens at week 2 and no *Salmonella* were detected in these tissues by week 3-4. Recovered *Salmonella* were resistant to ampicillin (>98%) and were shown by western blot to express the recombinant protein. Splenocytes from immunized mice demonstrated significant and specific proliferative responses to OvGST but no response to Ov103. Cytokine assays revealed significant amounts of IFN-γ with no detectable IL-4 and IL-5, consistent with a Th1-like response. These mice, however, generated low levels of antibody to the recombinant proteins despite high levels of antibodies to total *Salmonella* protein. Mice immunized with *Escherichia coli* synthesized recombinant protein with Freund's adjuvant induced high levels of antibody to Ov103 and OvGST, but no proliferative response by splenocytes. These data suggest that *Salmonella* can be used as a vehicle to immunize with *O. volvulus* antigens and, in one case, can generate Th1-like immune responses. A similar Th1-like responsiveness has been described in individuals with apparent natural immunity to onchocerciasis, implying a role in protection against disease.

118 THE ROLE OF IL-4 IN DEVELOPMENT OF T HELPER CELL RESPONSES ASSOCIATED WITH PULMONARY EOSINOPHILIA AND RESISTANCE TO MICROFILARIAE IN MICE. Pearlman E*, Hazlett FE, Lizotte MR, Williams SA, and Kazura JW. Case Western Reserve University, Cleveland OH; and Smith College, Northampton, MA.

Tropical Pulmonary Eosinophilia (TPE) is a severe asthmatic manifestation of lymphatic filariasis associated with amicrofilaremia, eosinophilia and elevated IgE which are believed to result from hyper-responsiveness to microfilariae (MF) trapped in capillaries of the lung. To determine the role of IL-4 in the pathogenesis of an animal model of TPE, control (IL-4+/+ mice) and IL-4 knockout mice (IL-4-/-) were immunized subcutaneously with soluble MF Ag and injected iv with 200,000 *Brugia malayi* MF. Parasite clearance and Th responses in the lung were then assessed. Seven days after MF inoculation, IL-4+/+ mice had < 5 MF/80 µl blood, whereas IL-4-/- mice had 2 - 83 MF/80 µl blood (2 experiments with 5 mice /group: $p<0.05$). Unimmunized IL-4+/+ mice also cleared MF from peripheral blood more rapidly than IL-4-/- mice ($p<0.05$ for d7- 29 after inoculation). PCR analysis of lung biopsies using probes specific for *B. malayi* demonstrated that MF DNA was elevated in immunized animals. Cytokine gene expression in lungs showed that IL-4 and IL-5 were elevated in immunized and unimmunized IL-4+/+ mice compared with IL-4-/- mice, whereas there was no difference in IFN-γ expression. CD4, rather than CD8 cells were the primary source of IL-4 and IL-5. H&E stained lung sections showed extensive areas of inflammation in immunized IL-4+/+ mice compared with unimmunized mice, and that the inflammatory infiltrate contained mononuclear cells and eosinophils. These data indicate that IL-4 is required for optimal clearance of MF from the blood and that this cytokine contributes to the pathogenesis of pulmonary inflammation.

119 IgE RESPONSIVENESS TO A RECOMBINANT FILARIAL ANTIGEN (WB1.2) CORRELATES WITH TPE PATIENTS WITH HLA-DQB1*0301 IN THEIR DQB1 GENOTYPES. Zimmerman PA*, Wang J, Kubofcik J, Phadke PM, Raghavan NK, Mollis SN, Kumaraswami V, Vijayan V, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Tuberculosis Research Center, Madras, India.

To investigate the potential influence that genetic mechanisms may have on the induction of filarial-specific IgE responses, IgE-specific recognition of recombinant antigens was assessed in relation to the HLA class II loci, DQA1 and DQB1. Western blot screening of the *Brugia malayi* fusion protein, Wb1.2 (157kD β-galactosidase fusion protein / glutamine rich [15.7%] open reading frame of 153 amino acids [a.a.] / no sequence similarity with known proteins) with protein-G adsorbed individual sera from 58 *Wuchereria bancrofti*-infected patients from Madras, India (33 with tropical pulmonary eosinophilia [TPE]; 25 with asymptomatic microfilaremia [Mf+]) with known HLA-DQ genotypes was performed. Upon comparison of Wb1.2 - IgE responsiveness among all patients, with HLA-DQA1 and -DQB1 genotypic data, a positive correlation was observed with the presence of DQB1*0301 (Chi square=6.5; P-value=0.01). Further comparison illustrated that this association between IgE response to Wb1.2 and DQB1*0301 was most striking among TPE patients (Fisher's Exact=9.1; P-value=0.004). To examine the fine specificity of this response, antibody responses to 29 overlapping peptides spanning the length of the 153 a.a. filarial polypeptide were determined. By this methodology, a glutamine-rich (31.4%) sequence of 35 a.a. has been identified as a primary antibody epitope of Wb1.2. This 35 a.a. sequence will now be used to assess the significance of HLA-DQB1*0301 in presentation of this filarial antigen.

120 BOTH IL-4 AND IFN-γ mRNA ARE PRESENT IN THE INITIAL LYMPH NODE RESPONSES IN HUMAN CUTANEOUS LEISHMANIASIS. Barral A*, Bonfim G, Nascimento C, Barral MN, and Carvalho EM. Servico de Imunologia, Univ. Federal da Bahia, Bahia, Brazil.

The initial survival of *Leishmania* in the mammalian host depends on the initial balance of macrophage activating (IFN-γ and TNF-α) or deactivating (TGF-β, IL-10) cytokines (CKs). Early produced CKs may also influence in the determination of the Th1/Th2 balance which is correlated to a resistant or susceptible course of disease. The importance of lymph nodes (LN) in experimental leishmaniasis has been recognized. Presence of enlarged LN have been described in human cutaneous leishmaniasis a long time ago, but little is known of the importance of LN in human leishmaniasis. Here we describe the CK profile determined by reverse-transcriptase PCR for IL-2, IL-4, IL-10, IL-12, TGF-β and IFN-γ in LN cells from 10 patients with early cutaneous leishmanial infection. At time of diagnosis none of the patients had a skin lesion, but all had leishmania cultivated from the LN. During a 6-month follow-up 7 patients developed typical leishmanial ulcerations, and in 3 LN regressed without later development of skin ulceration. Cells were obtained by fine-needle aspiration and processed for mRNA. Patients who developed ulcerations were compared to patients who remained free of disease. Both groups exhibited mRNA for IL-4 and IFN-γ and none showed a signal for IL-10.

121 ANALYSIS OF *IN SITU* CYTOKINE EXPRESSION IN LESIONS OF ZOONOTIC CUTANEOUS LEISHMANIASIS. Louzir H*, Ben Salah A, Marrakchi H, Zaatour A, Ftaiti A, Dellagi K, and Melby PC. Pasteur Institute of Tunis, Tunis, Tunisia; and The University of Texas Health Science Center, San Antonio, TX.

Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* is polymorphic in its clinical evolution. To better define the role of the host immune response in disease expression, we evaluated the clinical evolution of 117 patients with ZCL from an endemic region of Tunisia, and studied the *in situ* cytokine response in a subset of these. Patients were evaluated at 14, 42, and 105 days after the initial examination and biopsy. 30% of the patients had a solitary lesion, 58% had 2-5 lesions, and 12% had 5-15 lesions. The lesions were 4-70 mm in diameter. At day 14, the lesions showed spontaneous regression or healing in 65% of patients, and the lesions were unchanged or had increased in size in the remaining 35%. In 8% of patients the lesions were still active at day 45. The expression of IL-4, IL-6, IL-10, IL-12, IFN-γ, and TNF-α mRNA was analyzed by reverse transcriptase-polymerase chain reaction in 75 biopsies from the lesions of 57 patients. Almost all of the lesions expresssed mRNA for TNF-α (74/75), IFN-γ (71/75), and IL-6 (70/75). IL-10 and IL-12 mRNA was detected in 40 and 36 biopsies, respectively. IL-4 mRNA was found at a low level in only 6 of the lesions. There was no association between IFN-γ expression and the size, number, or clinical evolution at day 14. High levels of IL-6 and TNF-α expression were preferentially found in biopsies from patients with large, multiple, or non-healing lesions. IL-12, which is associated with resistance and healing in murine leishmaniasis, was to our surprise found more commonly in the progressive lesions. These results suggest that the inflammatory monokines contribute the clinical polymorphism of ZCL.

122 CYTOKINE EXPRESSION IN LEISHMANIASIS PATIENTS TREATED WITH SODIUM STIBOGLUCONATE (PENTOSTAM). Endy TP*, King AD, Macarthy PO, Magill AJ, Aronson NE, and Oster CN. Infectious Disease Service, Walter Reed Army Medical Center, Washington DC; Department of Immunology, Walter Reed Army Institute of Research, Washington DC; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington DC.

Leishmaniasis is caused by parasites of the protozoan genus *Leishmania* which produce visceral, cutaneous, and mucosal disease. Sodium stibogluconate (Sb) has been the drug of choice for all forms of leishmaniasis but its mechanism of action or its immunologic effects are poorly understood. The purpose of this study is to examine cytokine expression and T helper cell responses as well as correlation of cytokine repsonses with the pancreatitis seen in patients treated with Sb. Sixteen patients with cutaneous leishmaniasis were prospectively studied and results compared to 10 normal controls. Lipase and amylase were elevated (5 to 10 times normal) with Sb therapy and peaked at day seven of therapy (Rx). There was no correlation with serum TNF-α, IL-1β, and IL-6 levels with duration of Sb Rx or with pancreatic enzyme levels. Cytokine expression of PMA and ionomycin stimulated peripheral blood mononuclear cells (PBMCs) demonstrated that IL-2 and IFN-γ were high pre-Rx and remained high throughout Rx. IL-10, IL-6, and IL-4 declined with Rx and were lowest at day 14-20 of Rx. Results of cytokine specific mRNA as detected by reverse-transcriptase polymerase chain reaction of unstimulated PBMCs revealed expression of TNF-β during Rx and down regulation of IL-6 and IL-4. This study confirms that Sb produces elevations in amylase and lipase during therapy and that this effect is not due to cytokine expression of TNF-α, IL-1β, or IL-6. This study provides evidence of a mixed TH1/TH2 response prior to Sb Rx. With treatment, TH1 response becomes predominant. This study supports the current literature on the importance of CD4 phenotypic responses in the control of *Leishmania* infection and offers unique insight on the immunologic changes of this response with Sb Rx.

123 IL-12 UP REGULATES CYTOTOXICITY IN HUMAN LEISHMANIASIS. Barral MN*, Brodskyn A, Barral A, and Carvalho EM. Servico de Imunologia, Univ. Federal da Bahia, Bahia, Brazil.

We have previously shown the presence of cytotoxic cells capable of lysing K562 or Daudi in the peripheral blood of cutaneous (CL) and mucocutaneous leishmaniasis (MCL) patients. Now we report on an assay using autologous macrophages (Mf) infected with viable *Leishmania* both as Ag-presenting and target cells in order to evaluate the parasite-specific cytotoxic responses in human leishmaniasis. In this system we have show: a) cytotoxicity is present in ML but not in CL patients, suggesting a role of the phenomenon in the pathology of leishmaniasis; b) cytotoxicity activity is present in both CD8+ and NK cell populations, as evaluated by cell depletion experiments using magnetic beads; c) exposure of lymphocytes from ML patients to infected autologous Mf induces the expression of mRNA for Perforin and Granzyme, as evaluated by RT-PCR; d) TGFβ down-modulates the autologous *Leishmania* -specific cytotoxic activity; e) exogenous IFN-β increased Ag-stimulated cytotoxicity in only 1 out of 5 MCL patients tested; and lead to response of only 1 out of 4 CL patients. Interestingly, the use of monoclonal antibodies against IFN-β, during the generation of effector cells lead an inhibition of cytotoxic responses; f) All five patients tested exhibited increased responses in the presence of IL-12 (from 1.2 to 10 times higher the levels observed in cultures without added cytokine).

124 PARASITE DRIVEN REGULATION OF ENDOGENOUS PARASITE ANTIGEN PRESENTATION BY MACROPHAGES INFECTED WITH *LEISHMANIA AMAZONENSIS*. Kima PE*, Ruddle NR, and McMahon-Pratt D. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT.

Leishmaniasis is an infection caused by protozoan parasites of the genus *Leishmania*. These parasites have a dimorphic life-cycle and productive infection of *Leishmania* occurs when promastigotes invade macrophages then undergo transformation in the parasitophorous vacuole into the amastigote stage. Our experiments have been guided by the hypothesis that only proteins that can exit the phagolysosome and access both the Class I and Class II pathways are going to serve as protective immunogens. To obtain proof for this hypothesis, CD4+ T cell lines raised against the protective antigens, GP46 and P8 as well as CD8+ T cell lines with specificity to GP46 were used to study the presentation of endogenously synthesized parasite antigens by infected cells. Using theI4.7 macrophage cell line which constitutively expresses I-Ak, we found that cells infected with amastigotes of *Leishmania amazonensis* or *L. pifanoi* do not present endogenously synthesized parasite antigens to CD4+ T cells. In contrast macrophages infected with promastigotes do present endogenous parasite molecules to CD4+ T cells transiently, with maximal presentation occurring within 24 hours of infection and decreasing to no antigen presentation being evident by 72 hours post-infection. Interestingly, antigen presentation of endogenous parasite antigen to CD8+ T cells subsequent to infection with promastigotes does not follow a similar time course. We conclude from these observations that differentiation of *L. amazonensis* within the macrophage results in sequestration of parasite antigens from the Class II pathway of antigen presentation. We discuss the impact of these observations on vaccination against cutaneous leishmaniasis.

125 NEUTRALIZATION OF *TRYPANOSOMA CRUZI* INFECTION BY FAB' FRAGMENTS OF A MONOCLONAL ANTIBODY TO A MEMBER OF THE FAMILY 2 OF TRANS-SIALIDASES. Villalta F*, Burns, Jr. JM, Chaudhuri G, Smith C, Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

Molecules involved in the process by which *Trypanosoma cruzi* invade mammalian host cells are prime targets for immunological intervention. We found that the soluble gp83 purified from PLC-treated membranes of *T. cruzi* trypomastigotes presents sialidase and trans-sialidase activities. Scatchard analysis indicate that this molecule binds to heart cells, Vero cell fibroblasts and macrophages by ligand receptor interaction. Monovalent Fab' fragments of a monoclonal antibody (Mab 4A4) inhibit binding of radioiodinated gp83 trans-sialidase to mammalian cells, block the parasite from attaching and entering myoblasts, fibroblasts and macrophages and remarkably neutralize trypomastigote infectivity in Balb/c mice by passive immunization. Oligonucleotide primers derived from protein microsequencing of gp83 were used to clone its gene by PCR. The cloned gene presents homology at the DNA and protein levels with the large multigene family 2 of trans-sialidases. Genomic clones expressing the recombinant protein in *E. coli* present sialidase and trans-sialidase activities and react with the Mab 4A4 on western blots. To identify the epitope required for parasite binding on the gp83 trans-sialidase recognized by the Mab 4A4, a series of carboxyl-terminally truncated recombinant peptides were produced. The Mab 4A4 does not inhibit the sialidase or trans-sialidase activities of the native or recombinant molecule. These results indicate that the epitope on the gp83 trans-sialidase recognized by the Mab 4A4 that is required for trypanosome binding to mammalian cells to promote trypanosome entry is an attractive candidate for immunological intervention in *T. cruzi* infection.

126 CALCIUM RELEASE FROM ACIDOCALCISOMES OF *TRYPANOSOMA BRUCEI*. Vercesi AE*, Catisti R, and Docampo R. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; and Departamento de Bioquimica, Universidade Estadual de Campinas, Campinas, Brazil.

In previous communications we provided evidence that trypanosomatids possess acidic vacuoles that we termed acidocalcisomes, where Ca^{2+} is accumulated via a vanadate-sensitive Ca^{2}ATPase. The acidic pH of this compartment is generated and sustained by a bafilomycin A^{1}-sensitive H^{+}ATPase and seems to be important for Ca^{2+} retention, since alkalinization induced by NH_4Cl, bafilomycin A^{1} or nigericin is followed by Ca^{2+} release. The data presented here indicates that Na^{+} stimulates Ca^{2+} release from the acidocalcisomes of digitonin-permeabilized *Trypanosoma brucei* procyclic trypomastigotes in a dose-dependent fashion; this effect being enhanced by increasing medium pH from 7.0 to 7.8. The hypothesis that this Na^{+} effect was mediated by alkalinization of the acidocalcisomes via a Na^{+}/H^{+} antiporter was supported by experiments showing that Na^{+} promotes release of acridine orange previously accumulated in these vacuoles. We have also demonstrated that monensin-induced increase in cytosolic Na^{+} concentration of intact cells loaded with fura 2 and suspended in Ca^{2+}- free medium containing 116 mM NaCl was followed by an increase in cytosolic Ca^{2+} concentration. We propose that the increase in cytosolic Ca^{2+} concentration evoked by activation of the putative Na^{+}/H^{+} antiporter present in the acidocalcisome membrane may be an important event related to signal transduction mechanisms in these parasites.

127 SIGNAL TRANSDUCTION PATHWAYS IN *TRYPANOSOMA CRUZI* INDUCED BY EPIDERMAL GROWTH FACTOR. Lima MF*, Moura P, Ager EP, and Williams KL. Department of Microbiology, Meharry Medical College, Nashville, TN.

The generation of secondary metabolites induced by growth factors and its regulation intracellularly leading to recruitment and activation of intracellular mediators involved in trypanosome growth is a vital area in our understanding of trypanosome proliferation and differentiation. In this work we have studied growth of *Trypanosoma cruzi* induced by EGF. We have observed that EGF induces DNA synthesis and intra and extra cytoplasmic amastigote proliferation. *T. cruzi* amastigotes specifically bind EGF, presenting 1 x 10^3 surface receptors for EGF with a Kd of 2.0 nM. An EGF surface receptor homologue of 90 kDa on *T. cruzi* amastigotes has been characterized by Western blot analysis and immunoprecipitation with monoclonal antibodies to different domains of the human EGF receptor. This 90 kD protein recognized by monoclonal antibodies against the human EGF receptor is phosphorylated on tyrosine residues in higher amounts after amastigotes were exposed to EGF. Other proteins associated with the complex were also found to be tyrosine phosphorylated after exposure of amastigotes to EGF. We have found that amastigotes possess shc or shc homologues as evidenced by Western blot analysis with anti-shc antibodies. Specific inhibitors of tyrosine phosphorylation have inhibited EGF-induced amastigote growth in axenic media and intracellularly in Vero cells. The study of the mechanisms of signal transduction induced upon the binding of EGF to its receptor in trypanosomes may elucidate the signals that lead to intracellular trypanosome multiplication.

128 CLONING OF FeSOD FROM *LEISHMANIA CHAGASI* AND *TRYPANOSOMA CRUZI*: ROLE IN PROTECTION AGAINST OXIDATIVE STRESS. Ismail SO*, Bhatia A, Paramchuk W, Omara-Opyene LA, Gedamu L. Dept. of Biological Science, University of Calgary, Canada.

Leishmania chagasi and *Trypanosoma cruzi* are the causative agents of visceral leishmaniasis and Chagas' disease, respectively. Activated macrophages are important for the host defense against these parasites. However, during *L. chagasi* and *T. cruzi* infections, amastigotes of these parasites survive inside the macrophages despite the generation of potentially lethal free radicals such as H_2O_2, O^{2-} and NO. Therefore, a key question in understanding the pathogenesis of these diseases is how these parasites resist intramacrophage killing. To address this question we have cloned FeSOD from *L. chagasi* and *T. cruzi*. To investigate the role of FeSODs, we overexpressed *L. chagasi* and *T. cruzi* FeSOD cDNAs in *L. chagasi* cells. Following stable transfection, *L. chagasi* cells were challenged with a number of O^{2-} and NO generating compounds. We found that the *L. chagasi* cells overexpressing T. cruzi FeSOD cDNA were better protected against paraquat and nitroprusside toxicity when compared with cells overexpressing *L. chagasi* FeSOD cDNA or the controls. Susceptibility towards H_2O_2 and copper ion toxicity was not affected. We are investigating whether the difference between *L. chagasi* and *T. cruzi* FeSOD lies in the amount of enzyme produced in transfected cells or the *T. cruzi* FeSOD is enzymatically more active than the *L. chagasi* enzyme. We are also testing whether the two enzymes differ in protecting the cells against oxyradical DNA damage.

129 STABLE INTEGRATIVE TRANSFORMATION AND AUTONOMOUSLY REPLICATING PLASMIDS IN *TRYPANOSOMA CRUZI*. Dos Santos WG*, Buck GA. Department of Biochemistry and Immunology, ICB, UFMG, Belo Horizonte, MG, Brazil; and Medical College of Virginia, Virginia Commonwealth University, Richmond,VA.

We have developed two transformation vectors for *Trypanosoma cruzi*. The first, ptopo2neoEco3.5, was constructed by fusing the reading frame of the *T. cruzi* topoisomerase II gene with the neomycin phosphotransferase gene(*neo*^{r}). The second, pBSpaneo, has the rRNA promoter and the 3'splice site acceptor driving the expression of *neo*^{r} gene. Each vector was transfected into *T. cruzi* CL strain epimastigotes by electroporation and *neo*^{r} cell lines were obtained. This phenotype was stably mantained even in absence of G418 for at least 6 months. Southern analysis of the ptopo2neoEco3.5-transformed clones indicated that each contained a single copy of the *neo*^{r} sequence integrated into the endogenous TOPO II gene. In contrast, the pBSpaneo-transformed cell lines had only non-integrated plasmids. After transformation of *E. coli* with total DNA from parasites transfected with pBSpaneo, the plasmid could be recovered, and, by Southern blot and restriction analysis, appeared identical to the input plasmid. However, plasmids from ptopo2neoEco3.5-transformed clones had undergone substantial rearrangements. Deletion of the promoter in pBSpaneo incapacitates this vector since no *neo*^{r} parasites could be obtained. In conclusion, the ptopo2neoEco3.5 vector preferentially integrated into the *T. cruzi* genome while pBSpaneo was mantained as an episome and maybe useful as shuttle vector for studying modified genes in *T. cruzi*.

130 GENE DISRUPTION OF THE ELONGATION FACTOR 1 α LOCUS IN *TRYPANOSOMA BRUCEI* BY HOMOLOGOUS RECOMBINATION: IMPLICATIONS FOR THERAPY DESIGN. Ridgley EL*, Kaur KJ, and Ruben L. Department of Biological Sciences, Southern Methodist University, Dallas, TX; and Department of Cell Bio logy, University of Texas Southwestern Medical Center, Dallas, TX.

Translation elongation factor 1α (EF -1 α) is a multi-functional protein which is involved in protein synthesis and the regulation of cell growth. Previously, our lab has cloned the gene for EF -1 α in *Trypanosoma brucei*. Unique regions of the EF -1 α locus may be a target for gene disruption therapy. To test this hypothesis the genomic organization and expression of EF -1 α was first examined. EF -1 α was shown by Southern analysis to be tandemly repeated 6 times per haploid genome. All gene copies are located at a single locus on a large 5.7 Mb chromosome. To identify the transcription unit of EF -1 α genomic sequences upstream of the repeat as well as the intergenic region were cloned for use in primer extension and RT-PCR experiments. RT-PCR was used to determine that the repeated genes are co-transcribed. Primer extension analysis identified 3 splice acceptor sites with the predominate one located -60 bp from the initiation codon. To determine whether disruption of EF -1 α expression would effect growth in *T. brucei*, trypanosomes were electroporated with the vector pTEF-RPLC designed to "knockout" copies of EF -1 α by homologous recombination. Clone 17 lacks a single copy of the EF -1 α gene but growth of the parasite was inhibited by 90% when compared to control cells. These preliminary studies indicate that *T. brucei* is extremely sensitive to disruption of the EF -1 α locus and in future experiments unique regions of this gene may be exploited for development of therapies.

131 SEQUENCE SPECIFIC RNA ENDONUCLEASE ACTIVITY OF *LEISHMANIA* RNA VIRUS CAPSIDS. MacBeth K*, and Patterson JL. Division of Infectious Diseases and Department of Microbiology & Molecular Genetics, Harvard Medical School.

Leishmania RNA virus 1 (LRV1) produces a short viral RNA transcript corresponding to the 5' end of positive-sense ssRNAs in both virally-infected cells and *in vitro* polymerase assays. We hypothesized that this short transcript was generated via cleavage of full-length (+) ssRNA. A putative cleavage site was mapped by primer extension analysis to nucleotide 320 of the viral genome. To address the hypothesis that the short transcript is generated via cleavage at this site, two substrate RNAs were created that possessed viral sequence encompassing the putative cleavage site. When incubated with sucrose-purified viral particles these substrate RNAs were site-specifically cleaved. The cleavage site of the *in vitro* processed RNAs also mapped to viral nucleotide 320. The short transcript generating activity could be specifically abolished by proteinase K treatment of sucrose-purified viral particles and high concentrations of EGTA, suggesting that the activity requires a proteinacious factor and possibly intact viral particles. The cleavage activity is directly associated with short transcript generating activity, since only viral particle preparations which were capable of generating the short transcript in polymerase assays were also active in the cleavage assay. Furthermore, the short transcript generating activity is independent of the viral polymerase's transcriptase and replicase activities. We present a working model whereby cleavage of Leishmaniavirus RNA transcripts functions in the maintenance of a low-level persistent infection. Recently we examined the cleavage sites within the 5' untranslationed region of three different viral isolates of Leishmaniavirus. We have identified a concensus sequence surrounding all cleavage sites for the three different isolates. Surprisingly in an *in vitro* cleavage assay, baculovirus-expressed capsid protein alone possessed a cleavage activity identical to that of native virions. The sequence-specific RNA endonuclease activity of this viral capsid protein is unique in two regards: namely, 1) no other viral capsid proteins are known to possess endonuclease activity and 2) no RNA endonucleases are known to be sequence-specific.

132 CHARACTERIZATION OF A MULTI-DRUG RESISTANCE GENE (*lemdr1*) IN *LEISHMANIA ENRIETTII*. Chow LM*, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Drug resistance is becoming a major problem in the treatment of cancer, bacterial and parasitic diseases. In case of the drug resistance in cancer, we know that the mechanism by which drug is exported out of the resistant cells is by over-expression of a transporter P-glycoprotein, the product of the multidrug-resistance (*mdr*) gene. We propose that the mechanism of drug resistance in *Leishmania* is similar to that of cancer. We tested our hypothesis by cloning the P-glycoprotein homologue of *Leishmania* (*lemdr1*) and showed that it is over-expressed in a vinblastine resistant *Leishmania* cell line. Using functional complementation and gene-knockout experiments, we demonstrated that the *lemdr1* gene is responsible for vinblastine resistance in *L. enriettii*. To further understand the functional role of this gene in *Leishmania*, we studied the localization of this gene product. In order to facilitate localization, a hemaglutinin epiptope was engineered into a cloned copy of *lemdr1* gene and an antibody specific for this epitope was used to visualize the protein in IFA and Western blot analysis. Surprisingly, the IFA analysis

demonstrated that most of the protein was intracellular and likely to be associated with the ER. This contradicts our previous thought that this protein is a surface protein. Further characterization is still underway. The Western blot analysis of the purified membrane fractions from transfected *Leishmania* promastigotes demonstrated that the *lemdr1* protein is of about the expected size (140 kDa).

133 EXPRESSION OF A *LEISHMANIA CHAGASI* ANTIGEN IN *LISTERIA MONOCYTOGENES* FOR USE IN VACCINE DEVELOPMENT. Wilson ME*, Clark MA, Portnoy DA, and Jones S. Department of Internal Medicine and Microbiology, University of Iowa and VA Medical Center, Iowa City, IA; and Department of Microbiology, University of Pennsylvania, Philadelphia, PA.

Protective immunity against *Leishmania* sp. requires Th1 cells recognizing parasite antigens and producing macrophage activating cytokines such as interferon-γ. Since *Listeria monocytogenes* (Lm) elicits a THl-type response, we expressed *L. chagasi* antigens in Lm to study whether they could induce protective immunity. We used an avirulent Lm strain (DP-L2161) lacking the *hly* gene encoding listeriolysin. Prior studies showed the recombinant *L. chagasi* protein Lcri stimulates T cells and partially protects mice against *L. chagasi* infection. We constructed a plasmid (p*hly-Lcr*1) containing the Lcrll gene fused to the promoter, signal sequence, and N-terminal 6 amino acids of *hly* in a vector encoding CAT. DP-L2161 transformed with p *hly-Lcr1* secreted abundant Lcr1 into culture supernatants, seen on immunoblots developed with anti-Lcr1 serum. After i.v. infection with $1x10^7$ DP-L2161 containing p*hly-Lcr*1 or a control vector, we recovered DP-L2161 from BALB/c mouse spleens up to 4 days later. 50-100% of recovered clones retained their plasmid. We conclude that *hly*-- minus Lm survive for several days in a murine host, and retain plasmids encoding chloramphenicol resistance and a *Leishmania* antigen. Thus Lm is a reasonable organism for *Leishmania* antigen delivery, which may bias the immune system toward a Th1-type protective response.

134 MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION IN *ESCHERICHIA COLI* OF THREE DISTINCT CYSTEINE PROTEASE cDNAS FROM *LEISHMANIA DONOVANI CHAGASI*. Omara-Opyene AL*, Ismail SO, and Gedamu L. Department of Biological Science, University of Calgary, Canada.

There is mounting evidence to suggest that cysteine protease is the molecule which empowers *Leishmania* to survive inside the macrophages. We have recently isolated and characterized three distinct cysteine protease cDNAs from *Leishmania donovani chagasi*. We found that one of these cysteine protease genes is expressed abundantly during the promastigote stage of development and is turned off during the amastigote stage of development. The other two cysteine protease genes are expressed abundantly during amastigote stage of development and are turned off during the promastigote stage of the development. We have also examined the genomic organization of the two families of cysteine proteases from this species of *Leishmania*. We found that the amastigote specific cysteine proteases (Ldccys1) from *L. donovani chagasi* are encoded by tandemly organized genes located on chromosome 7. The promastigote specific cysteine protease (Ldccys2) appears to be a single copy gene and is located on chromosome 10 of *L. donovani chagasi*. The organization of the two families of cysteine protease genes in *L. donovani donovani* was also found to be similar. In this species, the family of amastigote specific cysteine protease genes (Lddcys1) is located on chromosome 5 while the family of promastigote specific cysteine protease gene (Lddcys2) is located on chromosome 8. These findings suggest that the products of the amastigote specific cysteine protease genes are important in the pathogenesis of *Leishmania*. We have expressed the three cDNAs in a bacterial expression system and purified large amounts of biologically active cysteine protease. The recombinant cysteine proteases are being used in a series of experiments designed to understand the functions of these genes.

135 IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* $(T1B)_4$ MAP VACCINE IN *AOTUS* MONKEYS. Moreno CA*, Rodriguez RJ, Oliveira GA, Calvo-Calle JM, Nussenzweig RS, and Nardin EH. Instituto de Inmunologia, Hospital San Juan De Dios, Santa Fe de Bogota, Colombia, South America; and Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

The immunogenicity and genetic restriction of the antibody response to a multiple antigen peptide (MAP) malaria vaccine candidate was determined in primate hosts. Two species of owl monkeys, *Aotus nancymai* and*Aotus vociferans*, which differ in phenotype and karyotype, were immunized with a MAP, $(T1B)_4$, containing T and B epitopes derived from the repeat region of the *P. falciparum* CS protein. Although primary responses differed significantly in the *A. nancymai* versus *A. vociferans* the administration of booster injections of $(T1B)_4$ elicited similar high levels of anti-repeat antibodies, with titers in excess of 10^5, in both *A. nancymai* and *A. vociferans* monkeys. The magnitude and specificity of the anti-sporozoite antibody responses in both species of monkeys was similar to that observed in the MAP simmunized murine responder strains. Five out of five monkeys immunized with a MAP/alum vaccine, a formulation acceptable for human use, developed anti-sporozoite antibodies, although titers were lower than those obtained with Freunds adjuvant. In addition, the MAP/alum was found to

elicit an anamnestic response in an owl monkey with a pre-immune low anti-repeat ELISA titer. These results suggest that the(T1B)4 MAP malaria vaccine may be immunogenic in individuals of diverse genetic background and that the administration of the MAP vaccine to individuals living in malaria endemic areas may effectively increase antibody levels and resistance to re-infection.

136 GENETIC RESTRICTION OF PRIMATE AND MURINE IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM* MAP VACCINE. Calvo-Calle JM*, Hurley CK, Clavijo P, and Nardin EH. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY; and Department of Microbiology and Immunology, Georgetown University, Washington, DC.

In order to design synthetic vaccines capable of eliciting an immune response in individuals of diverse genetic backgrounds, efforts have been made to identify parasite derived "universal" T cell epitope(s) for incorporation into multiple antigen peptide (MAP) vaccines. An epitope contained in aa 326-345 of the CS protein of *Plasmodium falciparum* has been identified as a potential "universal" T cell epitope based on the ability of human $CD4^+$ T cell clones to recognize the 326-345 peptide in the context of DR 1,4,7 and 9 class II HLA molecules. The correlation between genetic restriction of the human T cell clones and an *in vitro* peptide binding assay using transfected murine cells lines, or EBV transformed human B cell lines, was determined. Soluble DR and DQ molecules were also used in an ELISA based peptide assay to analyze the specificity of peptide interaction with class II molecules. Similar analysis was carried out using murine cell lines expressing defined class II molecules. MAPs containing *P. falciparum* CS repeat-derived B cell epitopes combined with the 326-345 sequence, have been tested for immunogenicity in mice of different backgrounds. These studies have been used to determine whether (1) *in vitro* peptide assays can be used to predict the immunogenicity of MAPs *in vivo* and (2) MAPs containing the 326-345 T helper epitope can function as "universal" vaccines and elicit immune response in hosts of diverse genetic background.

137 PROTECTIVE EFFICACY OF A LIPOSOME ENCAPSULATED R32NS1 *PLASMODIUM FALCIPARUM* MALARIA VACCINE IN A HUMAN CHALLENGE MODEL. Magill AJ*, Fries BT, Gordon DM, Wellde BT, Owens R, Krzych U, Schnieder IP, Wirtz RA, Kester K, Ockenhouse CF, Stoute JA, and Ballou WR. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC; Department of Entomology, Walter Reed Army Institute of Research, Washington DC; and Center for Immunization Research, Johns Hopkins University, Baltimore MD.

Animals and humans immunized with irradiated sporozoites develop antibodies (Abs) directed against the circumsporozoite (CS) protein and are protected against infection when challenged with sporozoites. Vaccines based upon immunodomiant CS repeats suffer from poor immunogenicity in humans and have been unreliable in protecting against sporozoite challenge. We have tested a vaccine preparation designed to maximize the Ab response to R32. R32 was expressed as a fusion protein with NS1, a nonstructural protein from influenza. In addition, R32NS1 was encapsulated in liposomes containing MPL, a potent adjuvant. Two groups of volunteers were vaccinated at 0,2, and 4 months with a "low dose" (LD) formulation (N=7) and a "high dose" (HD) formulation (N=6) and then challenged with infected mosquitos 2 weeks following the 3rd dose. Five of the 7 in the LD group were protected but 1 of the 3 infectivity controls failed to develop malaria. Only 1 of 6 in the HD group was protected and all 4 infectivity controls were infected. There appeared to be no significant difference in Abs to R32 as measured by ELISA between those protected and not protected. A third challenge study using only the LD formulation resulted in only 1 of 9 vaccines being protected and 4 of 4 infectivity controls infected. The protected individual did have a significantly higher Ab titer to R32 by ELISA and by sporozoite IFA. These results emphasize the importance of inoculum effect in the challenge model and support the concept that Abs to the CS repeat should play an important role in pre-erythrocytic vaccines.

138 HUMAN CTL RESPONSES TO THE *PLASMODIUM FALCIPARUM* SPOROZOITE SURFACE PROTEIN 2: IDENTIFICATION OF EPITOPES RESTRICTED BY SIX HLA CLASS I ALLELES. Wizel B*, Houghten R, Ballou WR, Paoletti E, Setti A, Coligan JE, Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Department of Molecular Microbiology & Immunol, The Johns Hopkins University, Baltimore, MD; Torrey Pines Institute for Molecular Studies, San Diego, CA; Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; Virogenetics Corporation, Troy, NY; Cytel Corporation, San Diego, CA; Laboratory of Molecular Structure, National Institute of Allergy and Infectious Diseases, Rockville, MD; Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Malaria Program, Naval Medical Research Institute, Bethesda, MD.

Vaccines designed to induce CTL against *Plasmodium falciparum* SSP2 (PfSSP2) are under development because $CD8^+$ CTL specific for the *P. yoelii* SSP2 protect mice against malaria by eliminating infected hepatocytes. Optimal development of PfSSP2 as a component of human malaria vaccines requires identification of multiple conserved CTL epitopes restricted by the most common HLA class I alleles. To this purpose, PBMC from 6 volunteers immunized with *P. falciparum* irradiated sporozoites were stimulated with recombinant vaccinia virus expressing PfSSP2, and with a panel of PfSSP2-derived 15-amino acid peptides bearing the binding motifs of 4 HLA-A (A1, A2, A3, A24) and 2 HLA-B (B7, B8) alleles. Antigen-specific, genetically restricted, $CD8^+$ T cell-dependent CTL activity against autologous target cells expressing PfSSP2 was demonstrated in all 6 volunteers. Two to 12 motif-bearing peptides per allele were capable of sensitizing HLA-class I-matched target cells for lysis by peptide-stimulated effectors. Peptide-specific, genetically restricted and $CD8^+$ T cell-dependent CTL activity was demonstrated in 5 of 6 volunteers. A limited set of shorter peptides representing the motif-bearing sequences within positive 15-mers were able to bind to their respective restricting element *in vitro*, and to sensitize target cells for lysis by effectors stimulated with the 15-mers. HLA-A2-, A-24 and HLA-B8-restricted peptide-specific CTL activity was also demonstrated using unstimulated PBMC. Available data indicate that from 1 to 6 of the positive A1, A2, A3 and B8 motif-bearing peptides are conserved among *P. falciparum* isolates. The identification of multiple CTL epitopes on PfSSP2 restricted by 6 common class I alleles provides important information to develop an epitope-based anti-liver stage malaria vaccine.

139 KILLING OF *PLASMODIUM YOELII* EXOERYTHROCYTIC STAGES, *IN VITRO*, BY NYLS3 MONOCLONAL ANTIBODY REQUIRES A HEAT INACTIVATABLE SERUM COMPONENT. Sacci Jr. JB*, Porrozzi R, de la Vega P, Charoenvit Y, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Rockville, MD; and Department de Ultraestrutura, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil.

Recent evidence has clearly established the capacity of a monoclonal antibody (NYLS3), directed against a *Plasmodium yoelii* liver stage antigen, to inhibit the development of exoerythrocytic (EE) stage parasites *in vitro* and *in vivo*. NYLS3 did not bind to sporozoites, thus, inhibition occurred through the action of the antibody upon the EE parasite using a mechanism distinct from that responsible for antibody inhibition of sporozoite entry. NYLS3, directly labeled with fluorescein, was able to enter *P. yoelii* infected hepatocytes and could be detected as early as 30 min. after addition to *in vitro* cultures. Antibody could not be seen in uninfected hepatocytes nor on the surface of infected hepatocytes, but vesicles containing strongly staining material were clearly evident (in infected cells) by utilizing confocal microscopy. The numbers of infected hepatocytes visualized in cultures fixed and then stained with NYLS3 were equivalent to those seen in cultures that were live stained. NYLS3 was able to gain entry to all infected hepatocytes, suggesting a mechanism specific to infected cells that allowed the antibody access to the schizont. NYLS3 inhibited EE parasites in the inhibition of liver stage development assay (ILSDA) when non-heat inactivated fetal calf serum was used in the media, but its capacity to clear parasites from the culture was reduced (65-80%) when heat inactivated serum was used. Therefore, a heat inactivatable serum component, possibly complement, was required for the inhibitory effects of NYLS3 upon P. yoelii liver stage parasites. Studies are underway to determine the effect of Fab fragments upon infected hepatocyte cultures.

140 INHIBITORY MONOCLONAL ANTIBODIES TARGET A CONFORMATIONAL EPITOPE WITHIN THE 83 kDa *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN (PF83/AMA1). Narum DL*, van der Wel A, Dubbeld M, and Thomas AW. Division of Parasitology, National Institute for Medical Research/MRC, Mill Hill, London, United Kingdom; and Laboratory of Parasitology, Biomedical Primate Research Centre, Rijswijk, The Netherlands.

The 83 kDa apical membrane antigen of *Plasmodium falciparum* (PF83/AMA1) is an analogue of the 66 kDa molecular forms seen in other species of malaria including the *P. knowlesi* 66 kDa apical protein PK66/AMA1. PK66/AMA1 is expressed in late-stage schizonts; is processed to a 44/42 kDa doublet at, or around, the time of schizont rupture; and is the target of Fab fragments which inhibit parasite development independent of schizont maturation, *in vitro*. PF83/AMA1 is expressed in late stage schizonts; is N-terminally processed to a 66 kDa species which at, or around, the time of schizont rupture may become associated with the merozoite's surface. Analysis of the sero-prevalence of naturally acquired antibodies (Abs) against PF83/AMA1, using a full-length recombinant baculovirus product (PF83-7G8-1), demonstrated a sero-prevalence rate of 94-100% in two different West African populations. We were interested to test whether Ab against PF83/AMA1 could inhibit parasite development. Therefore Louvain rats were immunized with 0, 1, 25 μg PF83-7G8-1 formulated in three different adjuvants: i) polyphosphazene, a polymer; ii) polyalphaolefin, a water in oil; iii) and Freund's as control. The Ab profiles were similar for each adjuvant. Purified polyclonal Abs were tested for growth inhibition using a two-cycle radiometric assay and inhibited parasite growth from 10-50%. Analysis of a subsequent panel of rat monoclonal Abs (mAbs) identified two competitive mAbs which recognized a conformational epitope that inhibited *P. falciparum* development by 60-70% at 2 mg/ml. This indicates that the 83 kDa molecular form of PF83/AMA1, is also a target for parasite inhibitory responses, and a potential component of a multivalent malaria vaccine.

141 IMMUNOGENIC CHARACTERISTICS OF THE *PLASMODIUM FALCIPARUM* RHOP-3 PROTEIN DEFINED BY A C-TERMINAL RECOMBINANT. Yang J*, Blanton RE, King CL, and Sam-Yellowe TY. Department of Biology, Cleveland State University, Cleveland, OH; Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH.

Plasmodium falciparum high molecular weight rhoptry protein complex (Rhop-H/110, 130,140 kD) has been implicated in the process of host cell invasion. The Rhop-3 protein (110 kD) is inserted into the erythrocyte membrane during merozoite penetration. To gain further understanding of the immunological importance of the Rhop-3 protein, we isolated and sequenced a cDNA clone RH3 3-5 which is a 1.2 kb insert that matched the 3' end of the reported Rhop-3 gene. A recombinant protein of 44 kD was expressed in *E. coli* using the RH3 3-5 cDNA, and purified on a Ni-resin column. From the recombinant protein, Rhop- 3 specific rabbit antisera were generated. The strong and specific reaction of this antisera with rhoptries in IFA confirms that the protein is of rhoptry origin. Further, the monospecific antisera recognized a single band of 110 kD in a *P. falciparum* extract. Unlike other Rhop-H specific antisera, no major "break-down" products were bound by this antisera nor were higher molecular weight proteins recognized. Therefore, at least at the C terminus, components of the Rhop-H (110, 130, 140 kD) are not immunologically related. In immunoblotting assays, the recombinant protein was recognized by sera from several malaria endemic regions. The reactivity of the recombinant protein in an ELISA with sera from an endemic area compared to North American control sera demonstrates that the recombinant has potential as an immunodiagnostic antigen. The recombinant protein will be used to evaluate the immunogenicity of the native Rhop-3 protein in populations living in endemic areas and the role of Rhop-3 in erythrocyte invasion.

142 ANTIBODY RESPONSES TO THE MEROZOITE SURFACE PROTEIN, MSP1 IN NAIVE *AOTUS* MONKEYS INFECTED WITH FALCIPARUM MALARIAS. Hui GS*, Hasiro C, Nikaido C, Kaslow DC, and Collins WE. Department of Tropical Medicine, University of Hawaii, Honolulu, HI; Laboratory of Malaria Research, National Institutes of Health, Bethesda, MD; and Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

We have shown that conserved B-epitopes were immunodominant in animals hyperimmunized with parasite-purified or recombinant MSP1 of *Plasmodium falciparum*. Cross-priming studies also suggested that conserved T-helper epitope(s) are efficient in inducing the anti-MSP1 antibody response. In this study, we determine whether a similar profile of immune responses were induced during live falciparum infections. Naive *Aotus* monkeys were infected by blood-stage challenge with either one of the two dimorphic MSP1 alleles represented by the FUP and FVO parasites. Sera collected after parasite clearance were analyzed by ELISAs. Monkeys infected with parasites carrying one allelic form of MSP1 had antibodies that were equally reactive with homologous or heterologous MSP1s. This preferential recognition of conserved epitopes of MSP1 was confirmed by competitive binding ELISAs. Studies with *P. yoelii* and *P. falciparum* show that the C-terminal 19 kDa fragment of MSP1, MSP1-19 is the target of protective immunity. Thus, monkey sera were assayed for recognition with recombinant MSP1-19s expressing variant and conserved B-epitopes. Results of direct and competitive binding ELISAs showed that the anti-MSP1-19 antibodies were also primarily directed against conserved determinants. The similarities between vaccine- or infection-induced antibody responses suggest a possible reciprocal enhancement of the two populations of anti-MSP1 antibodies when a subunit MSP1 vaccine is introduced into populations living in malaria endemic areas. This, together with previous observations that conserved determinants are important in MSP1-mediated immunity provide an optimistic outlook that a subunit MSP1 vaccine may be effective and practical for field applications in malaria-exposed populations.

143 NATURAL IMMUNE RESPONSES TO RECOMBINANT C-TERMINAL 19 KDA ANTIGEN OF *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 (MSP-1) IN IMMUNE ADULTS FROM KENYA. Shi YP*, Udhayakumar V, Sayed U, Anyona D, Roberts JM, Hightower AW, Oloo A, Hawley WA, Kaslow DC, Nahlen BL, and Lal AA. Division of Parasitic Disease, NCID, Centers for Disease Control and Prevention, Atlanta, GA.; Vector Biology andControl Research Center, Kenya Medical Research Institute, Kisumu, Kenya; and Laboratory of Malaria Research, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

In this study, we have characterized the natural immune responses against the 19 kDa antigen in 57 immune adults from western Kenya, a malaria holoendemic area. T-cell proliferative response and IgG antibody isotypes were determined using three known variant forms of recombinant 19 kDafragment, which are referred to as E-KNG, Q-KNG and E-TSR yMSP1$_{19}$ antigens. We found that 54% of subjects showed positive proliferative response to Q-KNG yMSP1$_{19}$ antigen,while only 37% and 35% of subjects responded to E-KNG and E-TSR yMSP1$_{19}$ antigens respectively. In IgG isotype analysis, it was shown that IgG1 and IgG3 antibodies were the major isotypes against all

three variant antigens. The IgG1 responses were predominantly directed against the B-epitopes that are conserved between the variant yMSP1$_{19}$ antigens, whereas IgG3 antibody responded the variant specific B-epitopes in addition to the conserved B-epitopes of the antigens. Among the three variant antigens tested, IgG3 antibody responses against E-KNG, and Q-KNG antigens were found to be more prevalent than to E-TSR antigen in western Kenya. There were higher IgG3 response but lower proliferative response in the parasitemic group when compared to the nonparasitemic group with all three variant antigens. This information on the natural immune responses to the 19 kDa fragments will be beneficial in the development of a MSP-1 based malaria vaccine.

144 IMMUNE RESPONSES TO CONSERVED ASEXUAL-STAGE T CELL EPITOPES IN CHILDREN AND ADULTS RESIDING IN ETOA, A VILLAGE WITH HOLOENDEMIC MALARIA IN CAMEROON. Quakyi IA*, Leke R, Befidi R, Bomba-Nkolo D, Manga L, Njeungue E, Fogako J, Eno A, Sama G, Djokam R, Achidi E, and Ngu J. Department of Biology, Georgetown University, Washington, DC; and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon.

In order to develop an efficacious vaccine for *Plasmodium falciparum*, the dynamics of infection and acquisition of natural immunity, especially to vaccine-candidate antigens, need to be understood. Thus, in Fall 1994, a longitudinal study was initiated in the rural farming village of Etoa (located near Yaounde) where malaria is holoendemic. The goal is to understand the relationship between clinical outcome of infections and specific cellular and humoral responses. A census determined that there are 485 residents in the village. The principal vectors for malaria transmission were found to be *Anopheles funestus, A. nilli,* and *A. gambiae* with an estimated 47-49 bites per person per night. Prevalence results showed that 98% of malaria infections were due to *P. falciparum,* 2% were mixed infections of *P. falciparum* and *P. malariae,* and 4% had concurrent *Loa loa* microfilaraemia. Peripheral blood samples were collected from a large cross-section of the population and all donors had high antimalarial IgG titers, with a mean of 1:1000. Lymphocytes were tested *in vitro* for proliferation in response to conserved T cell epitopes present in MSP-1, MSA-2, AMA-1, and SERA. Adults showed a more restricted pattern of T cell proliferative responses to conserved epitopes compared to responses in children. These studies provide baseline data with which results from subsequent studies can be compared.

145 INDUCTION OF TRANSMISSION BLOCKADE IN MICE INFECTED WITH *PLASMODIUM BERGHEI.* Beetsma AL*, van de Wiel T, Sauerwein RW, and Eling W. Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands.

For the analysis of the mechanism of transmission blocking immunity and the identification of immune factors involved two models were developed.The transmission-blocking immunity was targetted to gametocytes, and not to gametes. In the first model mice were immunized by a chemotherapeutically controlled infection. Immunized mice are protected against challenge but remain carriers of the parasite. Mice were either immunized against *Plasmodium berghei* Anka, which produces gametocytes, or against *P. berghei* K173, a non-producer. Anka immune carrier mice are producers of low numbers of gametocytes that possibly support transmission-blocking immunity. The K173 strain was used as a negative control. Transmission capacity was tested 4 hours after the intravenous injection of Anka-infected blood containing 10^6 gametocytes per inoculum. In these analyses Anka immune mice exhibit a profound transmission reduction capacity in comparison to the K173 immune mice. In the second model mice were immunized by the injection of purified gametocytes mixed with Freund's Adjuvants or by adjuvant alone. These mice are not immune to the asexual stages of the parasite. The transmission reducing capacity can be determined on day 2, 3 and 4 after infection with the gametocyte-producing Anka strain. Gametocyte-immunized mice reduced transmission activity considerably in comparison to the adjuvants controls.

146 STUDIES OF NON-CONVENTIONAL FORMULATIONS AND VACCINE DELIVERY SYSTEMS TO ELICIT ANTI-Pfs25 TRANSMISSION-BLOCKING ANTIBODIES IN RODENTS AND PRIMATES. Kaslow DC*, Price V, Keister DB, Gupta S, Min-Ying, Kumar S, Cleland J, Tine J, Lanar D, Wellde B, Shiloach J, and Paoletti E, and Muratova O. Malaria Vaccine Section, LPD, NIAID, NIH, Bethesda, MD; Immunex Corp., Seattle, WA; NIH Clinical Center Pharmacy, NIH, Bethesda, MD; Biotechnology Unit, NIDDK, NIH, Bethesda, MD; Virogenetics Co., NY; Pharmaceutical R & D, Genentech Inc., South San Francisco, CA; Division of Immunology, WRAIR, Washington, DC.

Mice vaccinated three times with clinical-grade yeast-produced Pfs25 (TBV25H/ alum) elicit antibodies that completely block infectivity to mosquitoes (transmission-blocking actvity, TBA); however, sera from rabbits or primates likewise vaccinated reduce but do not completely block infectivity. It's likely, therefore, that clinical-grade TBV25H/alum will elicit, in vaccinated humans, antibodies that reduce but not block infectivity. Non-conventional formulations or delivery systems may be required to elicit TBA. We have studied glycolide-lactide pulsatile release microspheres of TBV25H (PRM-TBV25H) and a variety of delivery systems, including Salmonella,

direct DNA injection and attenuated vaccinia virus (NYVACPf7) each delivered alone or in combination with a booster injection of TBV25H/ alum. A single injection of PRM-TBV25H, either w/ or w/o TBV25H/ alum, elicits TBA in vaccinated mice. Primate studies of PRM-TBV25H are now planned. None of the other delivery systems, given either alone or (with the exception of NYVACPf7) in combination with a booster of subunit TBV25H/ alum elicited TBA in rodents. Three priming doses of clinical-grade NYVAC- Pf7 followed by boosting with clinical-grade TBV25H/alum elicits potent TBA in rabbits and a significant response in primates. Further studies are needed to determine if fewer priming doses or even a single injection of NYVACPf7 with TBV25H/alum will elicit TBA in rodents and then primates.

147 CELLULAR AND HUMORAL RECOGNITION OF CANDIDATE MALARIA TRANSMISSION-BLOCKING VACCINE ANTIGENS IN NATURALLY EXPOSED PERSONS. Ohas EA*, Kaslow DC, and Duffy PE. US Army Medical Research Unit- Kenya/Kenya Medical Research Institute,Kisumu, Kenya; and Laboratory of Malaria Research, National Institutes ofHealth, Bethesda, MD.

Malaria transmission-blocking vaccines may be useful in a number of control or quarantine modalities. Two *Plasmodium falciparum* surface proteins, Pfs25 and Pfs 28, appear as the parasite develops in the mosquito midgut.Yeast-expressed forms of both proteins (called TBV25H and yPfs28) elicit transmission-blocking antibodies in animals, but issues of human immunogenicity and vaccine boosting during infection remain. In a holoendemic area of western Kenya, 15/33 and 9/33 frequently exposed individuals had proliferative responses (S.I.>2) to TBV25H and yPfs28,respectively. 18/33 people responded to one or the other antigen, with 9/33 responding to TBV25H alone, and 3/33 responding to yPfs28 alone. Except for one individual who recognized both proteins at 2 μg/ml and 10 μg/ml, there was discordance in the recognition of the two antigens at similar concentrations, suggesting the response was specific to the recombinant protein and not directed against a by-product of the yeast expression system. Sera preadsorbed with yeast-expressed MSP1 demonstrated little, if any, recognition of either TBV25H or yPfs28 above that of non-immune sera. Within the group, relative antibody titers against either protein failed to correlate with the presence or absence of a proliferative response. The results suggest that these sexual stage antigens, or some cross-reactive protein(s), appear while the parasite resides in the human host. Inclusion of both antigens may increase the proportion of individuals who respond to the immunogen, and perhaps the proportion which may boost during subsequent infection.

148 ANALYSES OF PARASITOLOGICAL AND ENTOMOLOGICAL PARAMETERS IN BANACOUMANA, MALI, A POTENTIAL SITE FOR TESTING MALARIA TRANSMISSION-BLOCKING VACCINES. Doumbo O*, Toure YT, Diallo M, Sakai D, Bagayogo M, Kouriba B, Muratova O, Keister DB, and Kaslow DC. Department of Epidemiology and Parasitic Diseases, Mali National School of Medicine and Pharmacy, Bamako, Mali; and Malaria Vaccine Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.

In an effort to define an area with seasonal malaria transmission that might serve as a site for malaria vaccine testing, a group of adults (18-68 yo), adolescents (10-17 yo) and children (1-9 yo) residing in avillage, Banacoumana, located 60 km east of Bamako, Mali were studied for parasitemic and gametocytemic rates by analyses of Giemsa-stained bloodsmears and for mosquito infectivity by direct feeds of laboratory-reared F1 progeny of locally caught mosquitoes. Analyses of parasitological parameters by distribution in the village revealed that, for reasons thatare presently unclear, gametocyte-positive volunteers appeared to be clustered geographically. Comparison by age groups revealed that thechildren had higher parasite positivity, higher gametocytemic and higher mosquito infectivity rates than did the adolescents or adults (65.9% v56.4% v 35.0%; 11.3% v 10.4% v 8.1%; 90.0% v 82.3% v 63.2%, respectively), although the differences in the gametocytemic rates for all ages and the mosquito infectivity rates for the children and adolescents were not statistically significant ($P > 0.05$). Serum collected from a subset of the volunteers at the time of direct mosquito feed were studied in the standard membrane feeding assay. Infectivity by direct feed on the volunteer did *not* correlate with the effect serum samples from volunteers had on infectivity by membrane feeds. The data suggest that Banacoumana may be a feasible site for studying malaria transmission-blocking vaccines and that the standard membrane feeding assay may not be a perfect predictor of what will be observed in an *in vivo* setting.

149 MALARIA INCIDENCE MEASUREMENT AND VACCINE TEST SITE DEVELOPMENT IN VIETNAM. Doan H*, Richie TL, Nguyen DT, Tran TU, Luc NT, Church CJ, Le XH, Vu DC, Corwin AL, Le PT, Le DC, and Hoffman SL. Institute for Malariology, Parasitology and Entomology, Hanoi, Vietnam; U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; and Naval Medical Research Institute, Bethesda MD.

Multiple malaria vaccine testing sites are needed to evaluate the many vaccines under development and because of the expectation that efficacy will vary geographically. This expectation is based on differing transmission intensities, heterogeneity in vaccine antigens due to genetic differences among *Plasmodium falciparum* strains, and differences

in HLA type among vaccinees. Epidemiological data such as malaria incidence must be collected at test sites in order to evaluate their potential for vaccine testing. Between June, 1993 and January 1995, we measured malaria incidence at two potential vaccine test sites in Vietnam: Bai Chanh Commune, Thanh Hoa Province, northern Vietnam, and Khanh Nam Commune, Khanh Hoa Province, southern central Vietnam. Baseline point prevalence positivity rates for *P. falciparum*, *P. vivax* and *P. malariae* were 3.2%, 0% and 0% in Bai Chanh (July, 1993) and 14.4%, 11.3% and 1.0% in Khanh Nam (June, 1994). At Bai Chanh and Khanh Nam respectively, 187 and 134 people given radical cure to eliminate all stages of the malaria parasite. The regimen consisted of quinine sulfate, 10 mg/kg every eight hours for four days, doxycycline hyclate 2 mg/kg every 12 hours for ten days, and primaquine phosphate 0.5 mg base/kg daily for fourteen days. At neither study site were any positive smears recorded within 28 days of initiation of radical cure, indicating that this regimen was highly effective in eliminating malaria infection in these sites. Each cohort was followed for six months using weekly smears and daily visits, including the high transmission (wet) season at both sites. At Bai Chanh, the crude attack rate of *P. falciparum* and *P. vivax* at six months was 4.3% and 2.1% respectively. At Khanh Nam, the crude attack rate of *P. falciparum*, *P. vivax* and *P. malariae* at six months was 43%, 22% and 13% respectively. Sample size calculations indicate that the Khanh Nam site could distinguish a *P. falciparum* vaccine with 50% efficacy from placebo with a power of 0.8 and significance level of 0.05 if approximately 75 people were followed in each group for six months (whereas more than 900 per group would be needed at Bai Chanh). Additional data needed to characterize the Khanh Nam study site are being collected.

150 THE 3' NONCODING REGION OF WILD-TYPE STRAINS OF YELLOW FEVER VIRUS HAVE SIGNIFICANT DIFFERENCES: GENETIC AND BIOLOGICAL IMPLICATIONS. Wang E, Tesh RB, Shope RE, Weaver SC, and Barrett AD*. Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX.

The nucleotide sequences of the 3' noncoding regions (NCRs) of 12 wild-type strains of yellow fever (YF) virus have been compared. The results show that there are significant differences between them. Of three repeating conserved sequences (CS) in the 3'NCR, one was deleted and another modified in the viruses isolated from South America and regions of Africa other than West Africa. Thus, only one copy of the CS is required for replication of YF virus. On the basis of the 3'NCR, we could classify YF strains into viruses from West Africa which contained 511 nucleotides in the 3'NCR and those from other regions which contained 445 nucleotides in the 3'NCR. All YF strains studied have the same 19 nucleotides following the NS5 protein gene stop codon at nucleotides 10389 to 10399. Beyond this sequence 45 nucleotides have been deleted. These results suggest that the 3'NCR of YF viruses will be very useful for genetic and epidemiologic studies of YF virus. Interpretation for genetic and epidemiologic studies of YF virus. Interpretation of the evolution of YF virus will be presented by comparison of the 3'NCR sequences with those from other regions of the genomes of the same strains.

151 EFFECTIVENESS OF LIVE-ATTENUATED HAMSTER KIDNEY CELL CULTURE DERIVED JAPANESE ENCEPHALITIS VACCINE (SA_{14}-14-2). Hennessy S*, Zhengle L, Tsai TF, Strom BL, Caoming W, Huilian L, Xiangtai W, Bilker WB, Quimao L, Karabatsos N, and Halstead SB. Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA; West China University of Medical Sciences, Sichuan Province, People's Republic of China; Centers for Disease Control and Prevention, Ft. Collins, CO; and Health Sciences Division, The Rockefeller Foundation, New York, NY.

Japanese encephalitis (JE) is a major cause of death and disability throughout Asia. Although an effective JE vaccine is available, many children at risk remain unimmunized because of the vaccine's expense. In 1988, an inexpensive, monovalent, live-attenuated primary baby hamster kidney-cell culturederived JE vaccine was licensed in the People's Republic of China. Although the vaccine's efficacy is reported to be as high as 98%, there are concerns regarding the methodology of these studies. In order to measure the effectiveness of this vaccine, we conducted a case-control study of JE occurring in rural Sichuan Province during the summer of 1993. Cases consisted of children under 15 years who were hospitalized for an acute febrile central nervous system illness, and were confirmed by JE neutralizing antibody tests. Village- and age-matched controls were identified, and vaccination histories determined from preexisting records. Vaccine efficacy was calculated as 1-odds ratio. Forty serologically confirmed cases and 965 matched controls were identified. The effectiveness of two doses was 98% (95% confidence interval, 83%-99.8%). Adjustment for potential confounders did not affect the results. There were insufficient data to measure the effectiveness of one or three doses. We conclude that a two-dose regimen of this vaccine is highly effective in reducing the risk of acquiring JE.

152 INTRANASAL CHALLENGE MODEL FOR TESTING JAPANESE ENCEPHALITIS VIRUS VACCINES IN RHESUS MACAQUES. Raengsakulrach B*, Nisalak A, Myint KS, Thirawuth V, Ngampochjana M, Young GD, Ferguson LM, Innis BL, and Vaughn DW. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Veterinary Medicine, Armed Forces Research Institute

of Medical Sciences, Bangkok, Thailand; Connaught Laboratories Inc., Swiftwater, PA; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

An alternate method to evaluate the efficacy of second generation Japanese encephalitis (JE) vaccines is needed since customary methods are either unethical or impractical. Building on the method of Harrington that used intranasal inoculation of JE virus (Beijing strain) to produce a lethal infection in monkeys, we investigated if the model could be used for efficacy tests of JE vaccine candidates using an alternative JE virus strain (KE93). In a pilot study, three JE-naive rhesus macaques were inoculated intranasally with an undiluted virus suspension (2.0×10^{10} pfu). All three developed JE encephalitis 9-14 days post infection (p.i.) as confirmed by isolation of JE virus from nervous tissues and the presence of JE-specific IgM in the CSF. The rise in JE-specific IgG, IgM, and neutralizing antibody in the sera were documented on days 10-12 days p.i. JE virus in the sera and JE antigen on peripheral blood mononuclear cells were also detected intermittently throughout infection. To determine the 50% lethal dose (LD50) of the virus, 3 additional groups of macaques (3 per group) were inoculated with dilutions of virus. The LD50 of JE virus was 6×10^7 pfu. Time course of infection and immunological responses in animals who developed JE encephalitis were similar to those seen in the three macaques in the pilot study. In a vaccine efficacy test, one LD90 of virus was used. Four of 4 animals formerly receiving a licensed JE vaccine were protected while 4 of 4 JE-naive macaques succumbed to the challenge. This study demonstrates that the virus strain, route of inoculation, dose, and the outcome measure (encephalitis) are suitable for protective efficacy studies of JE vaccines.

153 EVALUATION OF IMMUNOGENICITY OF NYVAC-JEV AND ALVAC-JEV ATTENUATED RECOMBINANT JAPANESE ENCEPHALITIS VIRUS - POXVIRUS VACCINES IN HUMANS. Kanesathasan N*, Smucny JJ, Konishi E, Kurane I, Shope R, Vaughn DW, Mason PW, Paoletti E, Pincus S, Marks DH, Ennis FA, and Hoke CH. Division of Communicable Diseases & Immunology, Walter Reed Army Institute of Research, Washington, DC; Yale Arbovirus Research Unit, Department of Epidemiology & Public Health, School of Medicine, New Haven CT; Division of Infectious Diseases & Immunology, Dept of Medicine, Universtiy of Massachusetts Medical Center, Worcester MA; Deptment of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Virogenetics, Inc., Troy NY; and Connaught Laboratories, Inc., Swiftwater PA.

A controlled, randomized, double blind Phase I study evaluated responses to NYVAC-JEV or ALVAC-JEV poxvirus vaccines in volunteers distinguished by vaccinia immune status. Groups of ten volunteers received each experimental vaccine. Immunogenicity was measured by antibody (neutralization, radioimmunoprecipitation) and T cell(proliferation assay) responses. NYVAC-JEV vaccine induced antibodies to JEV envelope (E) and nonstructural-1 (NS-1) proteins in volunteers (9/10 and 7/10, respectively). However, vaccinia-nonimmune (V-N) recipients of NYVAC-JEV vaccine seroconverted with higher JEV neutralizing antibody titers (5/5, GMT day 58 1:61) than vaccinia-immune (V-I) volunteers(seroconversion 0/5, GMT <1:10; V-N vs V-I, p=0.01). ALVAC-JEV was less immunogenic in volunteers (4/10 with E or NS-1 antibodies, seroconversion 1/10, GMT 1:6 on day 58). Both experimental vaccines induced T cell responses to JEV antigens (4/10 NYVAC-JEV, 3/10 ALVAC-JEV) with no clear differences between V-N and V-I volunteers. However, all 5 V-N and 1 V-INYVAC-JEV recipients had proliferative responses to NYVAC virus vector while 1 V-N and 3 V-I ALVAC-JEV recipients had responses to ALVAC virus vector. The 3 V-I ALVAC-JEV recipients also developed responses to NYVAC virus vector. NYVAC-JEV poxvirus vaccine appears immunogenic, but vaccinia immune status has a significant effect upon immune responses.

154 THE DENGUE AND DENGUE HEMORRHAGIC FEVER EPIDEMIC IN PUERTO RICO, 1994-1995. Rigau-Perez JG*, Vorndam AV, and Clark GG. Dengue Branch, Centers for Disease Control and Prevention, San Juan, PR.

From June 1994 to February 1995, 25,266 cases of dengue were reported to the laboratory-based surveillance system in Puerto Rico (PR), in comparison with an average 8,800 cases annually in 1991-93. Possible risk factors for epidemic disease in 1994 included closure of municipal sanitary landfills; water rationing in many cities; and three years of dengue-1 (DEN-1) as the predominant virus serotype. The earliest indicator of exceptional disease activity was the higher than usual positivity rate for virus cultures in May (15-18%, compared to 0-4% in 1992). Epidemic dengue first occurred in west-central mountain areas, then the north, west, and east regions. DEN-2 was the predominant serotype isolated (590, 63.1%), followed by DEN-4 (197, 21.1%) and DEN-1 (148, 15.8%). The male to female ratio among reported cases(preliminary results) was 52:48; two-thirds of cases were younger than 29 years of age (the 10-19 year age-group had the highest incidence,11.4/1000 population); 5,309 (21.0%) showed hemorrhagic manifestations, and 4,457 (17.6%) were hospitalized. From October 12 to December 15,1994, the average daily bed occupancy for dengue or dengue-like illness in the 56 general acute-care hospitals was 232, with markedly uneven geographic distribution. Infection control nurses provided 2,059 reports of hospitalized cases, of which 139 (6.5%) fulfilled the clinical criteria for dengue hemorrhagic fever or dengue shock syndrome(12, 0.6%). Twelve fatal cases had positive diagnostic samples for dengue. Ecologic analysis of risk factors showed that 23 of 27 (85.2%)of

municipalities with water rationing had a higher rate of dengue in 1994-95 than they had in 1992-93, compared to 31 of 51 (60.1%)municipalities without rationing (relative risk 1.40; 95% confidence interval 1.07-1.84, $p<0.05$).

155 A STUDY OF THE SPATIAL AND TEMPORAL DISTRIBUTION OF DENGUE CASES DURING AN OUTBREAK IN PUERTO RICO (1991-1992) USING A GIS APPROACH. Morrison AC*, Santiago M, Reiter P, Rigau-Perez JG, Clark GG. Dengue Branch, Centers for Disease Control and Prevention, San Juan, PR; and Water Resources Division, Caribbean District, U.S. Geological Survey, Guaynabo, PR.

Control strategies for dengue need to target an appropriate geographic area. We studied the distribution of dengue cases in Florida, Puerto Rico (pop. 8,689) using a Geographic Information System (GIS). We identified 377 cases from a laboratory-based dengue surveillance system and georeferenced their residential addresses by digital zoning maps and a geopositioning system (GPS). We then used GIS software (ARC/INFO™) to map the cases and to determine the distance between cases with onset dates separated by 0-10, 11-20, and 21-30 days. Weekly plots showed clustering within housing developments. The interval between nearest cases ranged from 0-240 days; only 20% were separated by 11 to 30 days, a period roughly compatible with the sum of the intrinsic and extrinsic incubation periods of dengue. More than 80% of the neighboring case pairs were within 500 m of each other. Fifty-one houses had multiple cases (n=123 cases). Of the 2nd to 6th cases within a house (n=72), 37(51%) occurred within 5 days of each other. The small size of the study area (26 km^2) may have restricted virus dispersal. Nevertheless, our results indicate that control programs which react to individual dengue cases are unlikely to be effective unless they account for the rapid temporal and spatial dissemination of the virus.

156 A GRAPHIC METHOD FOR DETECTING REGIONAL INCREASES IN DENGUE INCIDENCE IN PUERTO RICO, 1994-95. Millard PS*, Rigau-Perez JG, Deseda CC, and Clark GG. Epidemic Intelligence Service, Centers for Disease Control and Prevention, Atlanta, GA; Dengue Branch, Centers for Disease Control and Prevention, San Juan, PR; and Epidemiology Division, Puerto Rico Department of Health, San Juan, PR.

The prompt detection of dengue outbreaks is important to alert communities and vector control programs. The Centers for Disease Control and Prevention's (CDC) graphic method to demonstrate significantly increased reporting (used in the Morbidity and Mortality Weekly Report's Figure 1) was retrospectively applied to laboratory-based surveillance data to evaluate its signals of increased dengue activity during the 1994-95 epidemic in Puerto Rico (PR). Data were analyzed by 4-week periods for the 8 regions of the PR Department of Health. We defined a significant increase in incidence as an excess of suspected cases >2 standard deviations over the mean for all 4-week periods from April to June, 1989-93 (the period of lowest seasonal incidence). No region showed a significant increase in incidence until the period ending June 25, when the system detected a significant increase in the Aguadilla region, corresponding to an epidemiologically confirmed outbreak there. By July 9, the system documented that the dengue outbreak continued in Aguadilla and outbreaks were detected in the regions of Arecibo,Mayaguez, and San Juan, while reports for all of PR also showed a significant increase. By the period ending October 7, significantly increased dengue incidence was shown in every region of PR. These results agree with the increases noted by routine analysis of surveillance data throughout the 1994-95 dengue season. This analytic method provides a timely, visually striking statistically-based signal for control efforts. Prospective use of this method with 1995 surveillance data will allow us to further assess its overall utility.

157 NATURAL VERTICAL TRANSMISSION OF DENGUE VIRUSES IN *AEDES AEGYPTI* (L.) IN FRENCH GUIANA. Fouque F*, Reynes JM, Carinci R, and Gaborit P. Laboratoire d'Entomologie médicale, Institut Pasteur de Guyane, Cayenne Cedex, French Guiana.

The infected status of the mosquito *Aedes aegypti*, vector of dengue in French Guiana, was studied during one year. The mosquitoes were collected in the field at all stages: eggs, larvae, pupae and adults. The immatures were reared in the laboratory until the adult stage. The adults were pooled and processed for virus isolation in AP61 cells culture. Two DEN-2 and one DEN-4 strains were isolated out of the 5108 individuals processed in 393 pools. The dengue strains were all isolated from adults collected at the immature stage, either eggs or larvae, in three different locations in French Guiana. One strain was isolated from the city of Saint-Laurent-du-Maroni, one strain from the devil islands and one strain from the city of Cayenne. The vertical transmission under natural conditions of dengue viruses in Ae. aegypti in French Guiana was demonstrated with a non negligible minimum infection rate of one infected mosquito per 1702 tested mosquitoes.

158 EARLY DIAGNOSTIC INDICATORS OF DENGUE. Kalayanarooj S*, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachu-eke S, Kiatpolpoj S, Innis BL, Rothman AL, Nisalak A, and Ennis FA Bangkok Children's Hospital, Bangkok, Thailand; Department of Virology,

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Division of Infectious Diseases and Immunology, University of Massachusetts Medical Center, Worcester, MA; Kamphaeng Phet Provincial Hospital, Kamphaeng Phet, Thailand; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

As part of a multi-center, prospective study of dengue pathophysiology, clinical predictors of dengue were sought. One hundred and eighty-nine febrile children (temperature >38.5°C for less than 72 hours) with a flushed face and without signs of localized infection were followed as in-patients. Sixty children were confirmed to have dengue; another 112 children had other, self-limited, febrile illnesses (OFI). Dengue patients were older (8.1 versus 6.2 years). Histories of anorexia and vomiting were elicited more frequently among dengue patients (odds ratios = 3.3, 95% CI 1.4-8.6 and 2.1, 95% CI 1.1-4.4 respectively). Early in the illness (2 and 1 days before defervescence) a higher percentage of dengue patients had a positive tourniquet test. Total WBC count and absolute polymorphonuclear cell (PMN) and monocyte counts were depressed, and plasma AST was elevated in dengue patients as compared to other patients as shown in the table. Early in the febrile phase of illness, a positive tourniquet test, depressed white blood cell count (especially decreased PMN and monocytes) and an elevated AST should increase clinical suspicion for dengue.

Test	Day -2 Dengue	Day -2 OFI	Day -1 Dengue	Day -1 OFI
Tourniquet	70%	44%	88%	38%
Total WBC	5320 cells/mm3	8739	4880	9479
Absolute PMN	3389 cells/mm3	5899	2676	6289
Absolute Mono.	88 cells/mm3	252	89	213
AST	97 U/L	46	90	43

159 DENGUE IN THE EARLY FEBRILE PHASE: VIREMIA AND ANTIBODY RESPONSES. Vaughn DW*, Green S, Kalayanarooj S, Innis BL, Nimmanitya S, Suntayakorn S, Rothman AL, Ennis FA, and Nisalak A. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Division of Infectious Diseases and Immunology, University of Massachusetts Medical Center, Worcester, MA; Bangkok Children's Hospital, Bangkok, Thailand; Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC; Center for Disease Control, Ministry of Public Health, Nonthaburi, Thailand; and Kamphaeng Phet Provincial Hospital, Kamphaeng Phet Thailand.

A multi-center effort to characterize the pathophysiology of dengue using new technologies, a study design that minimizes case selection bias (all cases of fever), and patients enrolled early in the febrile period (fever < 72 hours) began in 1994. Fundamental aspects of dengue are serum viremia and antibody response, described here for the first 189 children enrolled (age 8 mo to 14 years; mean = 6.7 years). Based on 3 diagnostic assays (IgM ELISA, hemaglutination inhibition (HAI), and virus isolation by intrathoracic injection of plasma into *Toxorrhyncites splendans* mosquitoes) 32% of these children had dengue. Among the 60 dengue patients, 4 (7%) had primary dengue infections. The virus isolation rate was 97%. The 2 patients from which a virus was not isolated were enrolled on the day of defervescence with high titers of antibody in the first blood specimen (HAI of 320 and 2560). Duration of viremia (from onset of fever to last measured day of viremia, mean = 4.7 days) did not correlate with disease severity, virus type, or antibody response (primary infections = 5.8 days, 95%CI = 4.2, 7.3; secondary infections = 4.6 days, 95% CI = 4.3, 4.9). Viremia correlated highly with temperature. All specimens collected 2 or more days before defervescence yielded a virus whereas all specimens collected 2 or more days following defervescence failed to yield an isolate. Mean percentages of inoculated Toxorrhyncites mosquitoes positive for virus decreased as fever day zero approached. All 4 dengue serotypes were isolated at both hospitals. This study confirms that all symptomatic cases of dengue as defined by serology experience a measurable viremia during the febrile phase and that as fever subsides, so does viremia.

160 PLASMA CYTOKINE LEVELS AND T CELL ACTIVATION MARKERS IN CHILDREN WITH ACUTE DENGUE. Green S*, Vaughn DV, Kalayanarooj S, Suntayakorn S, Nisalak A, Nimmanitya S, Hussem K, Innis BL, Kurane I, Rothman AL, and Ennis FA. Division of Infectious Diseases and Immunology, University of Massachusetts, Worcester, MA; Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Bangkok Children's Hospital, Bangkok, Thailand; Kamphaeng Phet Provincial Hospital, Kamphaeng Phet, Thailand; Center for Disease Control, Ministry of Public Health, Nonthburi, Thailand; and Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

Prior studies of the immunopathologic mechanisms in dengue hemorrhagic fever (DHF) have revealed elevated TNF α levels and elevations of T cell activation markers in plasma or serum of patients hospitalized with DHF.

The goal of this study was to investigate the events that occur earlier in the course of dengue infection in order to further elucidate the mechanisms of pathogenesis of DHF. We evaluated children in the outpatient departments at Bangkok Children's Hospital and Kamphaeng Phet Provincial Hospital with fever of unknown origin of less than 72 hours duration. Blood specimens were obtained daily until one day following defervescence and at convalescent follow up. We compared levels of IL-1 β, TNF α, interferon γ (IFN-γ), IL-4, IL-6, soluble CD4, soluble CD8 (sCD8) and soluble IL-2 receptor (sIL2R) in children with DHF, dengue fever (DF) and other febrile illnesses (ND). Levels of IFN-γ, sIL2R and sCD8 were higher in DHF and DF than in ND. Elevated levels in DHF occurred earlier in the course of illness. No differences between groups were noted for the other parameters. This study provides evidence that T cell activation in acute dengue exceeds that seen in other suspected viral illnesses and relates to disease severity. Further studies are warranted to determine the value of T cell activation markers as early predictors of severe dengue.

161 CYTOKINE PRODUCTION BY DENGUE-SPECIFIC MEMORY T LYMPHOCYTES. Gigstad JE*, Kurane I, Janus J, and Ennis FA. Division of Infectious Diseases and Immunology, University of Massachusetts, Worcester, MA.

There is evidence that the immune system plays a role in the pathogenesis of dengue hemmorhagic fever through the actions of serotype cross-reactive antibodies and memory T lymphocytes. Distinct subsets of T lymphocytes have been described on the basis of their cytokine production: Th1, associated with clearance of intracellular pathogens, and Th2, associated with help for antibody production and immune suppression. Both extremes of cytokine production have been associated with immunopathology. We studied cytokine production by CD4+ and CD8+ memory T lymphocytes from recipients of live attenuated dengue virus vaccines. Stimulation of peripheral blood mononuclear cells with inactivated viral antigen induced production of high levels of IFNγ and low levels of IL-4. When lymphocytes were stimulated in bulk culture with antigen in the presence of IL-4, production of IFNγ was decreased. We established 54 lymphocyte clones from 2 donors after initial stimulation in the presence of IL-4 and anti-IL-12. These clones produced more varied cytokine profiles with a trend toward decreased IFNγ. Thus, dengue-specific lymphocytes demonstrate a predominant Th1-like phenotype after primary infection. The cytokine milieu in which these memory T lymphocytes are activated during secondary infection may tip the lymphocyte response toward immunopathology.

162 INFECTIOUS DENGUE TYPE 2 VIRUS RNA MADE BY *IN VITRO* SYNTHESIS FROM A FULL LENGTH cDNA CLONE. Zhao B*, Warren R, Snellings N, Hoke CH, and Putnak JR. Departments of Virus and Bacterial Diseases, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

Dengue (DEN) viruses are important human pathogens causing periodic epidemics of DEN fever and the more severe DEN hemorrhagic fever and shock syndrome. In order to further study this virus and to develop future vaccine strategies, a full-length, infectious cDNA clone was constructed for DEN-2 (NGC strain) virus. Virus cDNA was cloned into pGEM-3z which contains a T7 RNA promotor. A silent mutation was introduced into the DEN sequence at nt position 9385 (A>T) which abolished a KpnI site. Full length RNA was made by *in vitro* transcription with T7 RNA polymerase and transfected onto Vero cells using the Lipofectin reagent. After 7 or 8 days the transfected cells were trypsinized, subcultured and overlayed with agarose. Virus was detected by the formation of plaques. The virus recovered from the infectious clone is being compared with the parent virus by immunofluorescence assay, SDS-PAGE analysis, and restriction endonuclease analysis to verify the absence of the Kpn site at nt 9385.

163 NUCLEIC ACID SEQUENCE POINT MUTATIONS ASSOCIATED WITH LIVE, ATTENUATED DENGUE-2 VIRUS VACCINES PREPARED IN PRIMARY DOG KIDNEY CELLS. Henchal EA*, Pedersen JC, Innis BL, Vaughn DW, Dubois DR, Eckels KH, and Hoke CH. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

We evaluated the biological and genetic characteristics of live, attenuated dengue-2 virus vaccines prepared after 30 (PDK30), 40 (PDK40) and 50 (PDK50) passages of the Sl 6803 strain in primary dog kidney (PDK) cells. Final production lots were prepared in diploid fetal rhesus lung cells. Pre-clinical and clinical data indicted that replication of PDK50 virus was limited in monkeys and humans. Human reactivity to the PDK50 vaccine was minimal. While PDK30 virus demonstrated delayed replication in the monkey model, all volunteers developed dengue-like symptoms with demonstrable viremia. The PDK40 virus, which is approaching clinical trials, had growth characteristics similar to PDK50 in the monkey model. Independent, overlapping reverse

transcriptase/polymerase chain reaction (RT/PCR) products prepared from infected cell RNA, purified virions or vaccine culture supernatants were cloned into TA plasmid vectors or sequenced directly using an automated DNA sequencer. We identified 10 unique point mutations in CDNA prepared from attenuated PDK50 virus. PDK50 and PDK40 viruses shared at least one point mutation (nt57; cytosine to uridine) in the 5'untranslated region (UTR). This point mutation was found also in dengue-2 16681 PDK53 vaccine and may disrupt an otherwise conserved 5' UTR stem-loop structure in the RNA. This mutation was not found in the nucleotide sequences of virulent dengue-2 viruses. While the attenuated dengue-2 PR 1 59 Sl lacked this unique mutation, similar sequence modifications disrupted a 5' UTR stem loop structure in the RNA of this vaccine candidate. These point mutations may contribute to dengue-2 virus attenuation after growth in PDK cells.

164 PATHOGEN DERIVED RESISTANCE TO DENGUE-2 VIRUS IN MOSQUITO CELLS BY EXPRESSION OF CAPSID AND PREMEMBRANE CODING REGIONS OF THE VIRAL GENOME. Olson KE*, Higgs S, Gaines PJ, Beaty BJ, and Blair CB. Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

The full-length capsid (C) and premembrane (prM) coding regions of dengue virus type 2 (DEN-2; Jamaica) genome were expressed in C6/36 (*Aedes albopictus*) cells in either sense or antisense orientations from double subgenomic Sindbis (dsSIN) viruses. Northern blot analysis confirmed the expression of sense or antisense DEN-2 prM or C RNA in infected C6/36 cells. C and prM proteins were demonstrated in cells infected with dsSIN viruses expressing DEN-2 sense RNAs by radiolabeling and immunofluorescent assays. C6/36 cells were infected with each dsSIN virus at a multiplicity of infection (MOI) of 50 and challenged 48 h later with DEN-2 NGC (New Guinea C) at 0.1 MOI. Whereas C6/36 cells infected with control dsSIN viruses supported high levels of DEN-2 replication, C6/36 cells infected with the dsSIN viruses expressing C or prM antisense RNA were completely resistant to DEN-2 challenge. Cells expressing prM protein or untranslatable sense RNA were completely resistant to DEN-2 challenge. Cells expressing prM protein were not resistant to DEN-2 virus when challenged at an MOI of 10. However, untranslatable sense prM RNA was completely protective when challenged at the high MOIs. Heterologous interference was not observed in any dsSIN infected C6/36 cells after challenge with DEN-3 or DEN-4 viruses. The expression of full-length C protein was toxic to C6/36 cells. Sense suppression of DEN-2 virus by expressing untranslatable prM RNA may be an important strategy for inhibiting flavivrus replication in mosquito cells. Sense suppression of DEN-2 virus will also be analyzed in *Aedes aegypti*.

165 PUPAL SURVEY: AN EPIDEMIOLOGICALLY SIGNIFICANT SURVEILLANCE METHOD FOR *AEDES AEGYPTI*. AN EXAMPLE USING DATA FROM TRINIDAD. Focks DA*, and Chadee DD. Medical and Veterinary Entomology Research Laboratory, U. S. Department of Agriculture, Gainesville, FL; and Insect Vector Control Division, Trinidad and Tobago Ministry of Health, St. Joseph, Trinidad, WI.

Traditional measures of *Aedes aegypti* abundance (the House, Container, and Breteau Indices) do not correlate well with risk of dengue transmission because human densities, productivity of the larval habitat, weather, and seroprevalence of dengue antibody are not taken into account. We surveyed 16 urbanizations throughout Trinidad, recording the types and numbers of water-filled containers present and the associated average standing crop of *Ae. aegypti* in each type. The average number of foci per hectare was 287 and ranged from 65 to 499. The average standing crop of pupae was 10 per container and ranged 12-fold from 3 (indoor vases) to >30 in flower pots. The product of a container type's pupal production and abundance provides an estimate of its importance in producing *Ae. aegypti*. Seven of the 11 container types observed accounted for <10% of all production; 4 of the 11 types (outdoor drums, laundry tubs, buckets, and small containers) accounted for >90% of all production. The average number pupae of person was 157 and ranged 40-fold from 216 (St. George County) to 8,438 (Victoria county). There was absolutely no correlation between any of the traditional indices and *Ae. aegypti* pupae per person or per hectare. These results will allow recently-developed simulation models of dengue transmission to used to estimate how receptive urban and rural areas of each county of Trinidad are to new dengue introductions.

166 EVALUATION OF A SIMPLE BED NET TRAP FOR SAMPLING AFRICAN MALARIA VECTORS. Ofulla AV*, Hawley WA, Beach RF, Roberts JM, and Hightower AW. Kenya Medical Research Institute, Nairobi, Kenya; Division of Parasitic Diseases, NCID, CDC, Nairobi, Kenya; and Division of Parasitic Diseases, NCID, CDC, Atlanta,

Measurement of biting rates by African malaria vectors is often done either by all night biting collections at human bait (HB) or pyrethrum spray collections (PSC) in conjunction with ELISA identification of human blood fed mosquitoes. PSC and HB are too labor-intensive for large scale application. We have tested simple, inexpensive bed net traps (BNT) to sample African malaria vectors. Traps were evaluated in the field in Siaya District, western Kenya in two ways. First, numbers and species of mosquitoes captured by the BNT were directly compared with the

HB and PSC methods by collecting mosquitoes from the same houses using each method on successive nights. Forty widely scattered houses were sampled on a rotating basis over a 24-week period. Correlation coefficients for numbers of both *Anopheles funestus* and *An. gambiae* complex species were similar for all three pairs of collection methods. Second, the specificity of the BNT for collecting mosquitoes biting individuals sleeping under the trap was evaluated using DNA profiling to identify sources of individual blood meals. Results indicated that more than 90% of *An. gambiae* captured by BNT had fed on individuals sleeping under the traps. Further, nearly all *An. gambiae* captured resting outside the BNT had fed on individuals sleeping outside the BNT. Therefore, BNT is less labor intensive, highly specific, and correlates well with PSC and HB methods.

167 ABUNDANCE, BITING FREQUENCY, AND AGE STRUCTURE OF *LUTZOMYIA CRUCIATA* (DIPTERA: PSYCHODIDAE) IN A MEXICAN FOCUS OF LOCALIZED CUTANEOUS LEISHMANIASIS. Rebollar-Tellez EA , Fernandez-Salas I, Van Wynsberghe NR, and Andrade-Narvaez FJ. Department of Immunology, Center of Regional Research (CIR), University of Yucatan (UADY), Merida, Yucatan, Mexico; Fac. of Biological Science, Medical Entomology Lab., University of Nuevo Leon, San Nicolas de los Garza, NL, Mexico.

Localized cutaneous leishmaniasis is a serious disease in the State of Campeche, Mexico. The incidence rate is 5.08 per 1000 inhabitants. The high-risk population are men between 14 - 45 years old who overnight in the forest. Early studies incriminated *Lutzomyia olmeca olmeca* as the vector of *Leishmania mexicana* in the Peninsula of Yucatan. However, recent studies indicate the possibility of other sand fly vectors. During one year, human bait collections were carried out 10-nights monthly (except March, 23 nights) from 6 to 10 pm. in a sub-tropical forest of the State of Campeche. The only biting species was *Lu. cruciata*. A peak of abundance was found during February and March. *Lu. cruciata* was more active from 6 to 7 pm. The regressions between catches and two climatic factors showed that *Lu. cruciata* was more active under a humidity of 88% to 100% and a temperature of 19 to 22°C. In March, 347 females were dissected to determine the age structure of the population. The parity rate was 67%with the highest peak occurring the first day and lower peaks about four-days intervals. The estimated gonotrophic-cycle length and survivor- ship using a cross-time series were not found significant, but an alternative estimate of survivorship gave 0.684. Further studies are required to fully incriminate *Lu. cruciata* as a vector of localized cutaneous leishmaniasis at the State of Campeche, Mexico.

168 FITNESS COSTS ASSOCIATED WITH A STRAIN OF *AEDES AEGYPTI* REFRACTORY TO *PLASMODIUM GALLINACEUM*. Yan G, Severson DW, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Genetic control of mosquito-borne disease critically depends on introduction and spread of parasite-inhibiting genes in the vector population. Understanding the fitness effect of these genes will provide important information towards a strategy to influence vector competence at the population level. Two *Aedes aegypti* strains, refractory and susceptible to *Plasmodium gallinaceum*, were selected from the Moyo-In-Dry strain (MOYO) through intensive inbreeding. Reproductive success and survivorship of the two strains were compared, and the influence of the parasite on mosquito fitness also was evaluated. Several fitness components were studied, including fecundity, egg-to-adult survivorship and developmental time, blood-meal size and body size. The refractory strain has a significantly shorter egg-to-adult developmental time and a smaller body size, takes a smaller blood meal, and subsequently lays less eggs than the susceptible strain. The mean longevity of the refractory strain is significantly shorter than the susceptible strain. Exposure to the parasite exhibited little effect on the survivorship and fecundity of either strain. Several factors may contribute to the lower fitness of the refractory strain, including inbreeding depression, the effect of other genes linked to the genes conferring refractoriness and pleiotropic effects associated with these genes. We currently are mapping the genes conferring decreased fitness in the refractory strain, using quantitative trait loci (QTL) mapping techniques.

169 HYBRIDIZATION AMONG SPECIES OF THE SAND FLY GROUP RELATED TO *PHLEBOTOMUS PAPATASI* PARALLELS GENETIC PROFILES AND GEOGRAPHIC DISTRIBUTION. Munstermann LE*, Ghosh KN, Guzman H, Tesh RB, and Mukhopadhyay J. Yale School of Epidemiology and Public Health, Yale University, New Haven CT; and Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX.

The Afro-Asian leishmaniasis vector, *Phlebotomus papatasi*, is distributed in North Africa, the Middle East and across India. A close relative, *P. duboscqi* is found throughout Central Africa south of the Sahara. The genetic profiles of two laboratory strains of *P. papatasi* (ISRAEL and INDIA) and one of *P. duboscqi* (KENYA) were established for 22 enzyme loci. The two species were clearly identified by 7 diagnostic enzyme loci--*Mdh-2* (malate dehydrogenase), *Me* (malic' enzyme), *Tre-2*, *Tre-3* (trehalases), *Dia-3* (diaphorase), *Idh-1* (mitochrondrial isocitrate dehydrogenase) and *Est-3* (an esterase). Mass and single pair crosses among the three strains were successful at

normal levels for the *P. papatasi* strains. F_1 hybrids were obtained between the *P. duboscqi* and *P. papatasi* strains, but at a very reduced level. They were clearly recognized by isozyme patterns unique to the hybrids. All attempts at backcrosses failed. The geographic distribution of a third species, *P. bergeroti,* is centered in northeast Africa midway between the *P. papatasi* and *P. duboscqi* distributions. Previous workers have successfully hybridized *P. bergeroti* with *P. papatasi.* The morphological and isozyme patterns of *P. papatasi* contain elements of the two other species and are intermediate in character. Although the three species are clearly distinct taxa, they represent an evolutionary continuum of the Papatasi group in North Africa that reflects their role in the epidemiology of leishmaniasis.

170 MICROSATELLITE DNA VARIATION AND THE GENETIC STRUCTURE OF *ANOPHELES GAMBIAE* POPULATIONS IN MALI, WEST AFRICA. Lanzaro GC*, Zheng L, Toure YT, Petrarca V, Kafatos FC, and Vernick KD. Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX; European Molecular Biology Laboratory, Heidelberg, Germany; Malaria Research and Training Center, National School of Medicine and Pharmacy, Bamako, Mali; Institute of Parasitology, University of Rome, Rome, Italy; and Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD

Studies of the spatial and temporal distribution of chromosome inversions form the basis of all our current thinking concerning the population genetics of *Anopheles gambiae.* It is on the basis of the distribution of these inversions that *An. gambiae* has been subdivided into different subspecific forms, known as "ecophenotypes". It has been suggested that ecophenotypes represent incipient species, which are to some extent genetically isolated. The overall goal of our work is to determine the degree of reproductive isolation among these forms. Individual female *An. gambiae* were collected from human dwellings at two sites (Banambani and Selikenyi) in Mali. Karyotypes were determined for each individual. Carcasses were held and DNA extracted. Microsatellite DNA genotypes were determined for each. We found highly significant linkage disequilibrium for microsatellite loci and inversions *j* and *u* on chromosome 2. Individual locus genetic distances were calculated for 20 microsatellite loci. We found that the greatest degree of genetic divergence between the BAMAKO and MOPTI forms is associated with loci on chromosome 2, the chromosome carrying inversions which differentiate these two forms. These results fit the expectation that inversions are under selection and represent large blocks of genes which carry particular genotypes which favor adaptation to specific seasonal or regional habitats. If microsatellite loci were not affected by selection (in this case indirectly by "hitchhiking") then we would expect the level of individual locus genetic distances to be roughly equivalent over all loci.

171 DEVELOPMENT OF MICROSATELLITE MARKERS FOR *ANOPHELES MACULATUS,* A MALARIA VECTOR IN THAILAND. Rongnoparut P*, Sirichotprakorn N, Yaicharoen S, Rattanarithikul R, and Linthicum KJ. Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Department of Parasitology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.

Anopheles maculatus is a species complex that includes the major malaria vector of Malaysia and important secondary vectors in southern and western Thailand. Two geographic races, *B* and *E*, of *An. maculatus s.s.* have been found in southern, peninsular Thailand. These two races have different vectorial capacities. Research was undertaken in peninsular Thailand to study the genetic structure of natural population of this malaria vector, particularly in these two races, using microsatellite markers. We attempted to identify several known microsatellite markers of *An. gambiae* in *An. maculatus* populations because there were no sequence data available for microsatellites in *An. maculatus* group. The results showed that no such *An. gambiae* sequences were found in any of the *An. maculatus* specimens examined. We report here the characterization of dinucleotide repeats and the identification of 15 microsatellite markers for *An. maculatus.* The sequences flanking the repeat units were determined. Allele frequencies are being scored and the allelic distribution determined in several *An. maculatus* populations. Our preliminary survey on two of these markers revealed that these loci were polymorphic and numbers of alleles at these loci varied widely. The degree of microsatellite variability of these loci were compared among populations of *An. maculatus* races *B* and *E*.

172 THE *ANOPHELES GAMBIAE* COMPLEX IN SENEGAL: A VERY HETEROGENEOUS TRANSMISSION OF MALARIA. Fontenille D*, Faye O, LeMasson JJ, Lochouarn L, Simard F, Diatta M, Konate L, and Trape JF. ORSTOM, Dakar, Senegal.

Species from the *Anopheles gambiae* complex are the main malaria vectors in Senegal. *An. gambiae, An. arabiensis* and *An. melas* are present and are identified by PCR. Malaria transmission is very heterogenous depending on the regions and the local environment. In Senegal the climate ranges from Sahelian in the north (less than 300 mm of rain per year in 3 months) to Soudanian in the South (1200 mm of rain per year in 7 months). Since 1992

longitudinal surveys have been conducted in 6 areas representative of these climates. Three kinds of transmission are observed: (1) high and continuous: Dielmo (annual EIR: 100 to 200 infected bites per man per year) and Wassadou (annual EIR:200). (2) High and seasonal: Barkedji (annual EIR: 120) and Ndiop (annual EIR: 20 to 60). (3) Low and seasonal: banks of the Senegal river and Dakar (annual EIR: less than 1). *An. gamblae* and *An. arabiensis* are sympatric in 5 of the 6 stations, sometimes with *An. fenestus*. In Dakar *An. arabiensis* is the only species. *An. gamblae* is a better vector than *An. arabiensis* . Malaria transmission by *An. gamblae* (represented by its Savana cytotype) occurs during the rainy season, whereas transmission by *An. arabiensis* is longer, sometimes all year long as in Dielmo. It was observed that the main vectors and the level of transmission could be very different each year. In the Sahelian region, where breeding sites are a long way from each other, malaria transmission is isolated in time and space. For these regions Senegal is an interesting place to study gene flow between vector populations and genetic factors of malaria transmission.

173 IDENTIFICATION OF SURFACE MOLECULES OF MOSQUITO SALIVARY GLANDS WHICH MALARIA SPOROZOITES USE AS RECEPTORS FOR INVASION. Barreau C*, Touray M, Miller LH, and Vernick KD. Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD.

There is evidence which suggests that malaria sporozoites recognize mosquito salivary glands by specific ligand-receptor interactions. We are interested in identifying the putative salivary gland receptor(s) for sporozoite invasion. We use an *in vivo* bioassay for sporozoite invasion of salivary glands. In this assay, purified sporozoites from mature oocysts of *Plasmodium gallinaceum* were injected into *Aedes aegypti* mosquitoes and salivary glands were dissected at different time points after injection. This assay was used to determine the effect of experimental treatments with antibodies and lectins at 24 hours post-injection (where the maximum sporozoite invasion into glands was reached). We raised a rabbit polyclonal antiserum against female *Ae. aegypti* salivary glands which recognized tissue-specific determinants in the basal lamina of salivary glands. Purified IgG antibody fraction of the immune serum blocked sporozoite invasion *in vivo*. We tested a panel of 19 lectins and found seven which bound to salivary glands. Of these seven, S-WGA and WGA completly blocked sporozoite invasion, PSA and SBA partially blocked and Con A, DBA and PHA-E did not block. These observations suggest that there are glycoconjugates on the surface of salivary glands which sporozoites must specifically interact with in order to invade. Because the putative sporozoite receptors contain immunogenic determinants, it is feasible to identify them by an immunological strategy. We generated monoclonal antibodies directed against the surface molecules of salivary glands by immunizing mice with a salivary gland membrane preparation. Results from these studies will be reported.

174 A *IN VITRO* SYSTEM FROM *AEDES AEGYPTI* TO STUDY MIDGUT CELL PATHWAYS INVOLVED DURING HOST CELL INVASION BY VIRUS AND PARASITE. Poupel O, Vazeille M, Shahabuddin M, Tardieux IC*. Institut Pasteur, Ecologie des Systemes Vectoriels, Paris, France; and Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD.

We have developed an *in vitro* system from *Aedes aegypti* midgut tissue culture that maintains the midgut characteristics and functions for several days. The midgut cells display a typical polarized organization and after microinjection of a blood meal into the lumen, proteases are secreted into the lumen and provide complete blood digestion. We have been able to reproduce a viral infection using dengue virus serotype 2 as well as an invasion by *Plasmodium gallinaceum* ookinetes. Such *in vitro* system allows us to study which constitutive and inducible pathways of the midgut cells are critical for the establishment and persistence of the association between the midgut and the pathogen. Using a pharmalogical approach, we are currently looking at the influence of host intracellular calcium and host protein phosphorylation on the fate of the association between dengue virus and *Aedes aegypti* midgut cells as well as between malaria ookinetes and *Aedes aegypti* midgut cells. The *in vitro* system protocol and results on these studies will be presented.

175 MICE IMMUNIZED BY BITES OF *ANOPHELES STEPHENSI* DEVELOP ANTIBODIES THAT INHIBIT ACTIVITY OF MOSQUITO SALIVARY GLAND APYRASE. Mathews GV, Sidjanski S, and Vanderberg JP*. Department of Medical & Molecular Parasitology, New York University School of Medicine, New York, NY.

BALB/c mice were immunized to mosquito saliva by bites of *Anopheles stephensi* mosquitoes. Studies were conducted on the ability of these mice to develop antibodies against the apyrase component of the saliva. By immunoprecipitation procedures and Western blot analysis we demonstrated the presence of anti-apyrase antibodies in the immunized mice. Furthermore, these antibodies were able to inhibit apyrase enzymatic activity. Serum titers of 1: 20 were able to inhibit approximately 90% of salivary gland apyrase activity, while titers of 1: 160 retained the ability to inhibit more than 50% of apyrase activity. Parallel inhibition assays with IgG from immunized vs. non-immunized mice showed that the inhibitory activity of serum from immunized mice could be accounted for by its IgG component. Antibodies raised against *An. stephensi* saliva did not immunoprecipitate

apyrase from a homogenate of bovine endothelial cells; neither did these antibodies inhibit the enzymatic activity of the apyrase nor inhibit the apyrase activity of a monolayer of the bovine endothelial cells. Mosquito salivary gland apyrase has previously been shown to facilitate mosquito feeding by inhibiting hemostasis at the mosquito bite site. However, our studies have shown that mosquitoes feeding on immunized mice had no deficiency in probing these mice for a blood meal, even in the face of high titers of anti-apyrase antibodies.

176 TARGETED MAPPING OF LOCI LINKED WITH GENES AFFECTING DISEASE TRANSMISSION BY MOSQUITOES. Severson DW*, and Kassner VA. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Bulked-segregant analysis has been used for saturation mapping of specific genome regions in a wide variety of organisms. The method involves the evaluation of pooled DNA samples representing selected individuals from segregating populations. The individuals for each pool are selected to represent the two parental types for a character of interest. Within each pool the individuals are, in theory, identical for the gene of interest and, because of random segregation and recombination, arbitrary for all other genes. Markers that are polymorphic between the pools will reflect a high probability of being linked genetically with the character used to prepare the pools. We are using bulked-segregant analysis to identify RAPD markers linked with genes determining vector competence in *Aedes aegypti*. We initially evaluated an F_2 intercross population for RFLP marker genotypes at loci for two genome regions that contain major genes affecting vector competence. This includes a region on chromosome 1 that affects vector competence for the filarial worm parasites *Brugia malayi* and *Dirofilaria immitis* and a region on chromosome 2 that affects vector competence for *B. malayi* and the malarial parasite *Plasmodium gallinaceum*. Pools were prepared, consisting of 8 individuals each, that represented the respective parental genotypes at the RFLP loci. The pools were screened, using RAPD-PCR, for amplified fragments unique to one of the pools representing one of the two genome regions. Putative polymorphisms were evaluated against DNA preparations for the individual mosquitoes from the entire intercross population. We have used this technique to map several RAPD loci linked with the RFLP loci on both chromosomes 1 and 2, respectively. Therefore, bulked-segregant analysis with RAPD-PCR seems to be effective for fine-scale mapping of specific genome regions in mosquitoes.

177 SEX-LINKAGE OF PERMETHRIN RESISTANCE IN A FIELD STRAIN OF *AEDES AEGYPTI*. Mebrahtu YB*, Taylor MF, and Norem J. University of Arizona, Department of Entomology, Tucson, AZ.

The genetic mechanisms that confer permethrin resistance were investigated in two strains of *Aedes aegypti*, vectors of yellow fever and dengue haemorrhagic fever. Larval resistance to permethrin in the *Ae. aegypti* field strain "Couva-Trinidad" has been conclusively linked to the sex-determining locus by analysis of backcrosses to the susceptible "Rock" strain. Resistance was recessive in the F_1 hybrids when the male parent was resistant, as contrasted with co-dominance of resistance when the female parent was from the resistant strain. Moreover, there was about 1:1.79 sex ratio distortion in favor of males when the resistant parent was crossed with a susceptible "Rock" female. *Aedes aegypti* has an autosomal single locus di-allele heterozygote-male system of sex determination. Suppression of the resistance-determining gene by the male-determining gene is similar to previous studies which have reported the suppression of other marker loci near the sex-locus. The backcrosses, which remain to be concluded, bear out this interpretation. Studies are also underway to analyze linkage of permethrin resistance to two sodium channel markers, but will depend upon results of *in situ* hybridization of these probes to polytene chromosomes in *Anopheles gambiae*. This will enable us to place these markers on the chromosomal map and so determine whether either of them lie near the sex-locus on chromosome one.

178 DO MOSQUITOES ORIENT IN MAGNETIC FIELDS? Strickman D*, Weissman M, Novak R, and Estrada-Franco J. Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, DC; Department of Physics, University of Illinois, Urbana, IL; Illinois Natural History Survey, Champaign, IL; and Department of Entomology, University of Maryland, College Park, MD.

Numerous species of bacteria, birds, mammals, and insects have been shown to be capable of detecting Earth's magnetic field lines. The method of detection involves small ferromagnetic particles, the movement of which are apparently sensed by the organism. We examined 14 species of mosquitoes for the presence of ferromagnetic material which might be used in orientation during flight. Preliminary measurements in a super-conducting quantum interferometry device demonstrated two kinds of ferromagnetic material: Material which was apprently internal in *Psorophora columbiae* and material which adhered to the outer surfaces of living or dead mosquitoes. We also found that ferromagnetic dust is a nearly ubiquitous contaminant in the laboratory, corresponding to the presence of such dust in the the atmosphere. Behavior experiments were performed with mosquitoes in a chamber where the orientation of magnetic fields could be manipulated, simulating various orientations of a natural magnetic field. In preliminary experiments, we found that 17% less female *Aedes aegypti* were able to find a host in

a moving magnetic field than in a constant one. We also observed *Anopheles pseudopunctipennis* apparently resting preferentially at either the positive or the negative end of magnetic field lines. Further behavioral experimentation will be necessary to confirm these observations and to determine their importance.

179 IMMUNE RESPONSES OF MICE DEFICIENT FOR 5-LIPOXYGENASE OR 12-LIPOXYGENASE DURING INFECTION WITH *SCHISTOSOMA MANSONI*. Secor WE*, and Funk CD. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Division of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

Inflammatory immune responses to parasite eggs lodged in the presinusoidal capillaries of the liver cause the pathologic sequelae observed during schistosomiasis. Recent studies have described the central role of cytokines in generation and regulation of granulomatous and fibrotic responses to schistosome eggs. However, the mechanism(s) by which cytokines effect these changes remains to be elucidated. Potent mediators of inflammation that may be involved in this process are arachidonic acid and its metabolites. Previous studies have shown that general inhibitorsof lipoxygenase (LO), but not cyclooxygenase, significantly reduce the size of primary lung granulomas which form around schistosome eggs injected into mice. These studies were designed to more clearly definethe roles of 5-LO and 12-LO in acute (8 week) infections of mice. Mice deficient for 5-LO or leukocyte-type 12-LO genes were infected with *S. mansoni* and studied for responses to this infection in comparison with wild type mice. 5-LO knockout mice showed decreased immediate (5 hour) and delayed (24 hr) hypersensitivity to schistosome egg antigen comparedwith wild type or 12-LO knockout mice. Also, granuloma sizes in the 5-LO knockout animals were reduced with respect to the other two study groups. Thus 5-LO, but not 12-LO, appears to be important in effecting the acute immune response to experimental *S. mansoni* infection.

180 T CELL HYBRIDOMAS FROM HIGH AND LOW RESPONDER MOUSE STRAINS RECOGNIZE DIFFERENT SCHISTOSOMAL EGG ANTIGENS. Hernandez HJ*, Brodeur PH, and Stadecker MJ. Department of Pathology, Tufts University School of Medicine, Boston, MA.

Egg-induced pathology in schistosomiasis is mediated by schistosomal egg antigen (SEA)-specific CD4 + T helper cells which in humans may display polar forms of clinical disease. In the experimental murine system, identical infection protocols result in significantly larger egg granulomas and earlier death in the high responder C3H strain in comparison with the low responder C57BL/6 (BL/6) strain. Recent reports have shown that in a variety of systems it is possible to down-modulate specific T cell responses once the immunodominant antigens have been identified, and the same strategy should be applicable to attenuate disease in schistosomiasis. We compared the responses of specific T cell hybridomas from C3H and BL/6 mice to SEA components fractionated by FPLC. Southern blot analysis of rearranged T cell receptor β loci indicated that each hybrid was both clonal and clonally independent. C3H hybridomas exhibited stronger IL-2 responses to components present in several fractions, while various BL/6 hybridomas responded to antigens contained in single fractions. Moreover, a recombinant fragment derived from the p40 egg antigen was recognized by C3H, but not BL/6, hybridomas. All C3H responses were restricted by I-A^k except for one which was restricted by I-E^k. The data suggest that there may be a correlation between the recognized immunogenic T cell epitopes and severity of disease. The identification of the most pervasive egg antigens should lead to the implementation of protocols for targeted T cell tolerance and down-regulation of pathogenic T cell responses by antigen specific means.

181 A CLONED, 38 kDa *SCHISTOSOMA MANSONI* EGG PEPTIDE INDUCES TH1 TYPE LYMPHOCYTE RESPONSES. Cai Y, Langley JG, Smith DI, and Boros DL*. Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI; and Division of Hematology/Oncology Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI.

In schistosomiasis mansoni soluble egg antigens (SEA)-induce tissue granulomas around disseminated eggs. Though several egg-derived antigenic fractions have been isolated, there is a dearth of information on the granulomagenic activity of egg antigens. We targeted for cloning the 38kDa egg peptide because of its observed antigenicity. The prepared cDNA library was inserted into λ phage vector and screened by specific anti 38 peptide antiserum. Positive clones were selected and the genes were subcloned into pGEX vector that yielded the soluble r38. This peptide and its SDS-PAGE-separated native counterpart induced in microgram amounts unaided by adjuvant, TH1 type immune responses in mice as manifested by strong splenic IL-2, IFN-γ production, no IL-4 andtrace amounts of IL-10 secretion. This pattern was confirmed by RT-PCR that also revealed strong IL-2, IFN-γ mRNA expression in splenocytes of r38 sensitized mice. The r38, n38 peptides induced granulomatous responsiveness and elicited mononuclear pulmonary granulomas around peptide coupled beads or live eggs. The two peptides demonstrated extensive cross reactivity in immune responses. Gene sequencing revealed that r38 is

identical with the p40 egg antigen which has homology to the small heat shock proteins. The strong immunogenicity of this peptide is attributed to its relatedness to the heat shock proteins, that are known immunogens. The study of a TH1 type granuloma will shed a better light onthe granulomatous immunopathology in experimental schistosomiasis mansoni.

182 EXPRESSION OF TNFα AND INDUCTION OF SCHISTOSOME EGG GRANULOMAS IS DEPENDENT ON TH PRESENCE OF ADULT PARASITES. Leptak CL*, and McKerrow JH. Department of Microbiology and Immunology, University of California, San Francisco, CA; and Department of Anatomic Pathology, Veterans Affairs Medical Center, San Francisco, CA.

Granulomatous inflammation is key to the pathogenesis of many infectious diseases, including hepatic schistosomiasis. The granulomas which form around parasite eggs trapped in the liver of infected hosts were thought to be induced primarily, if not exclusively, by egg antigens. We now show that the presence of adult worms in the liver, prior to the production of eggs, primes local immune responses key to granuloma formation. When parasite eggs are injected into the livers of naive animals, only minimal inflammatory responses result. However, if eggs are injected into the livers of mice previously infected with single sex adult worms, granuloma formation is restored. Models of infection which isolate the effects of worms from those of eggs confirm that each stage of the life cycle induces a unique pattern of cytokine expression. Hepatic expression of TNFα a major cytokine signal for granuloma formation, is a reaction to adult worms, not eggs. The specific inciting agent may be regurgitated digestive products of adult worms which accumulate in and activate Kupffer cells. Since exogenous administration of TNFα is required for normal egg production *in vitro* and in SCID mice, the data suggests an evolutionary adaptation whereby *S. mansoni* utilizes the host immune response to its own advantage, improving the chance of parasite transmission to a new host. The contribution of adult worm products to the induction of egg granulomas explains differences observed in cytokine expression between natural hepatic infections and models which analyze pulmonary granulomas formed around injected eggs.

183 ASSOCIATION OF HYPERSPLENOMEGALY SYNDROME AND HIGH TNF-A LEVELS IN LIVER HOMOGENATES OF MICE WITH CHRONIC SCHISTOSOMIASIS. Adewusi OI*, Bosshardt SC, Colley DG, and Secor WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

In this study, we investigated what role TNF-α may play in the pathophysiology of chronic (20 week) experimental schistosomiasis; particularly with respect to the development of severe hepatosplenomegalysyndrome (HSS) versus the less serious moderate splenomegaly syndrome (MSS). To do this, we compared TNF-α levels in liver homogenates of male CBA/J mice. Liver homogenates of acute (8 week) and HSS mice hadsignificantly higher levels of TNF-α than MSS mice. In cross sectional studies, TNF-α levels remained low through six weeks of infection, increased sharply by the eighth week of infection, plateaued from weeks ten to fourteen, and then decreased through the twentieth week (for MSS mice). We also examined the ability of soluble schistosome egg antigen (SEA)-stimulated spleen cells from MSS and HSS mice to produce TNF-α and IL-10. SEA-stimulated spleen cells from HSS mice produced significantly more TNF-α than SEA-stimulated spleen cells from MSS mice or uninfected controls. Conversely, SEA-stimulated spleen cells from HSS mice producedless IL-10 than did SEA-stimulated spleen cells from MSS animals, demonstrating the reciprocal relationship between the two cytokines. Based on these findings, we hypothesize that failure to regulate TNF-α production through an IL-10 mediated mechanism may contribute to the development of the chronic HSS syndrome and/or early death.

184 THE IL-10/Fc FUSION PROTEIN INHIBITS EGG GRANULOMA FORMATION IN SCHISTOSOMIASIS. Flores Villanueva PO*, Zheng XX, Strom TB, and Stadecker MJ. Department of Pathology, Tufts University School of Medicine, Boston, MA; and Department of Medicine, Harvard Medical School, Boston MA.

IL-10 is now recognized as a key factor in the down-regulation of cell-mediated hypersensitivity reactions and thus has the potential of attenuating the CD4 + T helper (Th) cell mediated granulomatous inflammation in schistosomiasis. We have shown that macrophages from schistosomal egg granulomas produce IL-10 and that this autocrine secretion negatively regulates their expression of B7 costimulatory molecules as well as their ability to stimulate egg antigen-specific Th cells involved in the pathogenesis of egg granulomas. The present work was performed to assess egg-induced pathology in infected mice injected with exogenous rIL-10 during the early phase of the disease, at a time when natural IL-10 production is still low or absent. For this purpose we used a construct consisting of murine rIL-10 genetically fused to mutated murine Fcγ2a (IL-10/Fc). This compound has all the biological activities of the unconjugated cytokine with the advantage of a 25x longer halflife *in vivo*. Groups of schistosome-infected mice were given IL-10/Fc, control Ig, PBS or no treatment, and were sacrificed 7.5 weeks after

infection. Computer-assisted morphometric analysis of liver sections indicated that IL-10/Fc-treated animals displayed a 26.7-44.7% reduction in granuloma size when compared to Ig-treated controls. Cytokine analysis of supernatants from SEA-stimulated mesenteric lymph node cells showed in IL-10/Fc-treated mice a significant decrease of IFN-γ/IL-2, and an increase of IL-4/IL-10, in comparison with Ig-treated controls. In similarly treated groups of mice sacrificed after 5.5 weeks of infection, IL10/Fc caused a drop in IFN-γ and IL-2, as well as in IL-12, when compared to control Ig. Our data suggest that IL-10/Fc inhibits egg granuloma formation primarily by inhibiting the Th-1 component of the granulomatous response and that this compound represents a potent means to ameliorate schistosomal disease *in vivo*.

185 IN SITU ANALYSIS OF Th CELL-REGULATORY B7 MOLECULE EXPRESSION IN *SCHISTOSOMA MANSONI* EGG GRANULOMAS. Rathore A*, Ricklan DE, Flores Villanueva PO, and Stadecker MJ. Department of Pathology, Tufts University School of Medicine, Boston, MA.

It has been shown that schistosomal egg granulomas are mediated by MHC class II-restricted CD4 + T helper (Th) cells bearing αβ receptor chains. However, Th cells are also dependent on costimulatory signals for effective activation, clonal expansion and cytokine production, as well as for prevention of cell anergy. In schistosome-infected individuals, accessory cells, such as macrophages from egg granulomas, may either stimulate or inhibit egg antigen-specific Th cell responses *in vitro*. Such a regulatory role has been found to depend on their expression of B7 costimulatory molecules, which, in turn, correlates with autocrine secretion of IL-10. In the present experiments we investigated the expression of B7 costimulatory molecules by direct immunocytochemical analysis in situ. For this purpose, cryostat liver sections from groups of mice carrying schistosome infections of 6.5, 7.5, 9.5 and 1 2.5 weeks duration were stained with anti B7-1 and B7-2 mAb, as well as with mAb to MHC class II antigens and to other markers identifying macrophages, T cells and B cells. Results showed that 21.0% of cells in the larger and highly cellular granulomas from the 6.5 week-infected mice stained positive for B7-2. At subsequent times, however, progressively less cellular and more fibrous granulomas were characterized by a sharp reduction in B7-2 staining cells, which declined, respectively, to 8.4%, 6.7% and 4.9% in granulomas from 7.5, 9.5 and 12.5 week infections. In contrast, relative expression of MHC class II molecules suffered only a slight decrease during the same period. Macrophages, T cells and B cells also decreased variously during course of the infection. We were unable to stain for B7-1 molecules with this technique. The data suggest that, by virtue of high costimulatory molecule expression, the early schistosomal egg granulomas are rich in accessory cells with a phenotypic configuration that supports Th cell activation, whereas at subsequent times, granulomas contain increasing numbers of costimulatory molecule-poor accessory cells capable of leading to Th cell unresponsiveness. Such configurations correlate well with observed Th cell functions and granuloma size throughout the experimental infection.

186 UPREGULATION OF B7.2 EXPRESSION ON B CELLS CORRELATES WITH THE DEVELOPMENT OF Th2 PATHWAY IN MURINE EXPERIMENTAL SCHISTOSOMIASIS. Cayabyab M*, and Harn, Jr. DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The most severe clinical manifestation of *Schistosomiasis mansoni* is the egg-induced granuloma formation in the liver. Progression to this clinical form has been postulated to result from the induction of T helper type 2 (Th2) responses. In CBA/J mice, granuloma formation is most severe at 8-9 weeks post-infection. Similar to previous studies we found selective induction of Th2 responses during the period of severe granuloma formation. Soluble egg antigens (SEA) specifically induced the production of Th2 cytokines, IL-4 and IL-10 in the spleen; whereas, SEA did not induce the production of Th1 cytokines, IL-2 and IFNγ. Since the costimulatory molecules B7.1 and B7.2 have been implicated in the development of Th1 and Th2 pathways, respectively, their expression was ascertained during the period where Th2 responses were maximal. Flow cytometric analysis show that B7.2, but not B7.1, was upregulated markedly in the spleen and the draining lymph node during the acute stage of infection. B7.2 was selectively upregulated on B cells, in that macrophages and T cells did not demonstrate upregulation of B7.2 expression. B7.2 expression on B cells was increased four-fold at 9-weeks post-infection compared to normals. Immunohistocytochemical analysis of infected livers revealed a high level of expression of B7.2, but not B7.1, on infiltrating cells surrounding the egg granuloma. Furthermore, the number of B7.2 positive cells in the liver appeared to have increased dramatically in 9-week infected mice. Taken together, these results suggest that disease progression in *S. mansoni* may be associated with egg-specific induction of Th2 responses, mediated by preferential B7.2 costimulation.

187 FIBROSIN, A NOVEL FIBROGENIC CYTOKINE, IS ELEVATED IN BLOOD OF MICE INFECTED WITH *SCHISTOSOMA MANSONI*. Wyler DJ*, and Talebian P. New England Medical Center and Tufts University School of Medicine, Boston, MA.

Tissue fibrosis is the basis of serious morbidity in schistosomiasis, yet little is known of its pathogenesis. Hepatic egg granulomas isolated from infected mice produce a variety of cytokines that stimulate scar-forming responses in fibroblasts, suggesting a molecular basis for this pathological complication of infection. Fibrosin, a recently-identified, novel lymphokine produced by granuloma $CD4^+$ lymphocytes, was purified from among these cytokines. Fibrosin cDNA nucleotide base sequence shares no significant homology with other DNAs. Fibrosin can stimulate fibroblast chemotaxis, growth, and extracellular matrix production. We developed an antigen-capture ELISA to measure fibrosin, and determined that fibrosin is detectable in serum and plasma. The monoclonal antibody (Mab) that captures the antigen was prepared from immunized rats and neutralizes the biological activities of purified native fibrosin. The Mab can immunopurify biologically-active fibrosin (60kD protein) from serum. The reporter is monospecific polyclonal rabbit anti fibrosin IgG. There is a log-linear relationship between fibrosin concentration and $O.D._{405}$ in this assay, with limits of sensitivity in the pg/ml range. Mean fibrosin concentrations in serum or plasma of mice with 8-week *Schistosoma mansoni* infection was observed to be 40-fold higher than that in samples from uninfected control mice. This assay can be expected to facilitate further investigation of the role of fibrosin in tissue fibrosis, particularly when adapted for use in studies of patients with schistosomiasis and other fibrotic disorders.

188 CROSS-SECTIONAL ANALYSIS OF CELL POPULATIONS IN PATIENTS WITH INFECTION BY THE *SCHISTOSOMA MANSONI*. Martins-Filho OA, Cunha-Melo JR, Silveira AM, Prata A, Alves-Oliveira L, Lambertucci JR, Gazzinelli G, Correa-Oliveira R*. Laboratory of Immunology Centro de Pesquisas Rene Rachou-FIOCRUZ, Belo Horizonte, M.G. Brasil; Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte. M.G. Brasil; Faculdade de Medicina do Triangulo Mineiro, Uberaba, Minas Gerais, Brasil; Universidade do Vale do Rio Doce, Governador Valadares, M.G. Brasil; and Faculade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, M.G. Brasil.

Little attention has been given to the identification of the different cell populations, their activation and its correlation with the clinical forms of schistosomiasis. In this paper we describe the flow cytometric analysis of cells derived from the peripheral blood of *S. mansoni* infected patients with different clinical forms (acute, intestinal and hepatosplenic) of the disease and spleen cells from individuals with the severe decompensated hepatosplenic form of schistosomiasis. Our results show a decrease on the percentage of total T cells in the peripheral blood(PB) when compared to normal controls. On the other hand this decrease did not correlate with the observed increase with the severity of the disease,of the activated T cells. Analysis of the CD4+ compartment showed that the highest levels of activated cells are present in the PB of acute and hepatosplenic patients. The percentage of CD8 high+ T cells were decreased in PB of both compensated and decompensated hepatosplenic patients. Although no significant differences were observed on the percentage of CD8high+ cells during the acute phase, analysis of the fraction of activated CD8high cells expressing HLA-DR was significanty decreased in the acute patients and increased with the severity of the disease. When we performed the analysis of the cell population in the spleens of decompensated hepatosplenic (dHS) patients no differences on total percentage of T cell was observed. However, a decrease of CD19+ B cells was clearly detected. As observed in the PB, an increase in activated T cells and of CD4 and CD8 cells was evidenced. Interestingly, a dramatic increase in the NK cell population was observed within the splenocytes of the dHS patients. These results are important since they clearly show a compartimentalization of the immune response during the infection that maybe important for the understanding of the immune factors involved on the development of severity of the disease.

189 PREDICTIVE VALUE OF ABSOLUTE LYMPHOCYTE COUNT IN IDENTIFYING INPATIENTS WITH AIDS IN AN URBAN TEACHING HOSPITAL. Fisher EJ*. Infectious Diseases Division, Medical College of Virginia, Richmond VA.

At our hospital in the midst of the burgeoning heterosexual HIV epidemic, many patients do not have obvious risk factors for HIV. One aspect of trying to identify those with unrecognized HIV disease is laboratory results. Absolute lymphocyte count (ALC) could potentially be an inexpensive alternative to CD4 testing, despite its well-known day-to-day variability. In patients with known HIV disease, an ALC of < 1500 /μLiter has a sensitivity and specificity of approximately 85% for CD4 <200/μL (i.e., AIDS or at risk for serious opportunistic infections). This study analyzes admission ALC of inpatients on a General Internal Medicine service (excludes most Oncology admissions). Of 266 inpatients, 258 had ALC performed. Of these 258, 47 (18%) were HIV-seropositive, 59 (23%) HIV-seronegative, and 152 (59%) had not had HIV serology done. 39 (83%) of the seropositives had CD4 <200. In seronegatives, ALC was ≤1500 in 32 (55%), and ≤1000 in 14 (24%).In those with unknown serology, ALC was ≤1500 in 79 (52%), and ≤1000 in 46 (30%). Excluding patients with cancer (10), severe renal disease (21), and those on immunosuppressive therapy (12) did not significantly change these data. With regard to identifying persons with HIV having <200 CD4, ALC of ≤1500 had sensitivity of 82%, specificity of 51%, positive predictive value (PV) of 22% and negative PV of 94%. ALC of ≤1000 had sensitivity of 54%, specificity of 73%, positive PV of 33% and negative PV of 90%.

190 CURRENT CAUSES OF COMMUNITY-ACQUIRED SEPTICEMIA IN TANZANIA: ROLE OF HIV-1 AND TUBERCULOSIS. Archibald LK, den Dulk MO, Pallangyo KP, and Reller LB. Department of Medicine, Muhimbili Medical Centre, Dar es Salaam, Tanzania; and Clinical Microbiology Laboratory, Duke University Medical Center, Durham, NC.

Current causes of community-acquired sepsis in sub-Saharan Africa are largely unknown, especially as regards mycobacteria (AFB) and fungi. Therefore, we sought to delineate prospectively (over 8 weeks in early1995) the etiology of bloodstream infections in febrile (≥37.5°C axillary) adults (age ≥15 years) admitted to Muhimbili Medical Centre in Dar esSalaam, Tanzania. From a single 20-ml blood sample, 10 ml was cultured aerobically in a biphasic (broth and agar paddle) system and the sediment (after lysis-centrifugation) from 10 ml was cultured on Middlebrook 7H11and inhibitory mold agar slants and in 7H11 broth. All patients were tested for HIV-1 and had thick and thin blood films for malaria as did a control group of 150 matched afebrile patients. Of 513 consecutive patients, 132 (26%) had positive cultures with 138 microorganisms. Of these 132 patients, 104 (79%) were positive for HIV-1. Pathogens isolatedin HIV-positive/HIV-negative patients were as follows: AFB 46/2, non-typhi *Salmonella* 22/3, *Staphylococcus aureus* 6/6, *Escherichia coli* 7/4, *Cryptococcus neoformans* 10/0, and *Streptococcus pneumoniae* 6/4. Malarial parasitemia did not differ in patients (9%) and controls (8%). Mortality was highest (11 of 48; 23%) in patients with AFB. In this un-selected patient population with a high prevalence of HIV, *Mycobacterium tuberculosis* has become the foremost cause of documented sepsis.

191 MALNUTRITION AND TUBERCULOSIS IN A NATIONALLY REPRESENTATIVE COHORT OF ADULTS IN THE UNITED STATES, 1971-1987. Cegielski JP*. Center for Pulmonary Infectious Disease Control, University of Texas Health Center, Tyler, TX.

TB is strongly associated with malnutrition, and both have increased sharply in the USA in recent years. To determine relative and attributable risks for TB due to malnutrition, data from the NHANES-1 Epidemiologic Follow-up Study, a nationally representative cohort of U.S. adults, were analyzed with multivariable Cox proportional hazards methods. The present study cohort included the 10,049 (of 14,407 participants) on whom it was possible to determine TB incidence and who had no prior TB. Baseline data 1971-75 included body mass index (BMI), mid-upper arm muscle area (AMA), skin-fold thicknesses (SFT), and other risk factors for TB. Malnutrition was defined as the bottom decile on each scale of nutrition status. Follow-up 1982-87 provided data on TB incidence by self-report, hospital records, and death certificates. 47 cases of TB were identified in 88,966 person-years of follow-up. Crude incidence density ratios were 5.5 (SFT), 6.9 (AMA), and 8.1 (BMI) for the risk of TB in malnourished vs. well nourished groups. 39% of TB cases were attributable to malnutrition (as low BMI). Controlling for age, race, sex, ethnicity, immigration, income,urbanization, comorbidity and alcohol use, hazard ratios were 9.7 (5.2, 18.2) (BMI), 6.2 (3.3, 11.5) (AMA), and 6.2 (3.2, 11.8) (SFT) for TB due to malnutrition. Malnutrition is a strong and common risk factor for TBA among adults in the USA 1971-1987, independent of other risks.

192 GENERAL PRACTICE AND TROPICAL MEDICINE IN RURAL COSTA RICA. Adams DP*. Department of Family Medicine, Ohio State University College of Medicine, Columbus, OH.

The purpose of this study was to observe primary care in Costa Rica through participant observation. It involved qualitative analysis of the daily provision of basic health care in rural and urban contexts. These findings reflect field data collected in conjunction with the 5th International Tropical Medicine Course, offered jointly by the Louisiana State University Medical Center and the University of Costa Rica Faculty of Medicine. Data was collected during July 1994. Data analysis yielded several findings. Despite efforts by the Costa Rican government to provide primary care for its population, significant problems continue to plague the national health system. Rural regions remain isolated from secondary and tertiary facilities, overburdened border clinics provide care not only for Costa Ricans but for Nicaraguans and Panamanians who enter the country from the North and South. Despite an active control program, malaria continues to be endemic in the eastern coastal areas. Dengue has also reemerged as a renewed health threat. Clinicians, social scientists, and epidemiologists can learn much from the Costa Rican example. The data presented in this paper highlight primary care services in a nation that, despite many years of socio-political stability, continues to struggle with a variety of tropical diseases, malnutrition, and poverty.

193 EPIDEMIOLOGY, PREVENTIVE SERVICES, AND ILLNESSES OF INTERNATIONAL TRAVELERS. Scoville SA*, Bryan JP, and Tribble D. Department of Preventive Medicine and Biometics, Uniformed Services University of the Health Sciences, Bethesda; and Division of Infectious Diseases, National Naval Medical Center, Bethesda, MD.

In order to evaluate the pre-travel medical needs and the costs of immunizations (imm) and medications (meds) required to prepare international travelers, we reviewed records of travelers attending the Travel Medicine Clinic at the National Naval Medical Center (NNMC). A post-travel questionnaire (PTQ) was sent to selected travelers. From Feb. 1991 -Mar. 1995, 1416 patients with a median age of 48 years sought pre-travel care at NNMC. The number of men and women was equal. The most common reason for travel was pleasure; the median duration of travel was 21 days. The most common destinations were Asia (27%), Africa (1 5%), Europe (1 3%), S. Am. (1 1 %), C - Am./Carib. (1 2%), and the M id. East (6%). The median number of imm prescribed was 3. Immune serum globulin was prescribed to 71% at a cost of $2.35 per patient, oral typhoid vaccine to 49% at $2.28, tet/diph toxoid to 31 % at $0.1 9, and yellow f ever to 23% at $3.41. Expensive imm. for hepatitis B ($86.70 per patient), Japanese encephalitis ($103.72), and rabies ($167.16) were provided to 9%, 4%, and 4%, respectively. Therapy for diarrhea (a quinolone and loperamide) was prescribed for 73% at an average cost of $21.90. Anti-malarials were prescribed to 45% of travelers; of these, mefloquine was prescribed to 62% and chloroquine to 24% at an average cost of $30.32 and $11.44, respectively. The average total cost per person for imm. and meds was $56.86. Of the 263 (75%) travelers responding to the PTQ, 17% reported upper resp. inf., 23% diarrhea, and 9% sought medical treatment during travel. Pretravel assessment and counseling of travelers is a valuable service at a reasonable cost if indications for expensive vaccines and anti-malarials are followed.

194 PREVENTIVE MEASURES AND HEALTH PRECAUTIONS EMPLOYED BY TRAVEL MEDICINE ADVISORS BEFORE AND DURING TRAVEL TO MEXICO FOR AN INTERNATIONAL CONFERENCE.
Caputi RA*, Cetron MS, Keystone JS, and Kozarsky PE. Emory University School of Medicine, Atlanta, GA; Center for Disease Control and Prevention, Atlanta, GA; and University of Toronto, Toronto, Canada.

Travel Medicine Advisors (TMAs) give preventive information and health precautions to large numbers of travelers; however, there is little information on preventive measures that these advisors take for themselves. To assess those measures, in April 1995 we distributed a self-administered questionnaire to TMAs during registration at the fourth international conference on travel medicine in Acapulco, Mexico. Of 292 respondents, 191 (53%) were male; the median age was 45 years. Participants were from North America 62% [USA 158 (54%) and Canada 23 (8%)], Europe 28%, other 10%. Professional characteristics of this study population were as follows: physicians (65%), nurses (24%) and other providers (10%). Practice types were academic (28%), private practice (37%), public health (18%), with a median of 7 years in practice. The vaccination status of these providers at the time of the conference was as follows: diphtheria 89%, MMR 63%, polio 90%, tetanus 95%, hepatitis A 60%, immune globulin 13%, hepatitis B 82%, typhoid 45%; several received vaccinations specifically for this trip to Mexico (hepatitis A 27%, immune globulin 8%, hepatitis B 2%, typhoid 8%). Prophylactic measures taken to prevent traveler's diarrhea included: bismuth subsalicylate 9%, antibiotics 8%. In addition, 212 (75%) TMAs chose to carry antibiotics for self-treatment; 69% carried antimotility drugs. Water purification methods were used by 43 participants (15%), of these 20 used iodine chemicals, 5 used filtration devices and 8 used both. Food and beverage risk behaviors were as follows: 38% drank tap water, 45% ate salads, 48% ate cold buffet, and 10% ate raw seafood. The majority of the participants brushed their teeth with tap water (68%), and 25% with purified water. While only 7% of the travel advisors recommend antibiotic prophylaxis to Mexico, 75% chose to carry antibiotics for self-treatment. Interestingly, 28% of study participants acknowledge taking different health precautions that they would recommend to their patients. In general our results indicated TMAs were cautious and conservative for this trip, even so many take different preventive measures from those they usually recommend to their clients.

195 SATISFACTION OF TRAVELERS USING A TRAVEL MEDICINE SERVICE: A PRELIMINARY REPORT.
Shepherd SM, and Shoff WH*. Travel Medicine Service, Department of Emergency Medicine, University of Pennsylvania Medical Center, Philadelphia, PA.

The objective of this study was to determine factors promoting or detracting from traveler satisfaction in a University Travel Clinic. Telephone surveys were conducted upon return from travel. During pretrip visit(s) clients were extensively counseled, given handouts, and allowed to ask questions. The participants were first time clients of the service. 50 of 72 surveys were completed (69%) Of the 22 non-responders, 6 (27%) were out of the country. Of clients surveyed, 33 traveled purely for pleasure, 14 for business and 3 for both reasons. 98% of our clients were of college or graduate level education.6 travelers expertise lay in fields relevant to tropical public health and ecotourism. Trip length varied from 2 weeks (42%) to 6 months-year (10%). 74% of clients were very satisfied with their interaction. 24% were satisfied and 2% reported dissatisfaction. Responses to open-ended questions revealed 10 factors that significantly promoted or detracted from client satisfaction. Of these, ease of facility access, quality, readability, brevity, and precision of printed materials, presence of question/answer sessions with the staff, and price and availability of medications were the most frequently mentioned issues. 6 clients with related expertise noted discrepancies between information provided by travel authorities and realities found. Specific factors relating to information content, quality and delivery clearly determine client satisfaction.

196 FOCAL LESIONS OF THE SPLEEN IN PATIENTS WITH FEVER OF UNKNOWN ORIGIN: SONOGRAPHIC PATTERNS AND DIAGNOSIS. Farid Z*, Kamal M, Anis E, Karam M, Mousa M, and Mateczun A. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and Abbassia Fever Hospital, Cairo, Egypt.

A review of ultrasonic scans performed for 567 patients admitted at the Abbassia Fever Hospital between 1990 through 1994 for investigation of fever undiagnosed for over 3 weeks revealed 24 cases of focal splenic lesions. They included lymphoma in thirteen cases, tuberculosis in seven cases, abscesses in two, infarction in one and echinococcal cysts in one. Final diagnosis was obtained by correlating the ultrasonography and clinical findings, together with pathologic, bacteriologic, angiographic and serologic data. Splenic lymphoma was diagnosed by splenic puncture in one patient, bone marrow biopsy in one, cytological examination of the ascitic fluid in one and tissue diagnosis from lymph nodes in ten patients. Splenic tuberculosis was diagnosed by biopsy material from lymph nodes in three patients, after splenectomy in two patients and by response to anti-tuberculous therapy in two patients with a clinical illness compatible with tuberculous infection and positive tuberculin skin test. Cases of splenic abscesses were confirmed by needle aspiration of the spleen with ultrasonographic control and blood cultures. In the patient with polyarteritis nodosa, the scan revealed a splenic infarction with angiographic correlation. The patient with splenic cysts had also lung and liver cysts due to serologically proven echinococcal disease.

197 FEBRILE ILLNESS ASSOCIATED WITH HANDLING IMPORTED JERBOAS. Barnett BJ*, Rawlings JA, and Hendricks KA. Infectious Disease Epidemiology and Surveillance Division, Texas Department of Health, Austin, TX.

Each year, thousands of exotic animals are imported into the US without benefit of quarantine. In May and June of 1994, the Texas Department of Health (TDH) received telephone calls from persons claiming to be ill after handling jerboas. Large quantities of these rodents had been imported from Egypt earlier in 1994 to be sold as pets. Many animals died en route or shortly after arriving in the United States. Necropsies were performed at eight locations. Streptococci were cultured from animal tissues at six laboratories; four identified the isolates as either Group G β *Streptococcus* or *Streptococcus equisimilus*. TDH developed a questionnaire and interviewed 93 people from ten states who had handled jerboas. Twenty-two of the 93 met our case definition. Symptoms included fever (95%), sore throat (91%), myalgias (68%), diarrhea (59%), cough (59%), and arthralgias (55%). Thirteen (59%) sought medical attention and received antibiotics. Case-patients were more likely than controls to have handled sick animals, 68% vs. 27% (OR = 5.86, 95% CI = 1.87-19.02), but not more likely to have been scratched or bitten. This incident demonstrates that exotic animals may serve as reservoirs for unusual pathogens and suggests that the exotic animal trade should be regulated.

198 SEASONALITY OF NUTRITIONAL STATUS OF YOUNG CHILDREN IN A SHANTYTOWN NEAR LIMA PERU. Marin CM, Segura JL, Bern CL*, Freedman DF, Lescano AG, Benavente LE, Cordero LG, Clavijo L, and Gilman JB. Asociacion Benefica Proyectos en Informacion Salud Medecina y Agricultura, Lima, Peru; and Division of Nutrition, Centers for Disease Control and Prevention, Atlanta, GA.

Seasonality of nutritional status has been reported predominantly in rural populations dependent on an agricultural cycle. We examined seasonal variation in nutritional status among young children in an urban shantytown near Lima, Peru, from 1987 to 1993. Every child below 36 months of age was weighed and measured every 4 to 5 months. A total of 38,626 measurements were available from 11,333 children over the seven years. We calculated weight-for-height Z-scores (WHZ) based on the NCHS/CDC reference, and used generalized estimating equations to account for the within-person correlation among the WHZ scores. Weight- for-height was highest in September and lowest in March of each year; the estimated difference in mean WHZ between September and March was 0.37 units ($p < 0.0001$). This seasonality effect was seen in both boys and girls, and in each year of the study; although it was largest among 6- to 24 month old children, it was seen in younger and older children. The lower nutritional status seen in summer-autumn than in winter-spring may be related to lower intake due to seasonal availability of foods, or to the higher prevalence of bacterial and parasitic diarrhea in the summer months compared to the winter.

199 FALSE CHARCOT LEYDEN AND FATTY ACIDS CRYSTALS : THE PINEAPPLE CRYSTALS. Ardoin FG*, Petithory JC, and Ash LR Contrìle de Qualité National en Parasitologie, Centre Hospitalier, Gonesse, France; and School of Public Health, U.C.L.A., Los Angeles, CA.

Charcot Leyden crystals described by Robin in 1853 and fatty acid crystals found in feces are quite often mistaken in publications for some other crystals. We have been studying alimentary plants and we describe here the diagnostic

features between real and false parasitic objects which may be encountered in microscopic parasitic coprology. The pineapple crystals we have discovered exist in great quantity in the fresh fruit, *Ananas comosus*, they are also found in tinned pineapple and fruit juice and generally speaking in all preparations containing that fruit. They are characterized by their resistance to a temperature of 100°C and also to sterilization of 15 mn at 125°C. These crystals are not soluble in alcohol, ether, acetic acid or formol aldehyde. They are not digested in the alimentary tract of man. These reasons explain the fact that pineapple crystals are frequently found in feces.There is a size variation in length from 30 to 130 µm and 1 to 2 µm in width, they appear having parallel edges and are pointed at both ends. In colour, they show a greenish tinge like Charcot Leyden crystals. Thus confusion in distinguishing pineapple crystals from Charcot Leyden or fatty acid crystals may occur : "Some Charcot Leyden crystals may show a long acicular form in cases of amoebiasis "(Acton 1918). The control for these crystals is microscopic examination of a drop of pineapple juice.

200 COMPARATIVE EFFICIENCY AND EFFECTIVENESS OF VARIOUS SEDIMENTATION METHODS FOR CONCENTRATING INTESTINAL PARASITES AND EGGS. Cover EC*, and Price DL. Natural Science Department, Manatee Community College, Bradenton, FL.

The sedimentation methods commonly used for intestinal parasites and eggs are modifications of the formalin/ether methods. Differences in procedures used presently may include surfactant use, size of fecal specimen, straining procedure and screen mesh size, washing steps, type of centrifuge and centrifuge speed, removal of supernatant, how the cover glass was added, and the use of sealant. For this study materials and procedures of several manufacturers were used, including two with 50 ml centrifuge tubes and six with 15 ml centrifuge tubes. Fecal specimens were from individual patients. Bulk fecal specimens were divided into 6 or more 13 ml portions, consisting of one part feces and four parts solution. Direct counts were made of parasites and eggs in fecal specimens and the numbers compared with counts after concentration. The results of the two 50 ml tube procedures were compared. Comparisons were made using the same fecal specimen and procedure using 50 ml versus 15 ml centrifuge tubes. Each procedure using a 15 ml centrifuge tube was tested using 6 patients' specimens. No advantage was found using the procedures requiring a larger amount of feces and 50 ml centrifuge tubes. Results were impacted by: screen mesh size, fecal volume in centrifuge tube, centrifuge type and speed, how the fecal plug was transferred to the slide, and slide preparation. Results were reported as the number of trophozoites, cysts, eggs, and juvenile worms per slide (or average per row) prepared by direct examination of the specimen as compared to the numbers counted after concentration.

201 SPATIAL ASPECTS OF THE DISTRIBUTION OF *MYCOBACTERIUM LEPRAE* INFECTIONS IN THE US GULF COAST. Truman RW*, and Hugh-Jones ME. Laboratory Research Branch, G.W.L. Hansen's Disease Center, Baton Rouge, LA; and WHO Collaborating Center, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

Leprosy is lowly endemic in Texas and Louisiana with 20-40 new cases presenting each year among native born US citizens. Nine-banded armadillos, *Dasypus novemcinctus*, in this region are known to habor *Mycobacterium leprae* but the origin of this sylvan infection and the risk that infected armadillos present to man remains unclear. A geographical information systems (GIS) approach was used to identify significant spatial-related epidemiological factors. In examining more than 1400 armadillos we have found that *M. leprae* infections occur mainly among armadillos in low lying, poorly drained habitats. Seroprevalence rates in these areas average 15%. The delectability and transmission of leprosy in armadillos may be influenced by environmental factors but there are no directional trends in the prevalence that might suggest a nidal origin. In humans, the distribution of infections among native born citizens since 1894 shows a similar geographical pattern. Armadillos are frequently eaten in this region and the size of the reservoir suggests that they might contribute to some human infections. However, a comparison of armadillo contact histories reported by patients residing in areas suspected to be of either high or low sylvan prevalence failed to support an hypothesis that direct contact with infected armadillos is a significant risk factor for human leprosy. Armadillos may be a unique parallel population supporting the autocthenous transmission of sylvan leprosy and they may prove to be useful epidemiological models for human leprosy. Their impact on human health remains uncertain. At this time rainfall is apparently unrelated to prevalence. Soil and water table characteristics are presently being investigated for their possible contribution

202 ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS ISOLATED DURING AN OUTBREAK OF HIV-INFECTED PATIENTS BY DNA FINGER-PRINTING. Diaz R*, Montoro E, González R, Echemendía M, and Valdivia JA. Pedro Kourí Institute, Ciudad de La Habana, Cuba..

From July 1993 to August 1994, 24 HIV-infected patients were coinfected with *Mycobacterium tuberculosis* at the Santiago de las Vegas Sanatorium and Pedro Kourí Institute Hospital in Havana, Cuba. It was the first reported

tuberculosis outbreak in patients with HIV infection in our country. All isolates were susceptible to all drugs tested except one, which was resistant to isoniazid. We subjected these strains to DNA restriction fragment length polymorphism (RFLP) analysis using Pvu II enzyme and an insertion element IS6110 probe. Three different RFLP patterns with 4 to 7 copies were obtained. 22 isolates showed the same DNA fingerprint (7 copies). One strain isolated from a patient with tuberculous meningitis presented other 7 bands pattern. The third RFLP pattern (4 bands) was observed in the isoniazid-resistant isolate. All patients with drug-sensitive *M. tuberculosi* strains and the same fingerprint (22) coincided at least in one of two previously mentioned places and therefore it could be suggested that this infection derives from a common source. This DNA fingerprinting method can be very helpful in epidemiological investigations.

203 SEQUENCE TYPING OF *MYCOBACTERIUM AVIUM* COMPLEX (MAC) AS AN APPROACH TO VIRULENCE. De Smet KA, Brown IN*, Yates MD, and Ivanyi J. Dept Medical Microbiology, St.Mary's Hospital Medical School, London, UK; PHLS Regional Centre for Tuberculosis, Dulwich Hospital, London, UK; and MRC Tuberculosis Unit, Hammersmith Hospital, London, UK.

In the tropics, clinical reports of *Mycobacterium avium* complex (MAC) are rare despite the presence of the organisms in the environment. By contrast, in the USA and Europe, MAC cause lung infections in the elderly, lymphadenitis in children and disseminated infections in AIDS patients. It is not known whether these geographical differences relate to strain variation or the high prevalence of tuberculosis in many tropical countries.The possible association between these differing incidences and diverse clinical manifestations with yet unknown virulence markers represents an important topic for research. To discriminate among isolates, we have sequenced 280bp of the internal transcribed spacer between the 16S and 23S ribosomal RNA genes in a collection of 45 MAC isolates. Nine different sequence types were identified and there was a strong association of one of the types (Mav-B) with both AIDS and lymphadenitis in children. Similar associations were found between other sequence types and the other clinical manifestations. Sequence typing provides an accurate way of identifying distinct MAC strains. The differences observed between MAC from different clinical sources suggest that the sequence types may relect biologically relevent polymorphism among MAC isolates. Definition of sequence types to be found in tropical areas may thus help explain the rarity of clinical MAC.

204 GROWTH OF *MYCOBACTERIUM AVIUM*-INTRACELLULARE COMPLEX (MAC) STRAIN 101 IN MACROPHAGES INFLUENCES ITS COLONY MORPHOLOGY METABOLIC STATUS AND VIRULENCE. Kansal R*, Gomez-Flores R, and Mehta RT. Department of Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center, Houston, TX.

Factors influencing the colony morphology are of crucial importance for drug development as well as for understanding the virulence of MAC strains. The MAC 101 strain (Original-MAC; OG-MAC) used in the present study grows as smooth transparent (Sm-T) colonies which tend to become opaque and pigmented when incubated for longer periods. However, when cultured inside macrophages, two types of colonies could be recovered. The new colony morphology (Mø-MAC) appeared to outnumber the Sm-T colonies of original MAC. They looked more flat with a central spot, irregular edges, dry and granular appearance,more like the rough (Rg) mutants. Both colony types were isolated and their growth was measured by MTT assay which revealed that MTT uptake by Mø-MAC was significantly lower than OG-MAC. Morphologically also, these bacteria look different as visualized by Electron Microscopy. In animal studies, the Mø-MAC multiplied at a much faster rate than the OG-MAC, without causing any mortality. In vitro studies indicate that Mø-MAC are killed more efficiently by macrophages as compared to OG-MAC, suggesting that the growth of MAC in macrophages alters its virulence. Further experiments, however, are underway to provide conclusive evidence.

205 ENTEROBACTERIA PROMOTE THE GROWTH OF *MYCOBACTERIUM AVIUM* COMPLEX STRAIN 101. Gomez-Flore R*, Kansal R, Tamez-Guerra R, and Mehta RT. Department of Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center, Houston,TX; and Departamento de Microbiologia, Univer sidad Autonoma de Nuevo Leon, San Nicolás de los Garza, NL, México.

A mycobacterial growth factor was present in the conditioned media of *Escherichia coli* and *Enterobacter cloacae* cultures, but not in *Pseudomonas aeruginosa* culture. This factor was found to potentiate the growth of *Mycobacterium avium* complex (MAC), *M. avium*, and *M. intracellulare*. The growth factor appeared to be heat stable and to have a molecular weight of < 500 Da. Acetate production by enterobacteria was demonstrated to be responsible for the biological activities observed. Acetate was associated with mycobacterial growth promotion at a concentration of 3 mM, however, acetate was toxic for mycobacteria at levels higher than 4 mM. Growth promotion and toxic effects of acetate were reproduced by culturing mycobacteria in media containing acetic acid or sodium acetate. Mycobacterial growth promotion was directly associated with acetate levels regardless of the pH of the

media. Our data suggest that production of acetate by enterobacteria may regulate mycobacterial growth and therefore, intestinal acetate might be a key factor in the pathogenicity of MAC.

206 SURVEILLANCE FOR PNEUMONIC PLAGUE IN THE US DURING AN INTERNATIONAL EMERGENCY: A MODEL FOR CONTROL OF IMPORTED EMERGING DISEASES. Fritz CL, Dennis DT, Tipple MA, Campbell CL, McCance CR, and Gubler DJ. DVBID/CDC, Ft. Collins, CO.

In September 1994, the Centers for Diseases Control and Prevention (CDC) responded to reported large outbreaks of plague in India by implementing a national system of surveillance to identify and control imported pneumonic plague. Information was rapidly disseminated to public health officials, health professionals and the public nationwide. Public Health Service (PHS) regulations authorized CDC to inspect, detain, and isolate persons with a suspected communicable disease. First line active surveillance relied on airline crews to notify quarantine officials of sick passengers prior to arrival, on-board medical examination by quarantine officials and contract physicians, isolation and hospitalization of suspected cases, and surveillance of fellow passengers for 7 days. Practicing physicians and health departments participated in a passive surveillance system to identify plague cases not detected at airports. From September 27 to October 27, surveillance detected 13 plague suspects; none was confirmed as having plague. This coordinated response to an international health emergency may serve as a model to detect and control the importation of other emergent diseases.

207 ELECTROCHEMILUMINESCENCE DETECTION OF *YERSINIA PESTIS* ANTIGEN IN SERUM. Parker RW*, Horne MF, Ezzell JW, and Lofts RS. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Sensitive electrochemiluminescence (ECL) detection of *Yersinia pestis* F1 antigen in serum was achieved by using the ORIGEN Immunoassay System (IGEN, Inc.). The immunoassay format was a double antibody sandwich technique. The capture antibody, rabbit IgG anti-F1 antigen, was biotinylated and pre-bound to streptavidin-coated paramagnetic microparticles. The capture antibody, labeled with ruthenium (II)tris-bipyridal chelate, was also used as the reporter antibody. Reaction mixtures were incubated for 1 hr at room temperature before ECL assay. Standard concentration curves of F1 antigen demonstrated a detection range of 0.01-100 ng/ml. Because sera were tested in 25 µl aliquots, this equated to a total mass detection range of 0.25-2500 pg of F1 antigen.This detection range compared significantly better in terms of sensitivity and dynamic range than the standard microplate microplate ELISA detection range of 100-1000 pg. The sensitive ECL detection of *Y. pestis* antigen in serum should enable early confirmation of suspected plague cases.

208 DEVELOPMENT OF A SIMPLE 15 MINUTE ASSAY FOR THE DETECTION OF *YERSINIA PESTIS* ANTIGEN. Moss DW*, Parker RW, Ezzell JW, Kijek TM, Abshire TG, Rossi CA, Korch GW, and Lofts RS. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

A simple and rapid membrane-based EIA test (dipstick) was developed to detect *Yersinia pestis* antigen. A specially adapted ink-jet printer (Ivek, Corp.) was used to apply a buffered solution of purified *Y. pestis*-specific rabbit immunoglobulin to a polyvinylidene difluoride microporous membrane. The membrane-bound antibody captures antigen from sample matrixes for analysis, and is stable at room temperature when stored dry. A HRP-labeled preparation of the same rabbit immunoglobulin detects the captured antigen. The peroxidase produces a signal from its activity on the substrate 3,3',5,5'- tetramethylbenzidine (TMB), forming an insoluble blue precipitate. The substrate is ready to use in liquid form and is stable at room temperature. The test functions on a variety of sample matrixes, including serum, urine, saliva, whole blood, and extracts of insect and vertebrate tissues. Assays are completed in <20 minutes and require as little as 0.5 ml of sample. The system achieves a detection limit of 10 ng/ml of F1 antigen in spiked serum samples. Comparison with an ELISA-based system suggests comparable performance for experimental sensitivity and specificity. Room temperature stability of all assay components, combined with a fast and simple procedure, make this assay well suited for field use.

209 EVALUATION OF ELISA-BASED TESTS FOR IgM AND IgG ANTIBODIES TO *YERSINIA PESTIS* F1 ANTIGEN USING SERA FROM HUMAN VACCINEES. Lewis TE*, Roberts BA, Mangiafico JA, Hile JA, Danner DK, Pittman PR, Rossi CA, Korch GW, and Lofts RS. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

We developed ELISA procedures to detect *Yersinia pestis* F1 antigen-specific IgM and IgG antibodies in human serum. An evaluation of the ELISA procedures was done to assess their potential as alternatives to the WHO-

standard PHA diagnostic test for plague. All three procedures were evaluated by using one pre-vaccination, and 30- and 60-day postvaccination sera from each of at least 80 human plague vaccinees. Each of the three procedures achieved 100% specificity with the pre-vaccination sera. The ELISA IgM and PHA tests gave comparable sensitivity results,yielding respective rates of 72% and 68% on the 30 day samples, and 76%and 68% on the 60 day samples. The highest sensitivity rate achieved was 94% for the IgG ELISA on the 60 day samples. These results suggest that the ELISA may be the preferred diagnostic test for plague, especially when the high- volume sample testing capability and reagent stability of the ELISA are considered. Studies are currently under way to evaluate test performance on serum samples from naturally infected individuals, and to transition the ELISA reagents into a rapid 17 minute membrane-based EIA (dipstick) designed for field use.

210 CHARACTERIZATION OF *YERSINIA PESTIS* STRAINS ISOLATED FROM PNEUMONIC PLAGUE PATIENTS FROM INDIA (SURAT, 1994). Chu MC*, Rana UV, Yockey BM, Wilmoth BA, Bracher JE, Berrada ZL, Carter LG, and Sehgal S. Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Ft. Collins CO; and Zoonoses Division, National Institute for Communicable Diseases, Delhi, India.

Yersinia pestis isolates were recovered from sputum specimens reportedly obtained from 3 suspect pneumonic plague patients admitted to the New Civil Hospital in Surat between 9/22 to 10/1/94. Specimens were collected between 9/24 and 10/3/94, transported to Delhi in Cary-Blair medium, and processed for culture isolation between 10/14 and 10/26/94. Subcultures maintained at NICD were confirmed as plague bacilli by CDC in February, 1995, these strains were characterized by comparing them with 12 *Y. pestis* strains selected from the CDC plague reference collection. Comparative strains included 3 other Indian isolates (1948, 1953 and 1963, Maharashtra and Karnataka) as well as representative strains from China, Russia, Burma, Vietnam, Nepal, Peru and USA. The 15 strains were compared by protein profile, Fl antigen expression, Fl structural gene amplification/sequencing, and plasmid profile (uncut and restriction endonuclease digested). All strains were Fl positive by protein expression and genetic evaluation. Three strains (All22, Nepal 516, Rhamgiri) were missing the 70-kb plasmid. The most visible difference between the 1994 India isolates and all others examined was the appearance of doublet protein bands at 24,500 and 25,000 daltons, with the larger protein band being unique. The identification of the 25,000 m.w. band will be undertaken.

211 PASSIVE IMMUNIZATION WITH MONOCLONAL ANTIBODIES AGAINST THE F1 ANTIGEN OF *YERSINIA PESTIS* PROTECTS MICE FROM FATAL BUBONIC AND PNEUMONIC PLAGUE. Anderson Jr GW*, Worsham PL, Andrews GP, Bolt CR, Welkos SL, Friedlander AM, and Burans JP. Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Naval Medical Research Institute, Bethesda, MD.

The recent plague epidemic in India demonstrated the need for an improved plague vaccine that induces rapid immunity and protects against pneumonic disease. In this study, three IgG and one IgA monoclonal antibodies (MAb) to the fraction 1 (F1) capsular antigen of *Yersinia pestis* were administered to adult Swiss Webster mice intraperitioneally 6 or 24 hours before a lethal subcutaneous or aerosol challenge of *Y. pestis*. One IgG1 F1 MAb protected 10/10 mice challenged subcutaneously with 48 LD_{50}s and 15/20 mice challenged with 23-34 LD_{50}s by small particle aerosol challenge of the F1-expressing CO92 strain. The ability of this MAb to protect was dose dependent. This MAb failed to protect against a subcutaneous or aerosol challenge with the C12 nonpolar strain of *Y. pestis*, which is incapable of producing the F1 capsular antigen. The other two IgG and the only IgA MAb offered less and no protection, respectively. F1-antibody titers 28 days postchallenge suggested that the Mabs did not uniformly prevent infection. Only 1 of the 48 mice surviving the 28 day observation period had detectable *Y. pestis* organisms in its spleen. Twelve colonies isolated from this spleen culture were F1-minus by Western blot. One of the F1-minus mutants examined by PCR had a deletion in the F1 operon. This mutant was virulent when inoculated subcutaneously (LD_{50}, 3.5 colony forming unit). The data suggest that secretory IgA antibody and mucosal immunization may not be necessary to protect against pneumonic plague and confirm earlier suggestions that a plague vaccine should not rely solely on the F1 antigen.

212 COMPARISON BETWEEN IMMUNODIAGNOSTIC ASSAYS FOR BRUCELLOSIS DIAGNOSIS. El-Masry N*, Farid Z, Mohareb E, Shaheen H, Kamal M, Bassily S, Brown FM. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and Abbassia Fever Hospital, Cairo, Egypt.

In the evaluation of the febrile patient, brucellosis often poses a significant diagnostic dilemma and is easily confused with a number of other infections including tuberculosis, typhoid and/or Q Fever. Since isolation of the organism from the blood is a relatively insensitive and time consuming procedure, involving up to a six week waiting period, serologic assays often shorten the time to diagnosis and treatment. In this retrospective study the

role of tube agglutination (TA) in diagnosing acute and chronic brucellosis, was compared with ELISA and immunoblotting assays in detecting anti-brucella antibodies against whole cell sonicate. Thirty five patients referred for evaluation of fever of unknown origin were studied and found to have varying titersfor brucella antibodies using the tube agglutination test. Of the 20 patients with TA titers > 1: 640 (the cutoff for diagnosis of brucellosis at NAMRU-3), blood cultures were positive in 4 (20%) and ELISA testing to *Brucella melitensis* antigens for both IgM and IgG subclasses was positive in 15 (75%). Of the 15 patients with TA titers < 1: 340, ELISA testingwas positive in only 2 (13%). Serum from 22 of these 35 patients was tested by an immunoblot technique to the 68KDa protein of *B. melitensis*. Out of this 22, 9 of 11 with TA titers > 1: 640 revealed a 68KDa band, while all 11 patients with titers < 1: 320 did not reveal this band. These results indicate that both immunoblotting and ELISA could be used as confirmatory tests for patients diagnosed with brucella infection by the tube agglutination method.

213 CHARACTERIZATION OF MTA/SAH NUCLEOSIDASE AND MTR KINASE FROM ENTERIC BACTERIA: POTENTIAL TARGETS FOR CHEMOTHERAPEUTIC INTERVENTION. Cornell KA*, and Riscoe MK. Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR; and Medical Research Service, Veterans Affairs Medical Center, Portland, OR.

In contrast to higher eukaryotes, many lower eukaryotes and bacterial species metabolize 5'-methylthioadenosine (MTA) and S-adenosylhomocysteine (SAH) via a single bifunctional enzyme, MTA/SAH nucleosidase. S'-methylthioribose (MTR) produced by the degradation of MTA is further metabolized via MTR kinase to MTR- 1 -phosphate and hence recycled back to methionine. These two enzymes represent attractive chemotherapeutic targets because of their role in salvage of adenine and methionine, and in maintaining low levels of MTA and SAH. Elevated MTA and SAH levels are known to inhibit polyamine biosynthesis, transmethylation reactions, and cell proliferation. Ideally, substrate analogs could be developed to selectively inhibit or subvert the nucleosidase and/or kinase, yielding useful agents for the treatment of a variety of infectious diseases. To study of these enzymes as chemotherapeutic targets, they were purified to homogeneity from bacterial lysates by exploiting a novel affinity chromatography resin. Amino terminal sequence data from the purified protein led to the cloning and sequencing of the MTA/SAH nucleosidase gene from lescherichia colif. Sequencing of the MTR kinase gene is in progress. The MTA/SAH nucleosidase gene was further subcloned into the PGEX expression system and kinetic analysis of the resulting fusion protein yielded similar enzymatic activities to native protein. In summary, we describe the purification to homogeneity and amino terminal sequencing of two microbial enzymes involved in the methionine salvage pathway, and report for the first time the cloning, sequencing, and expression of a gene encoding MTA/SAH nucleosidase.

214 SAFETY AND IMMUNOGENICITY OF A LIVE, ORAL, ATTENUATED Bah-15 *VIBRIO CHOLERA* EL TOR OGAWA VACCINE. Coster TS*, Kenner JR, Arthur JD, Killeen KP, Spriggs DR, McClain JB, Barrera-Oro M, Mekalanos JJ, and Sadoff JC. Division of Medicine, USAMRIID, Ft Detrick, MD; Division of Comm Diseases & Immunology, WRAIR, Wash, DC; Department of Microbiology and Molec Genetics, Harvard Medical School, Boston, Mass; and Virus Research Institute, Cambridge, MA.

Previously we tested a live, attenuated 01 *Vibrio cholera* El Tor Ogawa vaccine candidate (Bah-3), attenuated from its Bahrain progenitor by deletion of the genetic element that encodes virulence factors and RS1. The deletion mutant was further modified by insertion of a construct that encoded the B subunit of cholera toxin thus generating Bah-3. When tested in humans, Bah-3 was found to be too reactogenic despite the absence of known enterotoxins. We speculated that loss of motility would further attenuate this reactogenic parent strain. Bah-15 vaccine candidate is a stable spontaneous nonmotile derivative of Bah-3. In a randomized, double-blind, placebo-controlled trial we evaluated Bah-15 as an oral, single-dose cholera vaccine candidate. Eight volunteers received $3x10^8$ cfu Bah-15 with buffer and 3 volunteers received buffer alone. One vaccinee developed frequent, small-volume diarrhea (5 loose stools totalling 268 ml) without other associated symptoms; one control had 16 loose stools totalling 1264 ml. Two vaccinees experienced emesis (with minimal other complaints) and vaccinees in general commented more often about mild to moderate abdominal gurgling. Vaccine organisms were shed in stool by 7 of 8 vaccinees. Vibriocidal titers are being determined. Motility reversion of mutant is being examined. Our results indicate that loss of motility is an effective method of attenuation as Bah-15 was given at 2 logs more than the highly reactogenic Bah-3. Bah-15 is a potential vaccine candidate. Further testing is planned.

215 PERSISTENCE OF ANTIBODIES AND BOOSTER RESPONSE AGAINST ANTHRAX AND BOTULINUM VACCINES IN MILITARY PERSONNEL VACCINATED DURING OPERATIONS DS/DS. Pittman PR*, Sjogren MH, Makuch RS, LaChance R, Hack D, Arthur J, and Franz D. Division of Medicine, U. S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, DC.

We determined the persistence of anthrax and botulinum antibodies and the response to a booster 2 years after a cohort of military personnel were immunized during Operations Desert Shield/Desert Storm. Due to the exigency, volunteers were primed with one, two or three vaccine doses as the situation permitted. Of 281 volunteers primed with anthrax vaccine, 13 received one dose, 197 two doses, and 71 three doses. Two years after immunization, antibody against anthrax protective antigen by ELISA persisted in 2/13 (15.4%), 48/197 (24.4%), and 36/71 (50.7%) of those who received one, two or three priming doses, respectively ($p = 0.0001$). Geometric mean titer (GMT) was 80, range was 50 to 400, median titer was 50. A booster response in the three groups was demonstrated in 12/13 (92.3%), 196/197 (99.5%), and 71/71 (100%), respectively, ($p = 0.154$). GMT was 9664, range was 50 to 10000, and median was 10000. Persistence of antibodies against botulinum type A toxin among a cohort of 327 volunteers administered the botulinum pentavalent (ABCDE) toxoid was determined by using the mouse neutralization bioassay. Of 327 volunteers who received botulinum toxoid, 10, 244 and 73, respectively, received one, two and three priming vaccinations. Two years after priming, 0/10, 27/244 (11.1%), and 8/73 (11%) volunteers had persistent antibodies ($p = 0.813$). Titers ranged from 0 to 0.28 IU/ml, median titer 0. Thirty days after a booster vaccination, antibody was detectable in 7/10 (70%), 238/244 (97.5%), and 72/73 (98.6%) of volunteers who received one, two and three priming vaccinations ($p = 0.003$). Titers ranged from 0 to 327.68 IU/ml, median titer was 14.88 IU/ml. Antibody persistence was greater than expected for individuals administered part of the standard series for at-risk laboratory scientists. A robust booster response was evoked in over 97% of the subset of volunteers who received two or three priming doses of either anthrax or botulinum vaccines several years earlier. These findings are important to military medical planners who must set vaccination policy for military personnel.

216 PCR FIELD TRIAL FOR THE DETECTION OF *CAMPYLOBACTER* DURING EXERCISES "COBRA GOLD 94", COBRA GOLD 95" AND "BALANCE TORCH #1". THAILAND. Lebron CI*, Echeverria P, Walz SE, and Wignall SF. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Armed Forces Research Institute of Medical Science, Bangkok, Thailand.

Campylobacter species, particularly *C. jejuni* and *C. coli* are common etiologic agents of acute gastroenteritis. Methods to isolate *Campylobacter* spp. require special growth conditions and very often, special transport media to ensure that these organisms remain viable before they reach the laboratory. During 1994 and 1995 exercises "Cobra Gold" and "Balance Torch" (Thailand) PCR technology was tested on the field under adverse field conditions in order to provive rapid diagnoses of *Campylobacter* related gastroenteritis to American serviceman attending sick call in various locations throughout southern and northern Thailand. Stool culture analysis showed that *C. jejuni* was the most frequently cause of diarrhea. An oligonucleotide primer pair from a conserved 5' region of the flaA gene of *C. coli* VC167 was used to amplify a 450-bp region. Using PCR technology we were able to obtain 75% agreement between a traditional culture method and PCR technology under adverse field conditions. This technology provides the potential for improved diagnostic capability in field type laboratories.

217 SEWERS: THE PRINCIPAL *AEDES AEGYPTI* BREEDING SITES IN CALI, COLOMBIA. Gonzalez R, and Suarez MF*. Departamento de Entomologia, Facultad de Ciencias, Universidad del Valle, Cali, Colombia; and Instituto de Inmunologia del Valle, Universidad del Valle, Cali, Colombia.

Dengue control strategies involve different approaches against vector control. Source reduction has been the most popular government operation for diminishing breeding sites throughout the Americas. This activity assumes that the majority of *Aedes aegypti* breeding sites are located inside houses and could easily be eliminated or covered. A preliminary survey carried out in 1993 showed that the 57% of the sewers in Cali were positive for *Ae. aegypti* immature forms. The present report compares sewers with the traditional indoor *Ae aegypti* breeding sites. In ten randomly selected blocks, houses were inspected and all indoor and outdoor containers as well as the sewer around them were evaluated. The total number of larvae and pupae were collected and preserved in 70% ethanol for counting in the laboratory. The sewer had 3.2 times more larvae and 6.7 more pupae than all domestic indoor breeding sites. Averages of positive indexes of containers, houses and sewers were 17.8%, 37.8% and 83.6 respectively. Although 1.7 domestic positive breeding sites were found by each positive sewer, the pupae production in sewers was 25 times greater than inside houses. Sewers were the most important *Ae aegypti* breeding sites in Cali in terms of pupae production. This particularly important breeding site is out of the scope of source reduction and community participation programs.

218 *AEDES AEGYPTI* PRESENCE IN CORDOBA PROVINCE, ARGENTINE (1995). Aviles B, Harrington ME, Cecchini R, Asis M, and Mios C. Ministry of Health, Cordoba Province, Argentina.

In Argentina the last major epidemic of yellow fever transmitted by *Aedes aegypti* mosquitoes was in Buenos Aires City. Besides, in 1916, there was an intense dengue epidemic transmitted by the mosquito species in the north of the country. In the opportunity of the continental campaign of *Ae. aegypti* eradication of 1955, the area infested was

estimated as 1,500,000 km^2; later on *Ae. aegypti* was considered eradicated from Argentina in 1963. The Argentine National Ministry of Health reported the mosquito presence at several provinces of the country in 1994. During the summers of 1994-95, the Ministry of Health of Cordoba Province, Zoonosis Department, Argentina, started active surveillance of the mosquito species. Cordoba Province is located in the temperate zone of Argentina, between the parallels 29 and 35 SL. For this program, selected municipalities and hospitals of different localities were involved as integrants of the surveillance system. Some of them were chosen because they were positive for *Ae. aegypti* until 1963, others because they were important communication centers, and others as representative localities of a determined area. This system allowed the detection of *Ae. aegypti* at two localities, Villa Maria and Villa Nueva in February, 1995. In a second step, surveillance was amplified and intensified to all municipalities in the province. As a consequence, 48 localities in total were tested, resulting in a percentage of positive localities (4.16%). After this finding, an active infomation to the program authorities, physcians, and population; cleaning measures; and mosquito control were carried out. In spite of all these measures and also the occurrence of the first freezing in the autumn, the mosquito was still present in May at these localities. The last time that an active surveillance of *Ae. aegypti* was carried out in Cordoba Province was 30 years ago.

219 *AEDES ALBOPICTUS* IN SOUTH CAROLINA. Mekuria Y*. International Center for Public Health Research, School of Public Health, University of S.C., McClellanville, SC.

After it was found breeding in Houston, Texas in 1985, the imported "Asian Tiger Mosquito", *Aedes albopictus*, has spread throughout the eastern and southern states, extending from Chicago to Florida. However, in some states, there are gaps in the known distribution of the mosquito by counties. Published information on the distribution and abundance of *Ae. albopictus* in South Carolina is limited and fragmentary. This paper draws on various published and unpublished sources and presents synoptic information on the occurrence of the mosquito in 15 counties, and the significant biting nuisance it is causing. More surveys are needed to establish the infestation status of the other 31 counties of the state.

220 SAND FLY SPECIES COMPOSITION, DISTRIBUTION AND ECOLOGY IN A NORTHERN AREA OF MOROCCO. Hamdani A*, Essari A, and Guessous-Idrissi N. Unite d'Etudes et de Recherche sur les Leishmaniaoses, Faculte de Medicine et dePharmacie, Casablanca, Morocco.

An entomological survey was conducted to investigate the species composition, distribution and seasonal variation of the sand fly fauna in a Northern province of Morocco, Khemisset 80 km far from Rabat (400 m altitude and semi-arid climate). During the extensive sampling of sand flies conducted between June and November 1994, 9,016 sandflies were collected, and 9 species were recorded. Of these, 8 were from *Phlebotomus* genus and one from *Sergentomyia*. *P. perniciosus* (55.6%), *P. sergenti* (18.3%), and *P. longicuspis* (12.5%) were the most abundant species in the studied area. *P. perniciosus*, the most represented specie was essentially trapped in caves, while *P. longicuspis* was abundant in stables and houses. On the other hand, *P. sergenti* was present either in houses, stables or caves. However, *P. langeroni* was only present in Hrira, a douar almost 25 km from Khemisset. The sand fly season in the study focus was found to be a long one. The maximum number of sandflies collected was in September and October. The seasonal pattern of *Larroussius* in the area is bivoltine showing two peaks, one initial and minor around July, and another major and late in September/October. The results of this survey will be confirmed by a second entomological study this year.

221 FIELD BEHAVIOUR OF SANDFLIES IN WADI FERAN, SINAI-EGYPT. Shehata MG, Doha S, El Hossary S, Swalem M, Abd El Mohsen A, and El Kadi G.

Field behaviour of sandflies in Wadi Feran, which lies about the mid way between Saint Catherine and Abu Rudeis with an altitude of 200-500 m, Sinai-Egypt, was studied. These include: nocturnal pattern, man-biting activity, exophilic and endophilic behaviour, sugar feeding and blood engorgement which represent as some epidemiological information essential for estimating and level of transmission. Results indicate that both *Phlebotomus bergeroti* and *P. sergenti* are exophilic and endophilic in behaviour. Blood engorgement rates of 36% of *P. bergeroti* and 25% of *P. sergenti* inside houses provide indirect evidence for blood feeding on humans. Positive fructose response observed for the collected sandflies in Wadi Feran: *P. bergeroti* (47%); *P. sergenti* (47%); *P. alexandri* (56%; *P. major* (50%) and *P. kazeruni* (91%) confirms that sugar is essential for the biology of the different types of sandflies. Dissection of 178 females of phlebotomine sandflies showed no leishmania parasites. In conclusion, this report demonstrated several behavioural differences between the different sandfly species in the area which could be of great use for the control of leishmaniasis.

222 ISOLATION OF A FAMILY OF SERINE PROTEASE cDNA SEQUENCES FROM THE CAT FLEA *CTENOCEPHALIDES FELIS*. Stiegler GL*, Gaines PJ, Sampson CM, Lupien S, and Rushlow KE. Paravax, Inc., Fort Collins, CO.

Degenerate antisense serine protease PCR primers were designed from active-site amino acid residues that are highly conserved in most species. The degenerate primers were used in conjunction with the vector M13 reverse primer and a fed-flea cDNA library template to amplify the 5'-terminal serine protease gene region. Serine protease PCR amplified gene sequences were identified by hybridization with a radiolabeled degenerate oligonucleotide probe designed from conserved residues proximal to the histidine active-site amino acid. The hybridization-positive serine protease PCR gene products were cloned and characterized by DNA sequence analysis. Twelve unique serine protease cDNA gene sequences have been identified. The sequenced clones contained an open reading frame that includes conserved regions characteristic of the serine protease gene family. The cDNA sequence information is being used to design specific oligonucleotide probes for isolating the complete cDNA gene sequence.

223 CHROMOSOMAL ABERRATIONS IN INTRASPECIFIC HYBRIDS OF THE SAND FLY *LUTZOMYIA LONGIPALPIS*. Mukhopadhyay J*, Munstermann LE, and Ghosh KN. Yale School of Epidemiology and Public Health, Yale University, New Haven, CT.

Intraspecific crossing between two geographic strains of a single species of sand fly *(Lutzomyia longipalpis)* indicated high sterility in certain backcrosses. A cytogenetic study was undertaken to explain this partial sterility. For this purpose, the meiotic configurations of the F_1 hybrid males of two strains were examined. For generating the hybrids, reciprocal crosses were made between Columbian and Brazilian strains of *Lutzomyia longipalpis*. The genetic incompatibility of the hybrid male was inferred from the degree of chromosomal aberrations observed in the testicular chromosome of F_1 hybrid males. Chromosomal constitution during meiosis in hybrid males reveals translocation configurations between heterologous pairs of chromosomes, forming meiotic rings. The presence of bridges and fragments indicated probable existence of paracentric inversions as well. These results, combined with the genetic map, supports the concept of *Lutzomyia longipalpis* as a species complex.

224 DIFFERENTIATION BETWEEN *CULEX PIPIENS* COMPLEX MEMBERS USING A PCR ASSAY BASED ON DIFFERENCES IDENTIFIED BY GENOMIC SUBTRACTIVE HYBRIDIZATION. Crabtree MB*, Savage HM, and Miller BR. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Ft. Collins, CO.

Mosquitoes in the *Culex pipiens* complex are involved in the transmission of pathogens including St. Louis encephalitis virus. The major taxa in the complex, *Cx. pipiens* and *Cx. quinquefasciatus*, exhibit differences in biological and behavioral characters, yet they are morphologically similar, making identification of specimens difficult. Morphological discrimination between these taxa requires time-consuming measurements of terminalia and is only applicable to identification of male specimens. To date, attempts to develop a reliable method for differentiation based on biochemical and molecular techniques have been unsuccessful. We report here the use of genomic subtractive hybridization to identify regions of nucleic acid heterogeneity between *Cx. pipiens* and *Cx. quinquefasciatus* and the development of a PCR assay for discrimination between these species. The PCR assay was used to differentiate between the species using extracted individual mosquito genomic DNA, crude DNA preparations from a mosquito head or legs, and DNA from triturated mosquito pools. This technique will facilitate improved surveillance and control efforts and further studies of the contributions of members of the *Cx. pipiens* complex to the transmission of pathogens.

225 PURIFICATION AND CLONING OF THE SALIVARY NITROPHORIN (NO-CARRYING HEMOPROTEIN) FROM THE BED BUG, *CIMEX LECTULARIUS*. Valenzuela JG*, and Ribeiro J MC. Department of Entomology, University of Arizona, Tucson, AZ.

Rhodnius prolixus and *Cimex lectularius* have been shown before to contain salivary nitric oxide that helps the bugs to feed on their vertebrate hosts by promoting vasodilation and inhibiting platelet aggregation. In these bugs, nitric oxide is associated with heme proteins (nitrophorins) that serve a function of storing and transporting NO from the insect salivary glands to their host's skin. In this paper, the salivary nitrophorin of the bedbug *C. lectularius* was purified to homogeneity, and partial amino-acid sequence data was obtained. The sequence was used to generate a PCR product to screen a salivary cDNA library which yielded a 1 kb clone whose sequence indicates homology to the human inositol polyphosphate 5-phosphatase, but no similarities emerged when *Cimex* nitrophorin was compared to the sequence of *Cimex* nitrophorin. Because *Cimex* and *Rhodnius* belong to two different families of Hemiptera that evolved independently to blood feeding, their nitrophorins must have arisen as a product of convergent evolution. The diversity of insect nitrophorins may provide insights into the associations of heme proteins with nitric oxide.

226 TOXIC ACTIVITY (LC_{50}) OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* STRAIN LFB/FIOCRUZ 710 ON *LUTZOMYIA LONGIPALPIS* (DIPTERA:PSYCHODIDAE: PHLEBOTOMINAE). Wermelinger ED, Zanuncio JC, Rangel EF*, and Rabinovitch L. Departamento de Entomologia do Instituto Oswaldo Cruz FIOCRUZ Rio de Janeiro, Brasil; Departamento de Bacteriologia do Instituto Oswaldo Cruz FIOCRUZ Rio de Janeiro, Brasil; and Departamento de Biologia Animal, Universidade Federal de Vicosa, Vicosa, Brasil.

Leishmaniasis is one of Brazil's major disease problems and over the past few years the problem has increased. In order to investigate the potentiality of *Bacillus thuringiensis* subsp. *israelensis* in the control of phlebotomine vectors, we established the LC_{50}s of some preparations of bacteria on *Lutzomyia longipalpis*, the vector of visceral leishmaniasis in Brazil. Strain used was *Bacillus thuringiensis* subsp. *israelensis* LFB/FIOCRUZ 710 isolated from Brazilian soil. This strain showed activity against the genera *Culex*, *Aedes* and *Anopheles* (Culicidae). For this purpose, eight concentrations (2, 4, 6, 7, 8, 10, 12 and 14 mg. of biomass bearing delta-endotoxin crystals and spores, mixed and homogenized with 1 gram of previously autoclaved larval food) were assayed with three repetitions for each concentration. The LC_{50} calculed by probit analysis was 5.7 mg/gram. This result confirmed some susceptibility of *L. longipalpis* to *B. thuringiensis* subsp. *israelensis* , although more investigations are necessary to verify the actual potentiality of sandfly control by *Bacillus* strains which are pathogenic for insects.

227 DEVELOPMENT OF A EXPLANT MODEL TO TEST FOR A MIDGUT BARRIER TO BLUETONGUE VIRUS INFECTION IN *CULICOIDES VARIIPENNIS* SUBSPECIES. Spielholz J*, Dubovi EJ, and Patrican LA. Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY; and Department of Entomology, Cornell University, Ithaca, NY.

Culicoides variipennis is the principle vector of bluetongue virus (BTV) in the continental United States. Genetic studies have confirmed the existence of at least 3 different geographic subspecies that vary in their ability to amplify and subsequently transmit the virus by natural means, via oral infection. The northeast US is virtually bluetongue-free, due to the exclusive presence of a refractory subspecies, *C. v. variipennis*. In concordance with field data, laboratory experiments have been unable to demonstrate per-os infection in the refractory strain, leading to the hypothesis that a midgut barrier to infection must exist in this incompetent vector. We are presently investigating mechanisms of viral pathogenesis within the insect using a posterior-midgut explant system. With this *in vitro* model, we can compare the refractory to the competent subspecies midgut under constant temperature and nutritional milieu, while controlling for opportunistic pathogens such as bacteria and fungi. The explant is removed from a midge between 2 and 4 hours post- bloodmeal. The intact migut develops a normal peritrophic membrane between 18 and 24 hours in L-15 serum-free media; the bloodmeal is contained exclusively in the newly-forming peritrophic membrane. The midgut epithelium and basal lamina remain healthy and intact for more than a week in our experiments. So far, our model system uses *C. v. sonorensis*, the competent vector. Utilizing immunohistochemistry and a time course analysis of infection, we have obtained baseline data to proceed with a comparative study.

228 55 AN IMMUNOLOGICAL STUDY OF RIFT VALLEY FEVER VIRUS IN A SENEGALESE MOSQUITO, *AEDES FOWLERI*. Romosor WS*, Lerdthusnee K, and Leon R. Tropical & Geographical Disease Institute, Department of Biological Sciences, Ohio University, Athens, OH.

Rift Valley fever virus (RVFV) infection, dissemination and tissue tropisms were studied in a Senegalese mosquito, *Aedes fowleri*. RVFV antigen was detected in serial paraffin sections by application of the avidin-biotin-peroxidase complex (ABC) technique. Female mosquitoes were provided a blood meal from a viremic hamster and held for 2, 3, 4 and 10 days prior to fixation in 5% para- formaldehyde. Mosquitoes allowed to feed on an uninfected hamster were used as negative controls. Female *Culex pipiens* mosquitoes were intrahemocoelically- infected with RVFV and used as positive controls. Viral plaque assay of dissected legs was used to determine the dissemination (movement of virus from the midgut into the hemocoel) status of each mosquito. The overall rate of infection was 25/31 or 80.7% and overall dissemination rate was 8/31 or 25.8%. Patterns of gut infection relative to dissemination status were consistent with the dissemination of RVFV via cells at the foregut-midgut junction and via the cardial/anterior midgut epithelium. The RVFV mosquito tissue tropisms observed were essentially identical to those observed in other mosquito species, *Cx. pipiens* and *Ae. mcintoshi*. RVFV antigen was detected in the following: all regions of the midgut, fat body, epidermis, ganglia of the central nervous system (cell bodies only), salivary glands, ommatidia of the compound eyes, the corpora allata/thoracic gland complex and Johnston's organ. Patterns of tissue infection suggest that dissemination from the midgut occurs sporadically, a factor which would affect the extrinsic incubation period of the virus in the mosquito species studied.

229 GARLIC - MOSQUITO REPELLENT OR ANTIMALARIAL? Goh LM*, Lowrie RC, and Wiser MF. Section of Peds. Infectious Diseases, Tulane University Medical Center, New Orleans, LA; Parasitology Department, Tulane Regional Primate Research Center, Covington, LA; and Tropical Medicine Department, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

Garlic *(Allium sativum)* has long been used by residents and missionaries in parts of East Africa as antimalarial prophylaxis instead of chloroquine. It is believed that the smell of garlic exuded in the sweat repels mosquitoes, thus reducing or eliminating exposure to an infective bite. We tested this hypothesis using both *in vitro* and *in vivo* experiments. For the *in vitro* component, aqueous garlic extract (AGE) was added to fresh monkey blood in 20 µl increments in a membrane feeder. Either 10 *Aedes aegypti* or 10 *Anopheles stephensi*mosquitoes per group were exposed to the garlic-laced blood for 15 min.at various concentrations (20-180 µl of AGE in 7 ml of blood). $(Chi)^2$ analysis of 1000 mosquitoes for each species showed a significant repellent effect of AGE against *Anopheles*, but not *Aedes* mosquitoes ($0.025 > p > 0.01$), which is directly proportional to the AGE concentration {$r=-0.90$ ($p<0.01$)}. The *in vivo* component consisted of feeding daily to a guinea pig, a crushed 300 mg garlic tablet dissolvedin Gatorade. *Aedes* or *Anopheles* mosquitoes were exposed to the garlic-fed and control guinea pigs simultaneously. Under present experimental conditions, the garlic-fed animal did not show significant repellent effect. We also tested garlic extracts and a derivative, allicin, for potential antimalarial activity against *Plasmodium berghei* using either the 4-day suppressive test (intraperitoneally) or the Rane test (orally). No antimalarial activity was observed in contrast to a single published report that claims another garlic derivative, ajoene, has antimalarial activity.

230 EFFECTS OF IVERMECTIN ON TRANSMISSION OF *BORRELIA BURGDORFERI* BY *AMBLYOMMA AMERICANUM*. Presley SM, Abbassy MM*, and Arthur RR. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and Johns Hopkins University, Baltimore, MD.

The effects of ivermectin (22, 23-Dihydroavermectin B_1, 1% injectable) on the transmission of *Borrelia burgdorferi* by *Amblyomma americanum* ticks feeding on New Zealand white rabbits were determined under laboratory conditions. Preliminary trials suggests the peak infectivity period of *B. burgdorferi*-infected rabbits to be ca. 9-11 days post-infection when rabbits were injected with $>10^6$ organisms intraperitoneally. *Amblyomma americanum* nymphs feeding onivermectin-treated (@ 400 µg/kg BW) NZW rabbits exhibited significantly decreased rates of feeding, as well as significantly decreased engorgement success. Within the first 36 hours post-infestation 76% (168/221) of nymphs recovered from the untreated rabbits had dropped-off, while only 12% (15/125) from the treated rabbits had engorged ($P<0.001$). Additionally, ca. 74% (221/300) of ticks on untreated rabbits successfully engorged, compared to only 42% (125/300) from the ivermectin-treated rabbits ($P<0.001$). Scanning electron microscopic examination of unfed and fed (both treated and untreated) nymphs to determine any histological/morphological effects, and bacteriological studies are in progress.

231 ACQUISITION OF LYME DISEASE SPIROCHETES BY CO-FEEDING *IXODES SCAPULARIS* NYMPHS. Patrican LA*. Department of Entomology, Cornell University, Ithaca, NY.

Uninfected *Ixodes scapularis* nymphs (recipients) were allowed to co-feed with *Borrelia burgdorferi* infected nymphs (donors infected as larvae) on uninfected naïve gerbils. The total number of infected:uninfected nymphs placed on individual gerbils was: 12:12, 6:18, 3:21 and 0:24 (control). Uninfected nymphs were placed on gerbils at the same time as the infected nymphs (day 0) or were placed on gerbils 1, 3 or 7 days later. Engorged ticks were examined under a microscope to determine whether they originated from the infected or uninfected (clipped tarsal claw) group of nymphs. Duration of tick attachment and engorgement weight were recorded. Nymphs were held at 21C and allowed to molt to adults. Adults were bisected with one half being tested for spirochetes using fluorescent antibody (FA) tests and the other half being tested by PCR. Preliminary FA results from three of the seven trials conducted on day 0 indicate that recipient nymphs acquire spirochetes disseminated in the skin while co-feeding with donor nymphs. The number of recipient nymphs acquiring spirochetes/the number of recipient nymphs recovered was 15/56 (26.8%), 5/28 (17.9%) and 2/36 (5.6%), which represents 28.3 (15/53), 45.5 (5/11) and 40.0% (2/5) of all infected ticks recovered from gerbils exposed to 12:12, 6:18 and 3:21 infected:uninfected nymphs, respectively. FA and PCR analyses of all trials are ongoing. Uninfected ticks acquiring spirochetes while co-feeding with infected nymphs potentially amplify the prevalence of infection in tick populations.

232 THE OUTER SURFACE PROTEIN A (OspA) LYME DISEASE VACCINE BLOCKS TRANSMISSION FROM THE VECTOR TO THE HOST. de Silva AM*, Barthold SW, Telford SR, Burnet L, and Fikrig E. Yale University School of Medicine, New Haven, CT; and Department of Tropical Public Health, Harvard University School of Public Health, Boston, MA.

We performed experiments to examine the expression of OspA on the Lyme disease spirochete, *Borrelia burgdorferi*, inside the *Ixodes* tick vector during transmission, and to relate OspA expression to antispirochetal immunity. Spirochetes in the midgut of unfed nymphs were stained by an OspA monoclonal antibody. In nymphs feeding on mice the majority of spirochetes in the mid gut and salivary glands did not stain with the antibody. Thus, the OspA protein appears to be cleared from spirochetes inside the vector during transmission. To examine the mechanism of protection afforded by OspA antibodies, mice were passively immunized with OspA antibody prior to the placement of infected nymphs. In the presence of OspA antibody in the blood meal spirochetal development events in the vector such as growth and salivary gland invasion that are an integral part of the transmission process were blocked and the mice were protected from *B. burgdorferi* infection. We also examined the survival of spirochetes in the vector and the host when OspA antibody was administered at different times after tick attachment. When the antibody was administered to mice during the blood meal, spirochetes persisted in the nymphs and the mice were not protected from *B. burgdorferi* infection in spite of the presence of circulating OspA antibodies in the host as well as the tick blood meal. OspA mediated immunity appears to be effective only during a narrow of window of time at the beginning of tick feeding when antibodies bind to spirochetes in the tick mid gut and blocking transmission from the vector to the host.

233 TICK-BORNE INFECTIOUS DISEASES IN A NORTHERN CALIFORNIA COMMUNITY. Fritz CL*, Conrad PA, Flores GR, Kjemtrup AJ, Campbell GL, Schriefer ME, Gallo D, and DJ Vugia. Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO; Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA; Public Health Division, Sonoma County Department of Health Services, Santa Rosa, CA; and Division of Communicable Disease Control, California Department of Health Services, Berkeley, CA.

In 1992-94, 32 of ~450 residents in a semirural Sonoma County, California subdivision reported symptoms attributed to Lyme disease. These data and results of environmental studies suggested a higher than expected risk for tick-transmitted diseases among these residents. To determine risk of contracting Lyme disease, ehrlichiosis, and babesiosis, a questionnaire was administered to and a serosurvey conducted among subdivision residents in August 1994. Serum samples from Sacramento County blood bank donors were used as controls. Questionnaires were completed by 230 (51%) residents, and blood s les obtained from 219 (49%) residents. Antibodies to *Borrelia burgdorferi* were detected in 3 (1.4%) residents, to *Ehrlichia chaffeensis* in 10 (4.6%) residents, and to *Babesia* sp. WA1 in 3.9 (18%) residents. None of 124. control samples was reactive to *B. burgdorferi* or *E. chaffeensis*; 25 (20%) control samples were reactive to *Babesia* sp. WA1. Seroreactivity was significantly associated with age <16 years (OR=5.0, 95% CI=1.7, 14.9) and with residence in the subdivision for 11-20 years (OR=4.4, 95% CI=2.0, 9.6). Fourteen (33%) of 43 symptomatic residents and 37 (21%) of 176 asymptomatic residents were seroreactive to 1 of the 3 organisms. Thus residents of a semirural California community were at risk of exposure to several tick-borne infectious disease agents. Infection with *Babesia* sp. and *Ehrlichia* sp. may be more common in California than previously recognized.

234 GENETIC ANALYSIS OF *RICKETTSIA TSUTSUGAMUSHI* ISOLATES OBTAINED FROM HUMANS, RODENTS, AND THEIR TROMBICULID MITE VECTORS IN AUSTRALIA. Dasch GA*, Jackson LM, and Chan CT. Viral and Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

Scrub typhus has been recognized in North Queensland for over 100 years and was also recently described in the Northern Territory of Australia. Only the chigger stage of *Leptotrombidium deliense* has been described as the vector for *Rickettsia tsutsugamushi* in Australia. Previous direct fluorescent antibody typing of Australian isolates of *R. tsutsugamushi* suggested their antigenic heterogeneity. We have investigated the genetic diversity of North Queensland isolates from chiggers (2), their rat (14) and bandicoot (4) hosts, and from humans (27) by amplifying a portion of the conserved groESL operon and all of the 22 kDa protein antigen gene by polymerase chain reaction and examining the amplicons for restriction fragment length polymorphisms. Ten groESL RFLP types were identified by digestion with eight restriction enzymes. These types were each unique to Australia since they were not identical to isolates from any of 12 other Asian countries examined. The most frequent RFLP genotype occurred in 5 rat, 2 bandicoot, both chigger, and 7 human isolates. The second most frequent groESL genotype was shared by 6 human and 4 rat isolates. One RFLP type each was unique to 2 bandicoot, 2 rat, and 1, 4 and 6 human isolates, respectively. The other three RFLP types were each shared by one rat and one human isolate. Less complete 22 kDa RFLP typing confirmed the genetic heterogeneity and uniqueness of Australian isolates of *R. tsutsugamushi* and permitted further subdivision of groESL types. Since these types of *R. tsutsugamushi* are all found only in Australia, they likely evolved during long geographic isolation and were not recently imported in chiggers on birds or rodents from other areas of Asia.

235 ENZYME IMMUNOASSAY FOR DETECTING *RICKETTSIA TYPHI* INFECTIONS IN FIELD COLLECTED RODENTS, MAUI, HAWAII. Kirschner KF*, Sasaki DM, Cope SE, and Olson JG. U.S. Navy Environmental and Preventive Medicine Unit No. 6, Pearl Harbor, HI; Zoonoses Section, Epidemiology Branch, State of Hawaii Department of Health, Honolulu, HI; Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, GA.

Hawaii annually reports cases of murine typhus on the island of Maui. We investigated the enzootic cycle in five locations on Maui that had reported cases of murine typhus. Box traps constructed of carpenter cloth were baited with coconut and placed in areas with good rodent harborage located near homes of reported cases. 167 rodents were trapped between March 15 and May 5, 1995. Blood and spleens were collected and frozen at -20°C until tested. Testing included enzyme immunoassays for *Rickettsia typhi* antibody and antigen. The overall prevalence of *R. typhi* antibody was 21%. *Rattus rattus* was the most frequently collected species, 129 (77%), and was most frequently *R. typhi* antibody positive, 32 (25%). Only two (6%) of 34 *R. exulans* were *R. typhi* antibody positive. None of the 4 *Mus musculus* were antibody positive. Four of the 5 localities had evidence of enzootic *R. typhi* activity in *R. rattus*. Puunene had the highest prevalence, 7 (50%) of 14, followed by Lahaina, 5 (45%) of 11, Kihei, 18 (23%) of 78 and Kahului, 2 (22%) of 9. None of the 17 *R. rattus*collected in Kula had evidence of *R. typhi* infection. Antigen detection assays for *R. typhi* were completed on blood and spleen homogenates of all rats. A total of 53 (32 %) of blood specimens and 15 (9%) of spleens were positive. Enzyme immunoassays provided data that confirm that *R. typhi* is currently enzootic in *R. rattus*.

236 *IXODES PACIFICUS* (ACARI: IXODIDAE): A VECTOR OF *EHRLICHIA EQUI*. Richter PJ*, Kimsey RB, Madigan JE, Barlough JE, Dumler JS, and Brooks DK. Division of Comparative Medicine, University of CA, San Diego, La Jolla, CA; Department of Entomology, University of California, Davis, CA; Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA; and Department of Pathology, University of Maryland Medical Center, Baltimore, MD.

We report transmission of an agent of granulocytic ehrlichiosis, *Ehrlichia equi*, from infected to susceptible horses via the bites of Western black-legged ticks, *Ixodes pacificus*. *E. equi*, a rickettsia described from horses in California thirty years ago, causes equine granulocytic ehrlichiosis (EGE) throughout the Americas and possibly Europe. A newly recognized and extremely similar *Ehrlichia*, the agent of a fatal human granulocytic ehrlichiosis (HGE) in north central United States, possesses almost identical 16S rDNA sequences to that of *E. equi* and causes indistinguishable disease when inoculated into horses. Exposure to bites of *Ixodes* ticks poses previously unrecognized and serious health risks to humans and animals.

237 ECOLOGICAL ASSOCIATION BETWEEN *IXODES PACIFICUS* (ACARI: IXODIDAE) AND THE SPATIAL AND TEMPORAL DISTRIBUTION OF EQUINE EHRLICHIOSIS IN NORTHERN CA. Vredevoe LK*, Richter PJ, Madigan JE, and Kimsey RB. Department of Entomology, University of California, Davis, CA; Division of Comparative Medicine, University of CA, San Diego, La Jolla, CA; Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA; and Department of Entomology, University of California, Davis, CA.

We asked if the biology of the western black-legged tick, *Ixodes pacificus*, might regulate the spatial and temporal distribution of equine ehrlichiosis infections in California north of Santa Cruz County. The rickettsial agent of equine ehrlichiosis, *Ehrlichia equi*, is responsible for the second most commonly diagnosed infectious disease of horses in northern California. Within this region we compared the spatial and temporal distribution of equine ehrlichiosis cases with the seasons of activity and life history of ticks which infest horses. Cases clustered significantly in the northern California region, with foci limited to the Sierra Nevada and coastal foothills. Cases also clustered seasonally; most occurred between September through May, though fewer coastal foothill cases were diagnosed during fall and late spring. The spatial and seasonal pattern of equine ehrlichiosis cases closely parallels the well characterized distribution of *Ixodes pacificus*, but not other ticks commonly associated with horses.

238 MOLECULAR CLONING AND SEQUENCING OF A 120 kDa IMMUNODOMINANT PROTEIN GENE OF *EHRLICHIA CHAFFEENSIS*. Yu XJ*, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX

Ehrlichia chaffeensis is an obligately intracellular bacterium that causes human monocytic ehrlichiosis in the United States, Africa, and Europe. A genomic library was constructed by using *E. chaffeensis* genomic DNA partially digested with Xba I in λZAPII vector. An antigen- producing clone designated γ5 was obtained by screening the library with canine anti-*E. chaffeensis* serum. γ5 was converted into pBluescript phagemid and expressed in *E. coli*. γ5 expressed a 120 kDa protein in *E. coli* that reacted with canine anti-*E. chaffeensis* serum. Monospecific antibody to

the γ5-expressed protein reacted with a 120 kDa protein in Western immunoblots of E. chaffeensis. E. coli JM101 transformed with recombinant plasmid containing the 120 kDa protein gene of E. chaffeensis can invade Hela and Vero cells. This protein is a leading candidate for playing an important role in the pathogenesis of E. chaffeensis and protective immunity in the host. DNA sequence analysis demonstrated an open reading frame with an ehrlichial promoter and more than half of the gene comprising multiple tandem repeat units encoding 80 amino acids each.

239 ULTRASTRUCTURE OF HUMAN GRANULOCYTIC EHRLICHIAE. Popov VL*, Han VC, Chen SM, Bakken JS, Dumler JS, Madigan JE, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX; Duluth Clinic, Duluth, MN; Department of Pathology, The University of Maryland,Baltimore, MD; and Department of Medicine and Epidemiology, School of Veterinary Medicine, The University of California, Davis, CA.

Human granulocytic ehrlichiosis (HGE) is a new, emerging human disease, presumably tick-borne, recognized originally in the upper Midwest of the United States. Its agent has not been cultivated in cell culture. We studied the ultrastructure of human granulocytic ehrlichiae in neutrophils purified from the peripheral blood of a horse inoculated with a blood sample from a patient with HGE. The majority of the granulocytes appeared to be infected with typical ehrlichiae. Infected neutrophils contained one or several parasitophorous vacuoles (morulae) filled with ehrlichial organisms having morphology similar to human monocytic ehrlichiae: smaller dense-cored cells and larger reticulate cells. Both cell types were surrounded by two trilaminar membranes - the cytoplasmic membrane and the cell wall, which usually manifested a wavy configuration. Some morulae contained enlarged reticulate cells, either demarcated by membranous septa, or small membrane-bound protoplasmic particles budding into the periplasmic space. Reticulate cells were also observed dividing by binary fission. These data demonstrate the ehrlichial nature of the etiologic agent of HGE.

240 EVALUATION OF AN ENZYME IMMUNOASSAY IN THAI SCRUB TYPHUS PATIENTS. Kelly DJ*, Suwanabun N, Eamsila C, Chaouriyagune C, Howard R, and Dasch GA. Department of Clinical Investigation, Walter Reed Army Medical Center Washington, DC; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Medicine, Chiangrai Prachanuchroa Hospital, Chiang Rai, Thailand; and Viral and Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

Previously our ELISA for *Rickettsia tsutsugamushi* used single antigens and dry milk or bovine serum albumin as a blocking buffer. We report an improved procedure using a mixture of Karp, Gilliam and Kato strain purified antigens in which background was lowered using a casein blocking buffer. The ELISA was tested in either single dilution or four-fold serial titration of the serum. Results were compared to a standard test, the indirect immunoperoxidase assay (IIP). Control serum samples were collected from 96 American soldiers and 198 Royal Thai Army soldiers with no history of clinical illness. In addition, sera were examined from 79 febrile Thai patients presenting at Chiang Rai and Bangkraui, Nontaburi hospitals suspected or confirmed to have scrub typhus (cases confirmed by elevated IIP IgG levels [>1: 800], IgM levels [> 1: 200], or presence of an eschar). The mean + 2 SD used for the upper limit of normal in the IgG ELISA was 0.10 for US soldiers and 0.43 for Thai soldiers. Using the 0.10 cutoff value, 29% of Thai soldiers would be diagnosed as disease positive. Variability of ELISA values was greater in the Thai soldier group than for American soldiers, possibly reflecting previous asymptomatic exposure to the agent. In the IgG assays in Thai patients, there was a significant correlation between IIP titers and single serum dilution ELISA values ($r= 0.76$, $P<0.0005$) and between IIP titers and ELISA titers ($r= 0.87$, $P<0.0005$). The single serum dilution IgG ELISA was as effective as the titration in determining presence of specific antibodies. The *R. tsutsugamushi* ELISA is a rapid and objective test amenable to accurately testing the large numbers of sera typical in a seroepidemiological study.

241 DEMONSTRATION OF CYCLOOXYGENASE-1 ENZYME IN *ENTAMOEBA HISTOLYTICA*: PARASITE PRODUCTION OF PROSTAGLANDIN E2. Belley AC*, Keller K, and Chadee K. Institute of Parasitology of McGill University, Macdonald Campus, Ste. Anne de Bellevue, Quebec.

Prostaglandins are produced from the oxygenation of arachidonic acid by the enzymes cyclooxygenase (COX)-1 and COX-2. These compounds have diverse effects in mammals, particularly in homeostatic processes, but are host noted for their pathophysiological effects in inflammatory states. Aside from their impact on host tissues, prostaglandins are potent immunomodulating agents. They inhibit macrophage and T and B cell functions that are critical in establishing an immunological response during an infection. The disease amebiasis is characterized by a down regulation of macrophage functions. The identity of the parasite-derived molecule that causes suppression is not known. In this study, we determined if the enteric protozoan parasite *Entamoeba histolytica*contains a homologous cyclooxygenase enzyme and produces prostaglandin E2(PGE_2). Using an affinity purified polyclonal antiserum against the human COX-1 enzyme for immunoblotting, an amebic protein of comparable molecular

mass to purified COX-1 standard was detected in the 2,000 X $_g$ protein fraction. An enzyme immunoassay for PGE_2 was used to detect and quantify PGE_2 production by *E. histolytica*. The parasite released significant levels of PGE_2 into the culture medium constitutively, in the absence of exogenous arachidonic acid substrate. Amebae preincubatedwith indomethacin, a pharmacologic inhibitor of cyclooxygenase activity,produced less PGE_2 as compared to amebae incubated without the inhibitor. Gas chromatography/mass spectrometry (GC/MS) analysis of culture supernatants corroborated the identity of the amebic PGE_2. These results demonstrate a putative cyclooxygenase-1 enzyme in *E. histolytica*and the production of the immunomodulator, PGE_2. These findings elucidate a potential mechanism by which *E. histolytica* overcomes host defenses and causes disease.

242 EPIDEMIOLOGICAL ASPECTS OF CYST PRODUCTION OF *ENTAMOEBA*. Zurabian R*, and Acuna-Soto R. Departmento de Microbiologia y Parasitologia. Facultad de Medicina. Universidad Nacional Autonoma de Mexico. M.

The protozoan parasites *Entamoeba coli, Endolimax nana, Entamoeba histolytica, Entamoeba dispar,* and *Entamoeba hartmanni* are exclusive human parasites that share many epidemiological and biological aspects. In a study of several semi-rural populations near Mexico City and based in morphology and PCR for speciation, we identified several aspects of cyst production relevant to the epidemiology of these parasites. The order of parasites is in decreasing order was: *Entamoeba coli, Endolimax nana, Entamoeba histolytica, Entamoeba dispar,* and*E. hartmanni*. This order turned to be the same when the species were listed according to the number of cysts/gram. Interestingly, this order of frequency is also described in most populations reported in the literature. We identified statistically significative cyclic changes in the number of cysts, the cycles were synchronized and simultaneous for all species. By studying the total and partial output of cysts/gram over time we detected significative individual variations in cyst production. Currently, we are evaluating possible effects of synergism or competition among the different species and studying individuals that produce constantly high numbers of cysts.

243 COMPARISON OF TWO IMMUNOASSAYS FOR THE DETECTION OF *ENTAMOEBA HISTOLYTICA/DISPAR* IN STOOL SPECIMENS. Rosenblatt JE* and Sloan LM. Division of Clinical Microbiology, The Mayo Clinic, Rochester, MN.

We compared two commercially produced enzyme-linked immunoassays (ELISAs) for the detection of *Entamoeba* antigens in 111 stool specimens submitted to the Mayo Clinic parasitology laboratory. The stools tested were collected fresh (unpreserved) and stored at - 70°C for periods of 1-8 years. ELISA results from both the *Entamoeba* Test (TechLab) and the ProSpecT *Entamoeba histolytica* Microplate Assay (Alexon, Inc.) were compared to those obtained by conventional microscopic examination (CME) of trichrome stained smears. 65 stools were negative by the *Entamoeba* Test and 38 were positive. Using the ProSpecT, 65 specimens were negative and 41 were positive. There were 5 "false negatives" (CME was positive) by the ProSpecT and 8 with the *Entamoeba* Test. The specimens tested by the *Entamoeba* Test went through one more freeze/thaw cycle than those tested by the ProSpecT which might account for the 3 additional "false negatives". Neither ELISA exhibited any false positives indicating that cross reactivity with other antigens was not occurring. ProSpecT and *Entamoeba* Test specificities were both 100%, positive predictive values were both 100%, and sensitivities were 89% and 83%, respectively. Both ELISA kits provide similar results in screening for the presence of *Entamoeba*. However, the *Entamoeba* Test has the additional advantage that positive specimens can be further tested to differentiate *Entamoeba histolytica* from *Entamoeba dispar*. In this study, the *Entamoeba* test indicated that all but one of the 38 positive specimens contained *E dispar*.

244 EXPERIMENTAL STUDIES ON THE KITTEN SHOWING THE DIFFERENCE OF PATHOGENECITY BETWEEN *ENTAMOEBA DISPAR* AND *ENTAMOEBA HISTOLYTICA* BY E. BRUMPT. Petithory JC*, Ardoin FG, Sargeaunt PG, and Brumpt LC. Contrìle de Qualité National en Parasitologie, Centre Hospitalier, Gonesse, France.

On the third of November 1925, at the Academy of Medecine, Paris, E. Brumptdescribed a new species of amoeba with quadrinucleate cysts : *Entamoeba dispar*. He based his conclusions on the differentiation of *E. dispar*from *Entamoeba histolytica* on firstly, epidemiological data, observing that in many countries and particularly in Europe numerous people were harbouring quadrinucleate cysts without any or few clinical symptoms of amoebiasis, secondly by numerous experimental studies on kittens. Unfortunately in 1993, many documents, specimens and archives were destroyed in what were E. Brumpt laboratories. We have, however, been able to save a small part of his important work with the collection of protozoa and especially some still very well preserved histological sections used by E. Brumpt to describe this new species. In these sections one can see the characteristic ulcers of the intestine in the case of *E. histolytica* with alteration of the *Muscularis Mucosae*, a thickening of the intestinal wall and the presence of hematophagous trophozoites, whereas with *E. dispar*, though sometimes numerous non-

hematophagous trophozoites can be seen there are small lesions without ulceration. The above observations have been confirmed by the study of zymodemes and more recently by molecular biology.

245 IMMUNODIAGNOSIS OF ALVEOLAR ECHINOCOCCOSIS BY ELISA USING PARTIALLY PURIFIED Em18 AND Em16 ENRICHED FRACTION. Ito A*, Ma L, Itoh M, Cho S, Kong Y, Kang S, Horii T, Pang X, Okamoto M, Yamashita T, Lightowlers MW, and Liu Y. Department of Parasitology, Gifu University School of Medicine, Japan; Institute of Infectious and Parasitic Diseases, Chongqing University of Medical Sciences, China; and Department of Medical Biology, Medical School, Nagoya City University, Japan.

Alveolar echinococcosis (AE), caused by the larval stage of the fox tapeworm, *Echinococcus multilocularis* is one of the most lethal helminthic infections of humans and often misdiagnosed as hepatic cancer. The development of improved methods for differential serodiagnosis is a critical need. Recently we found previously undescribed two epitopes, designated Eml8 and Em16, as good markers specific for AE by Western blot analysis. A new ELISA system was evaluated for serodiagnosis of AE using partially purified Em18/Em16 enriched fraction (PP-Eml8/16). The PP-Eml8/16 ELISA was compared with Em2^{plus}-ELISA using patients' sera from AE and cystic echinococcosis (CE) in China where both AE and CE are endemic, sera from CE in Australia where CE only exists, and sera from patients with cysticercosis, paragonimiasis, sparganosis in Korea. Antibody levels by ELISA using PP-Em I 8/1 6 were much higher in AE than in CE and this was also true for Em2^{plus}-ELISA. Some of CE from China showed exceptionally higher levels of antibody in comparison with those of CE from Australia where no AE existed. It is suggested that these strongly positive cases of CE from China may have been exposed to both species of *Echinococcus*. Although most of sera from paragonimiasis showed high antibody levels by Em2^{plus}-ELISA, they were negative by the ELISA using PP-Eml8/16. Therefore, PP-Eml18/16-ELISA is expected to be more reliable for differentiation of AE from CE and others especially in Asian countries where paragonimiasis is still not rare.

246 INDUCTION OF MUCOSAL ANTI-AMEBIC IgA ANTIBODIES BY IMMUNIZATION WITH *SALMONELLA TYPHIMURIUM* EXPRESSING THE AMEBIC SREHP MOLECULE. Zhang T*, Li E, and Stanley, Jr. SL. Washington University School of Medicine, St. Louis, MO.

The protozoan parasite *Entamoeba histolytica* causes amebic dysentery and amebic liver abscess, two major sources of morbidity and mortality world wide. One way to prevent *E. histolytica* infection would be to produce mucosal IgA antibodies capable of blocking the parasite's adhesion to intestinal mucosae. Previously, we described the expression of a protective amebic antigen, the serine rich *Entamoeba histolytica* protein (SREHP) in an attenuated vaccine strain of *Salmonella typhimurium*. However, levels of expression of the SREHP/MBP fusion protein in *S. typhimurium* were low, and oral immunization of mice with *S. typhimurium* expressing SREHP failed to induce mucosal anti-SREHP or anti-amebic antibodies. We have now produced a new construct which permits high level expression of SREHP/MBP in *S. typhimurium*. Mice orally immunized with this new *S. typhimurium*/SREHP vaccine produce salivary and stool IgA anti-amebic antibodies, and serum IgA and IgG anti-amebic antibodies. This represents the first *Salmonella*- based vaccine capable of producing mucosal IgA anti-amebic antibodies. We are now testing the efficacy of oral immunization with the *S. typhimurium*/SREHP vaccine in preventing intestinal amebiasis and amebic liver abscess in animal models.

247 *IN VITRO* SUSCEPTIBILITY OF METRONIDAZOLE-RESISTANT *TRICHOMONAS VAGINALIS* TO FURAZOLIDONE. Narcisi EM*, and Secor WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Trichomonas vaginalis is a common sexually transmitted protozoan parasite. Although often considered simply a nuisance infection, *T. vaginalis* has been implicated in premature rupture of placental membranes and possibly increases the risk for acquiring HIV. Metronidazole, a 5-nitro-imidazole is currently the drug of choice to treat *T. vaginalis*. However, several cases of drug resistant metronidazole infections are reported each year and the most commonly considered alternative, tinidazole, is also a 5-nitro-imidazole. This increase in clinical drug resistance, as well as an increase in patients with severe reactions to metronidazole, prompted us to search for alternative therapies. Furazolidone, which inhibits monamine oxidase, was investigated for effectiveness against 4 susceptible and 4 resistant patient isolates of *T. vaginalis*. The *in vitro* aerobic and anaerobic minimum lethal concentrations and the time for drug efficacy were determined for furazolidone, metronidazole, and tinidazole. Furazolidone killed both metronidazole susceptible and resistant trichomonads within 2 -3 hours of exposure. In contrast, the two nitro-imidazoles required > 6 hours before any killing effect was noted and they only acted on the metronidazole-susceptible isolates. Thus, furazolidone appears to be a good candidate for futher testing against metronidazole-resistant trichomoniasis.

248 IDENTIFICATION OF DEVELOPMENTALLY REGULATED TRANSCRIPTS IN *GIARDIA LAMBLIA* BY DIFFERENTIAL DISPLAY. Que X*, Aley SB, Meng TC, Hetsko ML, and Gillin FD. Department of Pathology, Division of Infectious Diseases; University of California San Diego Medical Center, CA.

To understand the molecular regulation of differentiation of *Giardia lamblia*, we examined gene expression during encystation and excystation, which we can efficiently reproduce *in vitro*. A differential mRNA display PCR technique was used to compare the mRNA populations at different stages of encystation and excystation. Gene transcript patterns in vegetatively growing and 24 hr-encysting trophozoites were compared in 10 groups by PCR with combinations of five arbitrary 5' primers and two anchored oligo-dT primers. Analysis of products in sequencing gels showed about 50 to 100 amplified cDNA bands ranging from 100 to 500 bp in each lane. Fingerprinting of mRNA populations during the time course of encystation and excystation showed clearly the appearance and disappearance of different bands at specific times, and encystation specific mRNA bands accumulated from 5 to 24 hours of encystation. To identify genes involved in encystation, six upregulated cDNA bands from 24 hr-encysting trophozoites were recovered and reamplified, yielding two stage-specific single copy genes whose transcripts increase greatly during encystation. Partial DNA sequence analysis demonstrated no correspondence with known genes. These studies suggested a role for complex transcriptional regulation of *G. lamblia* differentiation and allowed the direct identification of critical stages, as well as isolation of genes which may be important to development.

249 PATHOGENESIS OF MUCOSAL DAMAGE IN GIARDIASIS: POSSIBLE ROLE OF HOST IMMUNE RESPONSE. Sehgal R, Goyal R, Ganguly NK*, and Mahajan RC. Departments of Parasitology and Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh, India.

Despite the number of hypothesis put forward, the mechanism(s) related to pathogenesis of diarrhea and malabsorption in giardiasis are still unclear. The postulation that protective immune response by the host may play a role in pathogenesis of mucosal damage prompted us to study the role of gut macrophages, using BALB/C mice injected with strains of *Giardia lamblia* isolated from asymptomatic / symptomatic patients of giardiasis. It was observed that the cytotoxic potential of gut macrophages of mice injected with symptomatic strains was significantly higher as compared to those injected with asymptomatic strains. Using a co-culture system of enterocytes and gut macrophages and lactate dehydrogenase (LDH) levels as an indicator of enterocyte damage, it was again observed that there was a significant increase in LDH release in both the groups but more in animals infected with symptomatic strains. Further, when antioxidant status of small intestine of infected mice was compared with that of uninfected controls, it was observed that certain enzymes of redox cycle were decreased significantly in infected animals, indicating that these cells were more vulnerable to lipid peroxidation, which was observed to be significantly increased in infected animals as compared to controls. The lipid peroxidation of mice infected with symptomatic strains was significantly higher than that in mice infected with asymptomatic strains. The role of host and parasite factors in pathogenesis of mucosal damage in giardiasis will be discussed.

250 PURIFICATION OF A LECTIN FROM *GIARDIA LAMBLIA* AND CHARACTERIZATION OF INTESTINAL RECEPTOR(S) FOR THE LECTIN. Sehgal R, Sriniwas K, Ganguly NK*, and Mahajan RC. Departments of Parasitology and Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh, India.

Though, *Giardia lamblia* an intestinal protozoa, possesses intestinal surface lectin activity, the interaction of this lectin with intestinal receptors is a matter of speculation. The present study,was undertaken to evaluate the role of Giardia lectin in binding to intestinal receptors and to identify and characterize these receptors in experimental animals. A surface lectin of 130 KDa which consisted of a dimer of 65 kDa was isolated from Portland I strain of *G. lamblia*. The lectin identified a 56 kDa glycoprotein receptor on brush border membrane of mice. This receptor showed mannose, fucose and glycosyl residues. The density of receptor was related to the age of the animals with a high density in duodenum, jejunum and ileum of weanling mice and only in the jejunum and ileum of adult mice. The study shows that a 65 kDa lectin is present on *G. lamblia* which identifies a 56 kDa receptor on brush order membrane of mice, indicating thereby that this lectin-receptor interaction may be involved in parasite adherence.

251 GASTROINTESTINAL SYMPTOMS IN HAITIAN MIGRANTS - PARASITIC EVALUATION AND ETIOLOGY, AND RESPONSE TO ANTI-PARASITIC TREATMENT. Rusnak JM*, Reed K, Rodgers J, Pace E, Ross C, and Hayashi K. Department of Infectious Diseases, Wilford Hall Medical Center, Lackland Air Force Base, TX.; MacDill Air Force Base, FL.; Portsmouth Naval Station, VA; World Relief Organization.

Gastrointestinal (GI) complaints which overcrowded clinics in a Haitian migrant camp at Guantanamo Bay, Cuba, were evaluated by GI questionnaires and 3 serial stool specimens from 108 randomly selected Haitians of defined age groups to determine 1) parasitic prevalence as there are limited studies available in Haitians and 2) if GI symptoms of pain,diarrhea (D), gas/bloating (G/B) were secondary to parasites vs dietary change vs non-infectious causes (no response to parasite treatment). GI symptoms were common: 86% pain, 27% heartburn, 57% D, and 65% G/B. StoolO&P revealed *Ascaris* 32% (*Ascaris* or history of worms 59%), *Trichuris* 42%, *Giardia* 42%, Hookworm 4, *Strongyloides* 2,*Cryptosporidia* 1, *E. histolytica* 1. Onset of most symptoms occurred pre-arrival (58% > 2 months, unknown 19%) suggesting dietary change an unlikely etiology. The high response of 68% to parasitic treatment in children under age 10 suggests mainly a parasitic etiology in children. The low response rate in adults (17% total and 46% partial resolution) supports parasites to be a minor contributing factor in adults. Only older children and adults experienced "heartburn" (29%, 47%) or "burning"quality of pain. This data suggests non-infectious causes (gastritis, PUD, or reflux) or *H. pylori* as major etiologies in adults as opposed to *Giardia* (found in 54% Haitian adults) which may cause similar symptoms.

252 *IN VITRO* SUSCEPTIBILITY OF *GIARDIA LAMBLIA* TROPHOZOITES TO NEWLY SYNTHESIZED METHYL-BENZIMIDAZOLES. Cedillo RR*, Hernández CA, Tapia CA, Sánchez R, Castillo R, Muñoz 0. Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias. Hospital de Pediatríca, CMN, IMSS; Departamento de Farmacia, Facultad de Química, Universidad Nacional Autínoma de México.

Carbamate-benzimidazoles (CB) are used worldwide for the treatment of helmintic infections, and have a great activity against *Trichomonas vaginalis* and *Giardia lamblia*. However, these CB have poor solubility and low absortionfrom the intestine. In this work, we synthesized six methyl-benzimidazoles (MB). These compounds showed better solubility than CB, and should reach higher levels in plasma. In order to test the antigiardial activity of these MB, we developed an *in vitro* subculture assay. Serial dilution of the drugs, were prepared in 4 ml volumes of culture medium. Media were inoculated with 5 x 10^4 trophozoites/ml of *G. lamblia* strain IMSS-3. As control, we used media free of drug. After incubation for 48 h at 37°C, parasites were detached and 50 µl of each culture were inoculated into fresh medium, free of drug, and incubated for other 48 h. The finalnumber of trophozoites were determined by counting in an heamocytometer.The IC_{50} (µg/ml) for each benzimidazole (BZ) derivative, defined as the concentration of the drug that inhibited growth by 50% was: 5-chloro-1-methyl-2-methylthio (BZ): 0.12; 2-amino-1-methyl(BZ): 0.16; 2-amino-5,6-dichloro-1-methyl (BZ): 0.22; 5,6-dichloro-1,2-dimethyl(BZ): 0.22;5,6-dichloro-1-methyl-2-methylthio (BZ): 0.23; 5,6-dichloro-2-mercapto-1-methyl (BZ): 0.24. These results indicate that the methyl- benzimidazoles synthesized by us have a good activity against *G. lamblia* trophozoites.

253 TRICHOMONIASIS IN WOMEN FROM MISSISSIPPI; A FIVE YEAR RETROSPECTIVE REVIEW OF PAPANICOLAOU SMEARS. Acholonu AD, White JD, Lushbaugh WB*, Cason Z, and Lemos LB. Alcorn State University, Department of Biology, Lorman, MS.; University of Mississippi Medical Center, Department of Pathology, Section of Cytopathology; and University of Mississippi Medical Center, Department of Microbiology, Jackson, MS.

Since trichomoniasis is not a reportable sexually transmitted disease (STD) not much epidemiological work has been on it in the USA. PAP smears made from women who attended state owned health centers and clinics were sent to University of Mississippi Medical Center (UMC) where they were processed by standard procedures and examined microscopically for cellular abnormalities and *Trichomonas vaginalis*. Of a total of 559,454 females examined in the five year period reviewed, 31,797 (5.7%) were positive for *T. vaginalis*. The highest prevalence was recorded in 1990 when 7,850/120,336 (6.5%) of females examined were infected. The lowest was in 1992 when 5,691/118,423 (4.8%) were positive. Trichomoniasis was reported from all 82 counties in Mississippi with the exception of Issaqauena County from which no PAP smears were submitted for processing. The highest prevalence was recorded from Humphreys county (11.4%) and the lowest from Tishamingo (1.1%). Prevalence declined in the two years following the 1990 peak (1991 - 5.4%; 1992 -4.8%). The latest two years (1993 - 5.7%; 1994 - 6.0%) have shown increased prevalence. With this trend, another increase may occur by the end of 1995 and beyond. Clearly, trichomoniasis is a pervasive health problem that requires preventive measures, and further epidemiologic studies to determine the factors contributing to increasing prevalence.

254 CLONING AND IMMUNOLOCALIZATION OF A CALTRACTIN-LIKE GENE FROM *GIARDIA LAMBLIA*. Meng TC*, Aley SB, Kim J, Huang B, Smith MW, Harwood J, and Gillin FD. Departments of Medicine and Pathology, University of California San Diego Medical Center, San Diego, CA; Department of Cell Biology, The Scripps Research Institute, La Jolla, CA; and Molecular Genetics Laboratories, The Salk Institute, La Jolla, CA.

The calcium-binding protein caltractin is a major component of the basal body complex, the major microtubule-organizing center and functional equivalent of the centrosome, of the unicellular green alga *Chlamydomonas reinhardtii*. Using a probe derived from a *Giardia* genome sequencing project, we have cloned and sequenced a 1.2 kb genomic region containing a 528 bp ORF coding a 176 amino acid protein (predicted Mw 20 KDa) with homology to *C. reinhardtii* caltractin (53.4% overlap identity) and human caltractin (54.5%), as well as to other centrin-like proteins. Like other members of this calcium-binding protein subfamily, *Giardia lamblia* caltractin contains four evolutionarily-conserved, 29 amino acid, helix-loop-helix, calcium-binding EF-hand domains. By genomic Southern analysis, the gene is single copy and by Northern analysis, appears constitutively expressed in vegetative and encysting populations. Anti-recombinant human and *C. reinhardtii* caltractin antibodies react predominantly with the basal bodies of *G. lamblia* trophozoites and with a 20 kDa protein on immunoblot. Because of the close relationship between the flagella and the basal bodies, *G. lamblia* caltractin may play an integral role in cellular motility. In addition, like caltractin in *C. reinhardtii* and centrin in other organisms, *G. lamblia* caltractin may play a role in the division of the nucleus and cytoskeleton of this primitive eukaryote.

255 PURIFICATION AND PARTIAL CHARACTERIZATION OF A TRANSGLUTAMINASE FROM DOG FILARIAL PARASITE, *DIROFILARIA IMMITIS*. Mehta K*, Singh RN, and Chandrashekar R. Department of Bioimmunotherapy, University of Texas MD Anderson Cancer Center, Houston, TX; and Department of Medicine, Washington University Medical Center, St. Louis, MO.

Inhibition of transglutaminase-catalyzed reactions has been suggested to be an important biochemical target for effective control of filarial parasites. Therefore, the purification and characterization of the parasite transglutaminase (pTGase) would be an essential step for the identification or design of an appropriate inhibitor for pTGase. We recently purified and characterized a 56-kDa pTGase from *Brugia malayi* adult parasites. Here we describe the purification of pTGase from yet another filarial nematode, *Dirofilaria immitis*, using a simple three-step purification protocol. The three steps used were thermoprecipitation, ammonium sulfate precipitation and immunoaffinity chromatography. The antibody used for immunoaffinity chromatography was raised in rabbit against a synthetic peptide corresponding to the N-terminal sequence of pTGase from *B. malayi*. About 900-fold purification was achieved in a single immunoaffinity chromatography step that yielded a 56-kDa protein as the major band in a denaturing polyacrylamide gel electrophoresis. The protein eluted at this step was enzymatically active and had a specific activity of 2 U/mg protein. Several physico-chemical properties such as thermostability, substrate specificity, effect of various reagents and inhibitors suggested that this enzyme is very similar to the pTGase of *B. malayi*, but was quite distinct from the mammalian transglutaminases.

256 A SMALL HEAT SHOCK PROTEIN FROM *BRUGIA MALAYI* L4 STAGE LARVAE. Raghavan N*, and Scott AL. Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

It has been demonstrated previously that protective immunity in lymphatic filariasis is predominantly directed towards the L3/L4 larval stages. There is very little information available on the L3-L4 larval stage transition and L4 larval stage antigens. In order to identify L4 antigens important in the developmental regulation of the parasite and in host protection, a SL cDNA library of *Brugia malayi* L4 stage larvae (JHU93SL-BmL4) was constructed in a phage expression system. The library was immunoscreened using anti-L3 antibodies. One recombinant (BmNR13), which was represented in 0.01% of the transcript population and having an insert size of 741 bp was isolated and sequenced. The insert had an open reading frame of 152 amino acids with a spliced leader 16 bp upstream from the initiating codon ATG. The deduced amino acid sequence showed 36% sequence similarity to a small heat shock protein (hsp) from *Onchocerca volvulus* and 40% simlarity to mouse and human hsp27 molecules. Semi-quantitative RT-PCR analysis indicated the hsp transcript to be transcribed during all the developmental stages of the parasite. The recombinant protein (Bmhsp) was expressed as a ~55 kDa *TrpE* fusion protein in the pATH2 expression vector containing a 6XHis tag for ease of purification. The recombinant hsp antigen was used to study the T-cell responses of lymphatic filarial patients with various clinical manifestations. The small hsps, which also show similarity to α-crystallins may play an important role in the development of the filarial parasite by stabilizing and protecting the DNA against the traumas that are known to induce the synthesis of the heat shock proteins.

257 INITIAL CHARACTERIZATION OF THE MITOCHONDRIAL GENOME OF *ONCHOCERCA VOLVULUS*. Keddie EM*, and Unnasch TR. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL; and Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL.

As part of an ongoing project to characterize the respiratory pathways of *Onchocerca volvulus*, the mitochondrial genome of this skin-dwelling filarial nematode was cloned. A cytochrome b PCR product was generated using *O.*

volvulus genomic DNA and conserved primers derived from the cytochrome b sequences of the nematodes *Ascaris suum* and *Caenorhabditis elegans*. This 773bp product was cloned, sequenced, and used to screen an *O. volvulus* size-selected genomic library constructed in lambda EMBL4. A clone selected for continued analysis contained an approximately 14kb insert, consistent with the expected mitochondrial genome size as estimated by Southern blot. The excised genomic insert was digested with Sau3A and ligated to pBR322. Four plasmid subclones currently sequenced, representing 1.8kb of the *O. volvulus* mitochondrial genome, include portions of cytochrome oxidase subunit I, NADH dehydrogenase subunits 4 and 5, and cytochrome b. Analysis of additional subclones is underway to complete the mitochondrial genome nucleotide sequence.

258 DISRUPTION OF EMBRYOGENESIS IN *BRUGIA MALAYI* BY IMMUNIZATION WITH OVT1. Triteeraprapab S*, and Scott AL. Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; and Department of Microbiology & Immunology, Johns Hopkins University School of Hygiene & Public Health, Baltimore; MD.

As part of the initial efforst to identify molecules important for embryogenesis in filarial nematodes, OVT1, a gene encoding 2,022 amino acids, was identified by immunoscreen an *Onchocerca volvulus* cDNA female expression library. The nature of amino acid sequence and tissue localization of expressed matrix component. Homologous genomic DNA sequences and protein epitopes and OVT1 are found in other filarial nematodes including *Brugia malayi*. ELISA tests of anti-OVT IgG in Guatemalan patients showed low levels of IgG against the OVT1 although the mean level of putatively immune group was twice that of asymptomatic patients. 24 out of 25 of the sera fro *O. volvulus*-infected expatriates had detectable levels of anti-OVT1 IgG4 and 88% had detectable levels of anti-OVT1 IgE. Preliminary experiments in gerbil-*B. malayi* model showed that gerbils immunized with OVT1 IgE. had high antibody response against OVT1. At 14 weeks after challenge with *B. malayi* third stage larvae, the OVT1 immunized gerbils contained no detectable microfilaria despite the presence of male and female parasite. In addition, only two of 17 female parasites contained in utero microfilariae. One of the hallmarks if the immunity generated by irradiation attenuated larvae, the gold standard for immunization against filarial parasites, is that the parasites mature into stunted adults and do not achieve sexual maturity. Our data suggest that immunization with OVT1 can disrupt embryogenesis of *B. malayi* in OVT1-vaccinated gerbils by unknown mechanism. Inhibition of embryogenesis may provide and alternative approach of clinical pathology and transmission of onchocerciasis.

259 CONSTRUCTION AND CHARACTERIZATION OF *BRUGIA MALAYI* ADULT MALE, MICROFILARIA AND L3 cDNA LIBRARIES. Saunders LJ*, Lu W, Ling N, and Williams SA. Molecular and Cellular Biology, University of Massachusetts, Amherst, MA; Department of Biological Sciences, Smith College, Northampton, MA.

The nematode parasite *Brugia malayi* is the causative agent of human lymphatic filariasis, and affects more than 100 million people in tropical and subtropical regions of the world. Production of a vaccine that provides immunity against filariasis is a prospect for the future but will be a difficult task. An important step in identifying possible antigen candidates is the construction of cDNA expression libraries from selected developmental stages of the parasite. In this study, unidirectional cDNA libraries were constructed from *Brugia* microfilariae, adult males and L3 infective larvae with primary titers of $3.5x10^5$,$4.0x10^6$ and $1.6x10^6$ respectively. The average insert size in all of these libraries is approximately 900bp as determined by PCR analysis. Hundreds of random cDNA sequences were isolated, PCR amplified and partially sequenced to create an EST (expressed sequence tag) database. These sequences were compared to existing nucleic acid and protein databases. About 25% of the ESTs showed significant similarity to previously sequenced genes of *Brugia* and several other organisms including the free-living nematode *C. elegans*. Another 25% of these sequences showed less significant similarity to genes of known function and may represent matches to specific functional protein domains. The remaining 50% showed no homology to previously characterized genes and therefore represent unique parasite genes of unknown identity. A preliminary account of the genes identified and a comparison of genes expressed in each developmental stage will be presented.

260 DIRECT AMINO ACID SEQUENCING OF *ONCHOCERCA VOLVULUS* ANTIGENS SEPARATED BY TWO DIMENSIONAL GEL ELECTROPHORESIS. Bitter J, Erttmann KD, and Gallin MY*. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany.

Antigenic extracts of adult *Onchocerca volvulus* were subjected to two dimensional gel electrophoresis and immunoblot using sera from individuals exposed to *O. volvulus*. Reactivity of sera from putatively immune individuals (PI) was compared to that of patients with generalized onchocerciasis (GEN) as well as of patients with sowda (SOW). PI sera recognized fewer antigens compared to the other groups, several antigens were also recognized by GEN and/orSOW. Four of these antigens were directly subjected toN-terminal amino acid sequencing and an average of 15 amino acid residues were determined. The sequences, not related to each other,indicate that

one antigen corresponds to *O. volvulus* calreticulin,one may represent *O. volvulus* HSP60 and two represent enzymes, of which one is involved in glycolysis and the second is part of the pyruvate dehydrogenase complex. All sequences are strikingly similar to the corresponding human proteins, three of which are associated with different human autoimmune diseases. The results suggest a relationship between immune responses to *O. volvulus* and autoimmune-like reactivity in humans.

261 FURTHER CHARACTERIZATION OF OVGALBP, AN IgE BINDING PROTEIN OF FILARIAL ORIGIN. Klion AK*, Catmull J, Garraud O, and Donelson JE. Department of Internal Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA; Department of Biochemistry, University of Iowa, Iowa City, IA; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

The increased incidence of "allergic" symptomatology and clinical complications seen in non-endemic individuals with loiasis is thought to reflect a heightened immune response to filarial antigens. Using serum from one such non-endemic individual to screen an *Onchocerca volvulus* adult cDNA library, we previously identified an antigen, OvGalBP, with significant homology to the vertebrate S-type lectins, a family of thiol-dependent, metal-independent galactoside binding lectins, that includes an IgE-binding protein thought to be involved in IgE regulation. Like its vertebrate homologue, OvGalBP binds IgE in a lactose-inhibitable, isotype-specific manner. To explore further its potential role in the IgE elevation characteristic of loiasis, OvGalBP was subcloned into the pRSET vector, overexpressed and purified for use in cell culture. As has been reported for chicken S-type lectin, OvGalBP caused proliferation of normal mouse splenocytes (S.I.>2). This proliferation was abolished by removal of B cells, suggesting that OvGalBP may have B cell mitogenic activity. Furthermore, in preliminary experiments, OvGalBP was able to induce B cells from 1 of 4 filaria-infected patients and 1of 2 uninfected controls to produce IgE and IgG4 *in vitro*. Taken together these data support the hypothesis that OvGalBP may contribute to the polyclonal B cell activation seen in filaria-infected individuals.

262 *IN VIVO* AND *IN VITRO* CELLULAR IMMUNE RESPONSE TO RECOMBINANT FILARIAL PROTEINS OF *BRUGIA* SPP. Rao UR*, Nasarre C, Coleman SU, Horohov DW, and Klei TR. Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton, Rouge, LA.

Pulmonary granulomatous inflammation (PGI) of *Brugia pahangi* infected jirds (*Meriones unguiculatus*) to antigen coated beads has been shown to vary in intensity to fractions of somatic adult worm antigen (SAWA). In vivo PGI and *in vitro* lymphocyte blastogenesis (LB), however, have not always been comparable. Recently we observed that PGI to SAWA correlates temporally with LB of cells derived from renal lymph nodes (RLN) which drain the infected lymphatics indicating that distinct differences and compartmentalization of cellular responses occur during the course of infection. The objective of this study was to measure the cellular immune reponse to defined filarial proteins using these two assays. For this purpose, gp29, hsp70 (gifts of Dr. Selkirk), BpL4 (gift of Dr. Yazdanbakhsh) and chitinase (gift of Dr. Piessens) were used as fusion proteins of maltose binding protein (MBP). Control coated beads included were DEA, SAWA and MBP. PGI measurements were made at 14, 28, 56 and >150 days postinfection (DPI) in infected jirds; in jirds sensitized with SAWA and in uninfected jirds. LB was conducted at 14 and >150 DPI using RLN. The secretory homolog of glutathione peroxidase, gp29 was the only recombinant protein tested that induced a PGI response ($P<0.05$). This was seen at 28 DPI. All recombinant proteins and MBP were mitogenic at 10 μg/ml in LB, though these responses were reduced in infected animals. Further studies are in progress to define the role of recombinant proteins in filarial pathogenesis.

263 ANTIBODY RESPONSES TO RECOMBINANT *BRUGIA MALAYI* ANTIGENS IN EXPERIMENTALLY INFECTED RHESUS MONKEYS. Dennis VA*, Lasater BL, Lowrie, Jr. RC, Bakeer M, Chandrashekar R, Weil GJ, and Xu K. Departments of Parasitology and Microbiology, Tulane Regional Primate Research Center, Covington, LA; and Washington University School of Medicine, St. Louis, MO.

Identification of microfilariae (MF) in peripheral blood provides a definitive diagnosis of lymphatic filariasis. However, this method is insensitive for detection of prepatent or amicrofilaremic infections. Recent reports have shown that recombinant *Brugia malayi* antigens BmM5 and BmM14 may be useful for antibody diagnosis of lymphatic filariasis in humans. The purpose of this study was to examine the kinetics of antibody responses to these antigens in experimentally infected rhesus monkeys. Fourteen animals were infected with single or multiple injections of *B. malayi* L3. All infected animals produced antibodies to both recombinant antigens by 4-8 wks postinoculation, before the onset of MF patency at 10-12 wks. This included animals that failed to develop patent infections. Antibody levels declined over time, but increased again in animals that were reinjected with L3. No correlation was observed between antibody levels and MF densities or the development of lymhedema in rhesus monkeys. These encouraging results obtained in primates suggest that assays for antibodies to BmM5 and BmM14 may be very useful for detecting prepatent, patent, and mature amicrofilaremic *B. malayi* infections in humans.

264 THE KINETICS OF TYPE 2 CYTOKINE PRODUCTION IN BANCROFTIAN FILARIASIS DIFFERS BETWEEN CLINICAL GROUPS. de Almeida AB*, Maia e Silva MC, Maciel A, Freedman DO. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil.

Infection with *Wuchereria bancrofti* leads to several possible clinical outcomes. Using circulating antigen (CAg) in serum to define the presence of viable adult parasites (active infection), we investigated the kinetics and cellular origin of type 1 and type 2 cytokines in 6 individuals with clinical disease and active infection (Dis+CAg+), 7 with clinical disease but no active infection (Dis+CAg-) and 14 with clinically asymptomatic microfilaremia (Dis-). Cumulative production of IL-4 by 48h filarial antigen (BmA) stimulated PBMC did not differ between the 3 groups (Dis+CAg+ = 20.08; Dis+CAg- = 28.35; Dis- = 13.97 pg/ml), while γ-IFN was only produced by Dis+CAg- individuals (139 pg/ml). To further assess the kinetics of cytokine production, PBMC which had been stimulated with BmA for 24h were transferred to fresh media for a further 24h. In contrast to Dis- individuals where IL-4 production was greater in the first 24h (64.6% of total production), production in Dis+CAg+ and Dis+CAg- occurred mostly in the second 24h (61.3% and 69.5% respectively). In addition, in another cohort IL-4 mRNA expression at 4h was equivalent in 3 similarly defined clinical groups, yet at 24h it had ceased in 7/11 Dis- subjects but in only 1/10 Dis+ subjects. IL-4 production by positively selected BmA stimulated CD8+ cells was not significantly different from that of unfractionated cells in any of the 3 patient groups. CD8+ cells produced no g-IFN in any of the subjects. Thus, the IL-4 response is early and transient in Dis- individuals while being early and sustained in Dis+ individuals. In addition, CD8+ cells are the major source of IL-4.

265 ANTIFILARIAL IMMUNE RESPONSE IN JIRDS CONCURRENTLY INFECTED WITH *STRONGYLOIDES STERCORALIS* AND *BRUGIA PAHANGI*. Chisholm ES*, and Lammie PJ. Division of Parasitic Diseases, Center for Disease Control and Prevention, Atlanta, GA.

Previous studies have shown that oral exposure to nematode antigens influences the development of anti-filarial immunologic reactivity. In the present study, we investigated the effect of infection with *Strongyloides stercoralis* on the development of infection and anti-filarial immunity in the *Brugia pahangi*-jird model. Jirds were infected with 1000 *Strongyloides* L3 by the subcutaneous (s.c.) route, 1 month prior to s.c. infection with 100 *B. pahangi* L3. Control jirds were infected with either *Strongyloides* or *Brugia* alone. Animals were bled bi-weekly to monitor microfilaremia and to collect serum for antibody determinations. Both the onset and the mean microfilaremia were equivalent for groups of jirds concurrently infected with *Strongyloides* and *Brugia* (SS/BP) and for animals infected with *Brugia* alone (BP). Similarly, after 22 weeks of infection, mean recoveries of *Brugia* adult worms were also equivalent for the two groups (14.7 worms per SS/BP jird vs 15.2 worms per BP jird). In contrast, levels of anti-filarial antibody were significantly higher in SS/BP than in BP jirds by ELISA. Immunoblotting studies are in progress to define the antigenic specificity of this response. Concurrent infection with an intestinal helminth may influence the development of anti-filarial antibody without significantly affecting establishment of filarial parasites.

266 FIELD EVALUATION OF A NEW MICROSCOPIC TECHNIQUE FOR MONITORING FILARIAL INFECTIONS IN MOSQUITOES. Yates JA*, Green DF, Levesque MA, and Bockarie MJ. Department of Biological Sciences, Oakland University, Rochester, MI; and Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea.

Monitoring the effect of community-based chemotherapy on filariasis transmission requires dissection of large numbers of mosquitoes to detect changes in parasite infection rates and transmission potentials. We have previously described a technique in which mosquitoes are cleared and not dissected. The primary goal of the current study was to make a side by side comparison, under field conditions, of the classical dissection method and the clearing method. Parallel samples of mosquitoes were collected from human bait, man-biting catches, in 4 villages in the East Sepik Province where *Wuchereria bancrofti* is prevalent. From 5 paired samples collected in September, February, and May we concluded that the two methods yielded comparable infective rates. However, the clearing technique was faster and more sensitive in detecting infected mosquitoes. In every case higher infection rates were observed with the clearing method. We also found that when mosquitoes were immersed in water after drying and before ethanol fixation, undigested red blood cells within the mosquitoes digestive tract lysed. This allowed a remarkable view of microfilariae in the gut. To our knowledge no other method has been able to enumerate microfilariae within the residual blood in fixed mosquitoes.

267 MOLECULAR CHARACTERIZATION AND FIELD EVALUATION OF OV26, A NOVEL *ONCHOCERCA VOLVULUS* ANTIGEN. Lobos E*, Steiger S, Zahn R, Schneider D, Weiss N, and Chippaux JP. Swiss Tropical Institute, Basel, Switzerland; Centre Pasteur, Yaounde, Cameroon.

Recently a cocktail of 3 recombinant *Onchocerca volvulus* antigens (Trico) and 2 other Ags, OC3.6 and C27, have been used in antibody based assays for diagnosis of onchocerciasis. However, serious problems of sensitivity in detecting infection have arisen when these rAg are used in Central Africa or in areas outside of the OCP control area (West Africa). We have isolated and characterized a cDNA clone (OV26) using sera from patients with onchocerciasis in the pre-patent phase. Southern blot analysis suggests a single gene encoding OV26, and Northern blot analysis reveals a single transcript of 7.4kb. Human affinity purified antibodies as well as serum of mice immunized with recombinant OV26 (rOV26), which was produced as non-fusion protein in *E. coli*, indicate that the native parasite products are a high molecular weight complex (up to 160 kDa). Isolated rOV26 was used to analyze the reactivity of > 275 patients with intestinal nematodes, other filarial infections, or onchocerciasis. The results indicate that OV26 is a major antigen recognized by 92% of Mf+ patients (< 20 years) and shows a high specificity of 96%. More importantly, analysis of 110 patients from an endemic focus in Cameroon over a period of 2 years showed that anti-OV26 antibodies detected 69% of Mf+ patients, in contrast to 26% detected by Trico. The predictive value of infection occurring within the following year using anti-OV26 IgG as a diagnositic tool was 86% in contrast to 4.7% for Trico, 9.5% for C27 and 19% for OC3.6. Thus, rOV26 showed a striking diagnostic sensitivity in endemic areas of Central Africa where other antigens failed, and this novel antigen could complement and improve existing diagnostic tests.

268 CURRENT STATUS OF ONCHOCERCIASIS IN COLOMBIA. Corredor A, Palma GI*, Nicholls RS, Granada JF, Alvarez CA, and Guderian R. Inst. Salud en el Trópico, Univ. Nacional, Bogotá, Colombia; Department Microbiología, Univ. del Valle/ CIDEIM, Cali, Colombia; Lab. Parasitología, Inst. Nacional de Salud, Bogotá, Colombia; Hospital Regional Guapi, Colombia; and Hospital VozAndes, Quito, Ecuador.

Colombia, along with Ecuador, Brazil, Venezuela, Guatemala and Mexico, are commited to the implementation of the Onchocerciasis Elimination Program in the Americas (OEPA). Previous studies of the only documented focus in Colombia, located on the Micay River (Pacific Coast), suggested that onchocerciasis transmission was decreasing without any interventions. Over a 24 year period (1965-1989) prevalence measured by skin biopsy positivity fell from 15 to 4.1%. Before establishing a National Onchocerciasis Program in Colombia, a new survey was undertaken in April 1995 to assess current status of the disease in this focus. Communities along the upper Micay River and its tributaries were surveyed; physical examinations and skin snip biopsies were performed on 655 adults (>15 years). Individuals with positive biopsies were found only in the community of Nacioná, where prevalence was 40% (39/91). Punctate keratitis was present in 22% of skin snip positive patients and nodules in 17%. This community on the Chuare River, whose principal economic activity is gold panning, had not been evaluated in previous surveys, which could explain the discrepancies between this study and preceding ones. Although elimination of onchocerciasis transmission through ivermectin distribution seems feasible in this limited focus, further studies are required since absence of transmission in remote localities which have not been visited cannot be ascertained.

269 DEVELOPMENT OF A POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF *BRUGIA* MICROFILARIAE IN TISSUE SAMPLES. Lizotte MR*, Pearlman E, Kazura JW, and Williams SA. Department of Biological Sciences, Smith College, Northampton, MA; and Division of Geographic Medicine, Case Western Reserve University School of Medicine, Cleveland, OH.

A better understanding of parasite-host interaction will provide valuable insight to the study of lymphatic filaraisis. One aspect of this interaction is the pattern of migration of microfilariae into the various tissues of an infected host. For this purpose, a simple, genus-specific and inexpensive DNA assay would provide an attractive alternative to traditional dissection/microscopy techniques. While a few column systems for DNA isolation are commercially available, these systems are expensive and most involve the use of reagents and equipment not readily accessible in endemic laboratories. A simple, inexpensive method has been developed for the detection of *Brugia* microfilariae in various tissues of interest (lung, spleen, kidney, and liver). Tissue samples from mice were minced briefly, dissociated via trypsin digestion, and centrifuged to pellet cells and remaining tissue. The pellets were digested using Proteinase K, heat inactivated, and 1/200th of the resulting lysate was used as template for PCR amplification of the *B. malayi Hha* I repeat. Following standardization in reconstructed samples, lungs, spleens, livers and kidneys from 19 mice challenged intravenously with 2×10^2 *Brugia malayi* microfilariae were processed and used in PCR along with negative control tissues. The assay results show that this method is simple, sensitive, and specific for the detection of *Brugia* in tissue samples. The assay is currently being used in conjunction with traditional immunological methods to study microfilarial migration in Interleukin 4 "knock-out" and control mice with the goal of better understanding the host immune response to *Brugia* infection. Research is in progress to improve the PCR assay so that it will be quantitative.

270 IVERMECTINE AND DIETHYLCARBAMAZINE FOR THE TREATMENT OF HIGH MICROFILAREMIA *LOA LOA* AND *MANSONELLA PERSTANS*. Richard-Lenoble D*, Kombila M, Duong TH, Walker A. Department of Parasitology and Tropical Medicine, University School of Medicine, Libreville, Gabon and Tours, France.

A case history is presented of the effects of ivermectin for the treatment of chronic infections of *Loa loa* and *Mansonella perstans*. Mr B. is a 45 year old male who has live for 15 years in a forest region at Ntoum near Libreville, Gabon. He had a history of high microfilaria of *L. loa*. He was first treated 10 year ago with diethylcarbamazine but was discontinued after only 3 days because of painful secondary effects of the drug. He was examined in 1991 with severe oedema of the upper extremities. A count revealed 30520 *L. loa* and 38 *M. perstans* microfilaria/mm^3 of blood. He was given a doses of ivermectin (200 mg/kg) on Day 1 and on D30. On D90 he was given a treatment of DEC as a *L. loa* cure. The table presents the results of the microfilaria counts as well as eosinophils and antibodies to *L. loa* antigen.

Day post Tt Ivermectine	D0/1 200 mcg/kg	D8	D15	D30 200 mcg/kg	D45	D60	D90
mF Loa/ml	30520	5480	6880	8010	5610	2264	3604
mF M. perstans	38	29	38	33	36	8	8
eosino/mm3	2100	3400	3400	2600	2000	1800	900
Ab FAT	1/800	1/400	1/400	1/400	1/200	1/400	1/800

Day post Tt DEC (15 days)	D90	D95	D120	D180
mF Loa/ml	3604	840	0	0
mF M. perstans	8	6	12	24
eosino/mm3	900	1200	1800	600
Ab FAT	1/800	1/800	1/3200	1/3200

271 PARASITOLOGIC STUDIES OF *TAENIA SOLIUM* ADULT STAGE IN HAMSTERS. Aguilar L*, Avila G, and Flisser A. Departmento de Microbiologia y Parasitologia, Facultad de Medicina, UNAM, Mexico D.F.

We evaluated the golden hamster (*Mesocricetus auratus*) as an experimental model for adult-stage *Taenia solium* in order to identify parasitologic parameters, to study the effect of parasite load, and to maintain the infection for long periods. Ten-week-old hamsters were immunosuppressed with 2 mg of methylprednisolone, repeated daily for 10 to 13 days, and infected per os with one to five cysticerci. Infections were monitored by coproantigen ELISA. Tapeworms recovered at autopsy were counted, measured, fixed and stained. Average numbers of *T. solium* were 2 for males and 1.6 for females. Infection was maintained up to 110 days; the longest worm was 42 cm, had 381 proglottids and showed development of reproductive organs to sexual maturity. No gravid proglottids were seen. Dimensions of scolices, suckers, and hooklets were similar to to those of *T. solium* obtained from humans; however, the immature and mature proglottids were smaller in length and width. This observation may be related to phenotypic adaptation to the smaller intestinal environment of hamsters (35 cm) as compared to humans. Finally, coproantigen ELISA was a useful monitoring technique because infection was detected beginning three weeks after infection (in the prepatent stage) and was negative in all hamsters where no tapeworms were found at necropsy.

272 EVALUATION OF HEALTH EDUCATION FOR CONTROL OF *TAENIA SOLIUM* IN A RURAL COMMUNITY IN MEXICO. Bronfman M, Flisser A, Sarti E, Schantz PM*, Gleizer M, Loya M, Plancarte A, Avila G, Allan J and Fineblum W. Direccion General de Coordinacion Sectorial e Internacional and Direccion General de Epidemiologia, SSa; Facultad de Medicina, UNAM, Mexico D.F.; and Division of Parasitic Diseases, NCID, CDC, Atlanta, GA

A comprehensive field study was undertaken in a rural community in the state of Morelos, Mexico, to evaluate health education as an intervention measure against *Taenia solium*. Education was developed to inculcate recognition of the parasite and its transmission and to promote alteration of hygienic behavior and sanitary conditions that foster transmission. In-depth sociologic interviews and ethnographic observation were performed and a questionnaire was administered to a sample of 10% of the population to evaluate baseline knowledge and practices regarding transmission of the cestode. The health education strategy was implemented based on the

information obtained from the sociologic study. Prevalence of cysticercosis in swine was measured before and after the intervention by direct palpation of pig tongues for cysticerci and detection of cysticercus serum antibodies by immunoblot assay. There were statistically significant improvements in knowledge of the parasite, its life cycle, and how it is acquired by humans; however, changes in behavior related to transmission, e.g., defecation behavior and eating pork, were less dramatic and persistent. The prevalence of cysticercosis in pigs at the start of the education intervention was 2.6% and 5.2% by lingual examination and immunoblot positivity, respectively; one year after the end of the intervention, the rates were 0 and 1.4%, respectively. Although these changes were of only borderline statistical significance, they were accompanied by dramatic reductions in the reported access of pigs to human excrement and freedom to roam (22% to 11%). We conclude that health education, developed with community involvement, may reduce opportunities for transmission of *T. solium* in the human-pig cycle.

273 EPIDEMIOLOGICAL ASPECTS OF HUMAN INFECTIONS BY INTESTINAL CESTODES IN SANTIAGO, CHILE (1985-1994). Mercado R*, Arias B, Romero M, Peñaloza, and Castillo D. Departamento de Parasitología, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

A total of 70,064 parasitological fecal examinations (one per person) carried out in 1985-1994 period were analized. A stool exam for intestinal parasites was solicited to each patient attended in public outpatient clinics from the north section of Santiago city. Most patients presented gastrointestinal symptoms specially abdominal discomfort, lack of appetite and low weight in children. 59,960 out of the 70,064 examinations corresponded to 1-15 years old children, 4,280 to 16-30 years old subjects and 6,402 to up to 30 years old individuals. The infection rates (x 10^5) by intestinal cestodes were: *Hymenolepis nana*, 1,115; *Taenia* sp.,86; *Taenia saginata*,16; *Diphyllobotrium* sp.,6 and *H. diminuta*, 1. No *Taenia solium* infection was observed. The percentage of *H. nana* infections was 1.5 in 1985 and decreased to 0.3 in 1994. The frequency of bovine and porcine cysticercosis detected in 1989 in state abbatoirs was 0.1 %. At present over 96 % of Santiago city populations has potable water supply and sanitary sewage services. The improvement of sanitary conditions and the medico-veterinary control of slaughtered animals in abbatoirs in the last two decades have contributed to the decrease of the frequency of most of human intestinal cestodiases.

274 ULTRASTRUCTURAL ANALYSIS OF ADULT *TAENIA SOLIUM*-INTESTINAL WALL INTERFACE IN HAMSTERS.. Willms K*, Avila G, Merchant MT, Aguilar L, and Flisser A. Department of Microbiology & Parasitology, Facultad de Medicina, Universidad Nacional Autónoma de México, México.

Taenia solium metacestodes dissected from infected pork meat were fed to immunosuppressed hamsters following a previously described model. Taenia infection was diagnosed by an antigen capture ELISA in feces. Laparotomy was performed on animals after various periods of infection (10 to 100 days) in order to recover the adult worms from the small bowel by cutting open the intestinal wall. To obtain samples of the scolex-intestinal wall interface, the site was flooded in situ with Karnovsky glutaraldehyde-paraformaldehyde fixative, excised and processed for light and electron microscopy. Thin sections showed that the scolex was attached to the intestinal mucosa by at least one sucker containing epithelial tissue. The immediate surrounding brush border was almost completely destroyed, although intact in adjacent areas. Electron microscopic images revealed that the parasite surface was covered with the characteristic blade-like microtriches which are directly in touch with damaged host cells, most of which had no cell membranes. The outer borders of the sucker were in contact with the lamina propia of the intestinal wall. No inflammatory cells were observed. The large area of destroyed brush border suggests that the scolex attaches to the tissue by alternating suckers and secrets cytotoxic molecules or cytolytic enzymes to release nutrients.

275 GRANULOMA CYTOKINES IN MURINE CYSTICERCOSIS. Robinson P*, Atmar RL, Lewis DE, and White, Jr AC. Baylor College of Medicine, Houston, TX.

Neurocysticercosis, caused by *Taenia solium*, is one of the most common causes of seizures worldwide. The symptoms result from granulomatous inflammation associated with dying cyst forms of the parasite. While the invasive larvae can be killed by immune serum plus complement, immunity to the cyst stage depends on a cellular response. This dichotomy is reminiscent of the two extremes of cytokine profiles termed the T helper 1 (IL-2 and Interferon γ) and T helper 2 (IL-4, IL-5, and IL-10). To characterize the cytokine profile in active cysticercosis, granulomas were removed from the peritoneal cavity of mice infected intraperitoneally with *Taenia crassiceps* cysts. After inactivation of RNAse with paraformaldehyde, serial sections of the granulomas were examined for cytokine message by in situ hybridization using ^{35}S-labeled RNA probes. Antisense probes were prepared for IL -10 and IL-4 (T helper 2), as well as IL-2 and Inteferon γ (T helper 1). Control sections were probed with sense probes and no probe. Each granuloma revealed histologic evidence of a degenerating parasite with surrounding granuloma. Message for the T helper 1 cytokines IL-2 and interferon γ was present in the granulomas, in contrast there was no increase in the message for the T helper 2 cytokines IL-4 and IL-10 compared to controls. Granulomas in cysticercosis appear to result from Th1 cytokine stimulation. This contrasts with the humoral response that is

critical in immunity to the invasive larvae. The granulomas appear to play a role both in pathogenesis and clearing parasites.

276 *TAENIA SAGINATA* ONCOSPHERE PEPTIDASES. White, Jr. AC*, Baig S, and Robinson P. Baylor College of Medicine, Houston, TX.

There is an effective protective immune response to the oncosphere (invasive larva) stage and larval excretory/secretory products for larval cestode infections. Since the oncosphere invades through the host intestinal mucosa, we hypothesized that proteinases are important components of the oncosphere excretory/secretory products. To identify oncosphere excretory/secretory peptidases, *Taenia saginata* adult worms were collected from 3 patients treated with niclosamide followed by a purgative. Eggs were hatched and activated *in vitro*, and oncospheres incubated in medium RPMI containing antibiotics for 24 hours. Spent medium was assayed with peptide substrates coupled to 7-amino-4-trifluoromethyl coumarin (AFC), and free AFC was detected fluorometrically. The endopeptidase substrates Z-Phe-Arg-AFC and Z-Arg-AFC as well as the aminopeptidase substrate Arg-AFC were cleaved significantly when incubated with spent media from the oncospheres compared to control medium. Cleavage of Z-Phe-Arg-AFC was inhibited 78% by preincubation with the serine proteinase inhibitor PMSF. Endopeptidase activity was not enhanced in the presence of exogenous thiols, but was partially inhibited by the cysteine proteinase inhibitor E-64. There was no significant inhibition noted with pepstatin or phenanthroline. We could not reproducibly inhibit cleavage of Arg-AFC. The peak peptidase activity detected with Z-Phe-Arg-AFC and Arg-AFC were easily separated by gel filtration FPLC demonstrating that these were separate enzymes. These data demonstrate that *T. saginata* oncosphere produce excretory/secretory peptidases including a serine endopeptidase and an aminopeptidase.

277 ISOLATION AND CHARACTERIZATION OF A GENE FROM THE TAPEWORM *TAENIA SOLIUM* THAT ENCODES A PUTATIVE GLUCOSE TRANSPORTER. Rodriguez DD*, Shoemaker CB, and Laclette JP. Department of Immunology, Inst. de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico City; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The platyhelminth *T. solium* is an avid consumer of glucose, which lacks of a digestive tube and carries out all exchange with its host through the surface tegument. Therefore we would expect to find glucose transporters exposed to the external surface of the tegument. If exposed, this protein could be a potential target to induce a host's protective immune-response. Isolation of cDNA clones was carried out using probes generated by PCR using oligonucleotides that anneal with conserved sequence motifs of glucose transporters. A clone (TGTP1) of about 1,800 bp was obtained, which showed an open reading frame of 1,500 bp that encodes a protein of 500 amino acids. This protein is homologous to glucose transporters from *Schistosoma mansoni* showing 27-41% identity, and from human being showing 31-37% identity. Structural similarities include 12 alternating transmembrane segments interspersed with several extended hydrophilic segments. This strongly suggests that TGTP1 encodes a glucose transport protein. Interestingly, TGTP1 lacks the N- linked glycosylation sites typical of many eucaryotic transporters. Functional expression of TGTP1 is currently under process.

278 ARE BARREN FEMALE *STRONGYLOIDES STERCORALIS* FROM CHRONIC INFECTIONS REJUVENATED BY TRANSPLANTATION INTO PARASITE NAIVE RECIPIENT HOSTS? Schad GA*, Thompson F, Lee G, Lange AM, Nolan TJ, Holt D, and Bhopale VM. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

It is widely assumed that barren female *Strongyloides stercoralis*occurring in chronic carriers can return to fecundity when immunity wanes, but little direct evidence from experimentally manipulated infections supports this. To test this assumption, barren adult females were recovered from experimentally infected donor dogs when their fecal examinations turned negative. Groups of 100 active barren worms were transplanted into the small intestines of six naive canine recipients by laparotomy. Three recipients were examined at necropsy at 4-5 days post-infection (p.i.), before autoinfection could amplify the number of successfully transferred parasites. The remaining three recipients were examined 21-22 days p.i. when, if autoinfection had occurred, the worm populations should have increased. At 4-5 days p.i. adult worms were recovered from each of the recipients (19 ± worms/dog). By 21-22 days p.i. a remarkable population increase had occurred (522.6 ± 296). In the absence of reinfection, the increase must be attributed to autoinfection. Worms from chronically infected donors were stunted and electron microscopy revealed damage to the intestine and ovaries. Successfully transplanted worms recovered at day 4-5 p.i. were ovigerous, less stunted and showed repair of intestinal and ovarian tissues.

279 USING A BAYESIAN APPROACH TO ENHANCE THE ESTIMATION OF THE PREVALENCE OF INFECTION. Gyorkos TW*, and Joseph L. Division of Clinical Epidemiology, Montreal General Hospital, Montreal, Quebec, Canada and; and Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada.

In the absence of a gold standard, it is uncertain how to best use one or more diagnostic test results to estimate the prevalence of an infection. A practical user-friendly Bayesian approach is proposed which overcomes this problem by providing simultaneous inferences about the population prevalence and the sensitivity, specificity and predictive values of each diagnostic test in the form of a marginal density for each parameter over the range of possible values. This approach is demonstrated using the example of the stool O&P exam and serology in the diagnosis of *Strongyloides* infection. The data used to demonstrate this approach are drawn from published accounts of point prevalences obtained from a stool survey (24.7% prevalence) and a serological survey (77.2% prevalence) measuring *Strongyloides* infection at the same point in time in the same Cambodian refugee population (n = 162). It was found that the median prevalence was 76%, with a 95% credible interval for the true population prevalence of between 52% and 91%. Application of this approach to other situations is discussed (eg. to other diseases, to diagnostic tests where measurements are continuous or where patients are classified into more than two categories, and to the individual patient situation).

280 *STRONGYLOIDES STERCORALIS*: EOSINOPHIL-MEDIATED, TH-2 DEPENDENT PROTECTIVE IMMUNITY TO INFECTIVE LARVAE IN BALB/CBYJ MICE. Rotman HL*, Gleich GJ, Nolan TJ, Schad GA, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA; Department of Immunology and Medicine, Mayo Clinic and Foundation, Rochester, MN; and Department of Pathobiology, University of Pennsylvania, Philadelphia, PA.

Protective immunity was generated against *Strongyloides stercoralis* infective third stage larvae (L3) in BALB/cByJ mice when mice were immunized with live *S. stercoralis* L3. The goal of the present study was to describe the mechanism of immune-mediated killing of *S. stercoralis* L3 in diffusion chambers in mice. The role of $CD4^+$ and $CD8^+$ cells in immunity to L3 was investigated. Depletion of $CD4^+$ T cells by mAb treatment completely ablated killing of L3 in immunized mice. Further experiments were conducted in order to determine whether a TH-1 or a TH-2 type response was needed for immune-mediated killing of L3 to occur. Immunized mice were treated with mAb to eliminate granulocytes, IL-4 or IL-5 to assess the role these cytokines or their by-products play in larval killing. Treatment of mice with each of these mAb significantly reduced the protective effects of vaccination against larval *S. stercoralis*, indicating that a TH-2 type response was responsible for generating immunity to L3. The time and type of cell influx into the infection site was compared with the time challenge infections were killed. The only cell type that was found to increase in diffusion chambers in immunized mice was eosinophils; maximal levels of eosinophils were coincident with the time of parasite killing. Parasite development also controlled the time of larval killing as human eosinophil granule products were found to be toxic to host-adapted $L3^+$, but had no effect on infective L3. Thus in the murine host, larval *S. stercoralis* are killed by an eosinophil-mediated TH-2 type response.

281 IDENTIFICATION OF NOVEL GENES USING A cDNA LIBRARY FROM FILARIFORM AND RHABDITIFORM STAGES OF *STRONGYLOIDES STERCORALIS*. Moore TA*, Ramachandran S, Gam A, Neva FA, Saunders L, Lu W, Williams SA, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Department of Biological Sciences, Smith College, Northampton, MA.

The identification of biologically active molecules of the intestinal nematode *Strongyloides stercoralis* has been hindered by the limited availability of various stages of the parasite. cDNA libraries using 2000 rhabditiform larvae and 5×10^5 filariform larvae were constructed using a unidirectional cloning strategy that minimized RNA degradation and loss. The filariform library, with an unamplified titer of 1.5×10^6, had an average insert size of 975 bp (range = 500 bp to 1500 bp); the rhabditiform library, with 1×10^5 recombinants, had an average insert size of 675 bp (range = 100 bp to 1700 bp). Sequence analysis of 57 randomly selected plaques revealed an AT-rich genome (65% AT, 35% GC). Expressed sequence tag (EST) analysis of 32 filariform and 22 rhabditiform recombinants was performed. Of all sequences obtained, 4 were ribosomal in origin, 6 coded for structural proteins, 9 coded for proteins with enzymatic function, 11 exhibited homology with proteins of unknown function, and 24 were newly described proteins. While there was some overlap in the types of proteins encoded in the samples from each library, stage-specific proteins should be easily identifiable from these libraries.

282 ROLE OF ANTIBODY ISOTYPE AND COMPLEMENT IN IMMUNITY TO *STRONGYLOIDES STERCORALIS* L3. Brigandi RA*, Rotman HL, Yutanawiboonchai W, Nolan TJ, Schad GA, and Abraham D. Department

of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA; and Department of Pathobiology, School of Veterinary Medicine University of Pennsylvania, Philadelphia, PA.

Mice immunized against *Strongyloides stercoralis* L3 were shown to kill greater than 90% of challenge larvae contained within diffusion chambers. The objective of the present study was to identifiy the host components responsible for the immunity. Serum from unprotected, control mice and protected, immune mice in doses of 500-25 µl was transferred into naive mice at the same time and location as larval challenge. Transfer of as little as 50 µl of immune serum was able to confer protective immunity. The serum-transferred immunity was ablated by excluding cells from the larval microenvironment or by depleting granulocytes through monoclonal antibody treatment in the recipient mice. Specific antibody isotypes were isolated using protein G and isotype specific affinity columns. The resulting transfer experiments identified IgM as the isotype responsible for protective immunity. Antibody binding studies *in vivo* were performed and only IgM bound to the surface of L3 in immune animals. Elevated levels of C3 were also found bound to the surface of L3 in immune mice. Cobra venom factor treatment of immunized mice to deplete complement completely eliminated C3 binding to the surface of L3 and ablated immunity. Therefore, IgM, complement and granulocytes are necessary for immune elimination of *S. stercoralis* L3. Identification of antigens recognized by IgM may help select possible vaccine candidates.

283 ALBENDAZOLE PRODRUGS IN EXPERIMENTAL TRICHINELLOSIS. Yepez-Mulia L*, Morales-Hurtado R, Viveros N, Cedillo-Rivera R, Hernández F, Castillo R, Jung H, and Muñuz O. Unidad de Investigacion Medica en Enfermadades Infecciosas, Centro Medico Nacional, Instituto Mexicano del Seguro Social, Mexico City, Mexico.

Albendazole has been successfully used in chemotherapy of trichinellosis. Despite their efficacy, albendazole is highly insoluble and high dosis and long treatments are required to reach optimal plasma levels. Recently, new prodrugs have been produced which may undergo cyclization into albendazole after reduction of the nitro group by microflora of the intestine, allowing their slow releasing and therefore a better absorption. Our aim was to determine the reduction of two new albendazole prodrugs: M3 (N-methoxycarbonyl-NI(2-nitro-5-propylthio)phenylthio urea) and G2 (N-methoxycarbonyl-NI(2-nitro-4-propylthio)phenylthio urea) and to evaluate their activity against *Trichinella spiralis* in mice experimentally infected. Plasma from rats treated with G2 and M3 was analyzed by HPLC and the presence of albendazole and albendazole sulphoxide was detected. Regarding the antihelmintic activity of G2 and M3, no reduction in adult worm burden was observed at 25 mg/kg, however reduction in female fecundity of 62 and 45% was obtained at this dose. On the other hand, administration of 25 mg/kg at day 28 post-infection for 7 days, gave a reduction in muscle larva burden of 30 and 39% for G2 and M3 respectively. The data obtained in this study, demonstrated that G2 and M3 are reduced to albendazole having activity specially against *T. spiralis* female fecundity, nevertheless they did not have better bioavailability than albendazole.

284 THE PERSISTENCE OF ENZOOTIC DRACUNCULIASIS IN CENTRAL ASIA 50 YEARS AFTER THE ERADICATION OF GUINEA WORM DISEASE. Kliks MM*. Institute of Zoology, National Academy of Sciences, Akademgorok, Almaty, Republic of Kazakhstan.; CTS Foundation, Honolulu, Hawaii.

Dracunculiasis was eliminated as a human disease of public health importance in Central Asia during the period 1929-35 as a direct result of a campaign led by L. M. Isaev of tha Bochara Tropical Institute using a trained cadre of task dedicated public health and sanitation workers. The principle strategy of the program was the descruction of traditional covered stop wells and tbair replacement with protected water supplies where copepod vectors could not breed. The devastating sociopolitical impact of Stalinist collectivization and other agricultural programs of the First Five Year Plan of the USSR were coincidental and probably also played a role. Human infection in the most highly endemic areas ceased bv 1932. However, *Dracunculus medinensis* was repeatedly found in Central Asia between 1950-70 in a wide variety of domestic and wild animals, persisting as a rare, but firmly entrenched epizoonosis. In 1956 11.7% of domiciled and stray dogs in Qyzlorda town were infected indicating the potential for human exposure in a heavily populated urban area. An isolated human case occurred in 1966 in an adult male collective farm worker in Karalcalpakistan who drank from ground water sources, During 1993-95 the authors researched published and unpublished literature sources, reviewed records of the federal and regional epidemiological services, interviewed public health officials, field parasitologists, veterinarian and abattoir inspectors and used print. and electronic media to solicit reports of any subsequent cases in the Republics of Kazakhstan and Uzbekistan. Although many anecdotal reports were received and investigated no additional human cases were confirmed. Several accounts of infection in man and animals were judged to have been "prrobable" cases and are being actively investigated further. We did bring to light a 1983 case of infection in a domiciled dog from Qyzylorda town that was verified by the attending veterinarian, a public health official and several witnesses. These findings should have important implications for the selection of strategies and tactics for local, regional, and global eradication programs.

They should also be taken into consideration by officials of the WHO/UNDP/WB who are considering certifying that some Central Asian Republics have eradicated dracunculiasis.

285 CLONING AND EXPRESSION OF *ANCYLOSTOMA CANINUM* ANTICOAGULANT PEPTIDE-1 (ACAP-1): A NOVEL HOOKWORM-DERIVED INHIBITOR OF HUMAN COAGULATION FACTOR XA. Cappello M*, Hawdon JM, Jones BF, and Hotez PJ. Departments of Pediatrics and Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

Hookworm infection is a major cause of intestinal bleeding and iron deficiency anemia in the developing world. Each adult hookworm causes up to 0.2 cc of blood loss per day, with clinically significant hemorrhage found primarily in children and women of reproductive age. We have previously identified the major hookworm anticoagulant present in extracts of adult *Ancylostoma caninum* hookworms. This 8.7 kDa polypeptide, called *Ancylostoma caninum* Anticoagulant Peptide (AcAP), is a tight-binding (K_i^* = 323 pM) inhibitor of human coagulation factor Xa (fXa). Using PCR, a 400 bp gene product corresponding to the full length AcAP cDNA was amplified from an adult *A. caninum* cDNA library. The translated amino acid sequence of this PCR product matched the NH_2-terminal sequence of purified AcAP and showed putative structural homology to the *Ascaris* family of serine protease inhibitors. The AcAP-1 cDNA was subcloned into the pET-28a expression vector (Novagen) and used to transform competent *E. coli*. Soluble lysates of transformed cells induced with 1 mM IPTG contained potent fXa inhibitory activity. The expressed fusion protein, consisting of rAcAP-1 with a histidine tag at its NH_2-terminus, was purified to homogeneity using nickel resin affinity chromatography and rpHPLC. Purified recombinant AcAP-1 behaves similarly to native AcAP using SDS-PAGE and chromogenic assays of fXa inhibition, suggesting appropriate formation of its five disulfide bonds. Serum from experimental infection with *A. caninum* does not appear to recognize rAcAP-1 by Western blot, suggesting that antibodies to this molecule may not be acquired naturally during the course of infection. Work is currently underway to evaluate rAcAP-1 as a concealed antigen vaccine aimed at reducing the severity of hookworm associated gastrointestinal blood loss and anemia.

286 CD4+ CELLS REGULATE MUCUS PRODUCTION IN INTESTINAL NEMATODE INFECTION. Khan WI*, Abe T, and Yoshimura K. Department of Parasitology, Akita University School of Medicine, Akita, Japan.

Gastrointestinal mucus provides barrier that protects the epithelium from potentially injurious chemicals, enzymes, bacteria and dietary constituents. In addition to those, mucus is considered to take part in removal of parasites by binding and trapping. Goblet cells reside throughout the small and large intestine and are the main source of intestinal mucus. Goblet cell hyperplasia and copious production of mucus correlate well with the spontaneous expulsion of intestinal nematode, *Nippostrongylus brasiliensis*. However, information concerning a precise role of goblet cells or a mechanism of mucus production is still scanty. We therefore investigated the possible role of CD4+ cells in mucus production in mice infected with this intestinal nematode. Spontaneous expulsion of intestinal worms was interfered dose-dependently by a treatment with anti-CD4 antibody. In spite of interference in the spontaneous expulsion, treatment of mice with anti-CD4 antibody had no significant effect on number of intestinal goblet cells or on expression of terminal sugars of mucins. However, quantitative analyses of mucus oligosaccharides in the intestinal lumen revealed a significant reduction in the amount of mucus saccharides in anti-CD4 antibody treated and infected mice on Day 7 post-infection as compared to saline treated control. Recently, we also observed that anti-CD4 antibody inhibited antigen stimulated mucus production *in vitro*. These results suggest that CD4+ cells regulate mucus production and consequently amount of mucus probably has an important role in the spontaneous expulsion of this nematode.

287 EXPERIMENTAL *OESOPHAGOSTOMUM BIFURCUM* INFECTION IN MONKEYS. Eberhard ML*, Polderman J, Blokamp J, and Baeta S. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; Department of Parasitology, University of Leiden, Leiden, The Netherlands; and Department of Gynecology, University of Lome, Toga.

Recent studies have demonstrated that human infection with *Oesophagostomum bifurcum* occurs commonly in parts of Ghana and Togo. Larvae cultured from human stool collected from people in Ghana, were used to experimentally infect a rhesus monkey. Larvae cultured from eggs passed in the stool of this monkey were used 1) to infect other monkeys, 2) to conduct morphological studies, and 3) to establish that larvae retain their infectivity after periods of drying. Early growth of larvae and nodule formation in the gut was recorded, the prepatent period was determined to be 3 - 4 mo, and pattern of egg shedding was elucidated. Pre- and postinoculation sera were collected and tested in an ELISA formatted IgG4 specific assay. Thirteen of 16 (81%) of inoculated monkeys developed a positive serologic response, generally between 20 and 30 days after inoculation. Under laboratory conditions, drying of larvae for 2 - 3 wk followed by rewetting routinely resulted in reactivation of the desiccated larvae. Longer periods of drying resulted in more variable recovery with fewer larvae regaining motility. Two of

three (66%) monkeys inoculated with larvae which had been dried for 1 wk began shedding eggs at 4 mo postinoculation, demonstrating the viability of desiccated larvae. These studies have major implications for the natural transmission cycle in endemic areas.

288 MOLECULAR IDENTIFICATION OF *ENTEROBIUS VERMICULARIS* AS A CAUSE OF HUMAN EOSINOPHILIC ENTEROCOLITIS. Liu LX*, Chi JY, Upton MP, and Ash LR. Beth Israel Hospital, Harvard Medical School, Boston, MA; and UCLA School of Public Health, Los Angeles, CA.

In most cases of eosinophilic enterocolitis, a specific cause is never found. We evaluated a young Massachusetts native with severe abdominal pain, hemorrhagic colitis, and intense eosinophilic infiltration of the ileum and colon. Stool examination revealed numerous ~1 mm long nematode larvae of an initially unidentifiable species. No diagnostic adult helminth worms or eggs, protozoa, bacterial, or viral pathogens were isolated. The patient's symptoms resolved following treatment with mebendazole alone. Molecular speciation of the unknown nematode was performed by cloning nematode 28S and 5S spacer ribosomal RNA genes, using single-worm PCR and primers based on known nematode rRNA genes. The ~300 bp 28S rRNA target sequence from the unknown nematode was identical to that of *Enterobius vermicularis*, and differed from those of other human intestinal nematode species. The 5S rRNA spacer sequence, which varies widely in size and base composition among species, was 99% identical in nucleotide sequence between the unknown nematode and a reference *E. vermicularis* adult worm. Morphologic characteristics indicated that the nematode was an advanced fourth-stage larva of *E. vermicularis* , which has rarely been observed or associated with disease. Occult pinworm infection is prevalent yet often difficult to diagnose, and may be a cause of unexplained eosinophilic enterocolitis. A molecular phylogeny has been constructed based on multiple aligned 28S rRNA sequences from representatives of all major human parasitic nematode groups, which agrees well with traditional phylogenies and may provide a useful molecular tool for the speciation of individual nematode isolates.

289 EVALUATION OF LMD ELISA KITS FOR DETECTION OF HUMAN ANTIBODIES TO *ECHINOCOCCUS GRANULOSIS* AND *ENTAMOEBA HISTOLYTICA*. Wilson M*, Schantz PM, and Ware DA. Division Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

The LMD ELISA tests for detection of antibodies to *Echinococcus granulosis* and *Entamoeba histolytica* are commercially available and simple toperform. Specificity of the amebiasis kit was 95% when a panel of 120 specimens from persons with other hepatic and intestinal problems were tested. Sensitivity of the amebiasis kit was determined by testing 95 symptomatic patients with *E. histolytica*-documented infections: EIA results ranged from 67% to 100% positive and were related to the patient's clinical presentation. Specificity of the echinococcosis kit was 93% when a panel of 59 specimens from persons with other hepatic and parasitic problems were tested. Sensitivity of the echinococcosis kit was found to be 90% by testing 73 patients with surgery-documented hepatic cysts due to *E. granulosis* or *E. multilocularis*. The LMD ELISA kits performed as wellas the CDC IHA tests and can be used as a replacement for the standard IHA tests.

290 CYSTIC ECHINOCOCCOSIS IN JORDAN: A SIGNIFICANT DISEASE OF STABLE ENDEMICITY. Abdel-Hafez SK*, and Kamhawi SA. Department of Biological Sciences, Yarmouk University, Irbid, Jordan.

Retrospective analysis of case records of surgically confirmed hydatid patients over a ten year period revealed an overall mean annual surgical incidence (MASI) of 2.9 cases per 100,000 and reached as high as 8.2 per 100,000 in one locality. The most abundant cyst location was the liver (54.5 of cases) and multiple cysts were encountered in 5.2% of all cases. Surgical incidence in females was significantly higher than males among age groups over 20 years. Seroepidemiological surveys of the general population of Jordan confirmed the endemicity of the diseases with an overall seropositivity of 2.4 - 3.6% but was as high as 5.9 % in some localities at risk. Analysis of the nature of the infection in terms of fertility and viability in the various probable intermediate hosts showed that the sheep is the most significant host in the perpetuation of the life cycle of *Echinococcus granulosus* in Jordan. The prevalence, although varying in different locations, was repeatedly shown to be very high, exceeding 27.5% in some areas and reaching as high as 63.7% in ewes 4 years of age and older. Dog surveys revealed an infection rate of 9.8% but also varied from one locality to another, with the highest being 15.6%.

291 CLINICAL AND PATHOLOGICAL ASPECTS OF *LEISHMANIA* STRAINS FROM NATURALLY INFECTED DOGS IN MOROCCO. Berrag B*, Sahibi H, Natami A, Lasri S, Rhalem A, Bichichi M, Riyad M, and Guessous-Idrissi N. Departement de Parasitologie, Institut Agronomique et Veterinaire Hassan II, Rabat, Morocco; Unite d'Etudes et de Recherches sur les Leishmanioses, Faculte de Medicine & Pharmacie, Casablanca, Morocco.

Domestic dogs are considered to be a major reservoir host for human visceral leishmaniasis in the Mediterranean basin. The occurrence of canine leishmaniasis in this endemic area would maintain the infection. Hence, the knowledge of clinical and pathological signs are critical for practitioner veterinarians to diagnose the disease, particularly in rural areas. The study was carried out in Sid Elgandour locality belonging to Khemisset Province and located 80 km east of Rabat. This area was found to be highly endemic and the seroprevalence in local dogs in 1993 was 23.6% (estimated by IFAT at 1:80 serum dilution). All 13 notified seropositive dogs were examined at regular visits. Of those, 5 dogs (38.4%) died of severe leishmaniasis by 1994, which indicates the high virulence of local *Leishmania* strains. The strains isolated from sick dogs were typed as *L. infantum*. Two remaining seropositive dogs are still exhibiting clinical signs while 6 others (46.2%) are still asymptomatic. Clinical and pathological data revealed a large variability of signs of the disease including visceral and cutaneous changes. However, certain types are more frequent: exhaustion, loss of weight, alopecia, lymphadenopathy, onychogryposis, abundance of skin squama production, cutaneous ulcers. Fever and ocular impairment were inconstant. Necropsy revealed hepatomegaly and splenomegaly. Histological changes consisted of a massive infiltration by mononuclear cells into lymph nodes, spleen, liver kidney and lungs. Laboratory findings include anemia, lymphopenia, hypergammaglobulinemia and proteinuria.

292 NO TITLE. Guessous-Idrissi N*, Hamdani A, Dehbi F, Bichihi M, Sahibi H, Berrag B, and Rhalem A. Unite d'Etudes et de Recherche sur les Leishmanioses, Faculte de Medicine etde Pharmacie, Casablanca, Morocco.

While human cutaneous leishmaniasis (CL) is well known in Morocco where outbreaks due to *Leishmania major* emerged in the South during the eighties, human visceral leishmaniasis (VL) is still poorly investigated. The reported cases to the Ministry of Health show that VL cases proceed from the North of the country. Around one pediatric VL case from a Northern province of Morocco, an ecoepidemiological survey was carried out by a multidisciplinary team in order to elucidate the transmission mechanisms and reservoirs. The parasite isolated from the case was typed as *L. infantum*. Around this case, a clinical and serological survey were carried out in both human and dogs. Concomitantly an entomological sampling was done in order to incriminate a vector. The preliminary results suggest that Taounate is a well established focus of canine leishmaniasis with active cases and a high rate of seropositivity. The typing of isolated canine *Leishmania* strains is undergoing in order to assess the parasite reservoir. the sandflies species mainly represented in the area of the case are endophilic *P. sergenti* and *P. longicuspis* respectively demonstrated or suspected in other areas of Morocco as vectors of *L. tropica* and *L. infantum*. This suggest that the locality is a potential focus of human leishmaniasis, and an active and multidisciplinary survey is undergoing.

293 A SIMULATION MODEL OF THE INFECTION CYCLE OF *LEISHMANIA MEXICANA* IN *NEOTOMA MICR OPUS*. Kerr SF*, Grant WE, and Dronen NO. Biology Department, Incarnate Word College, San Antonio, TX; and Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX.

A simulation model of transmission of *Leishmania mexicana* among *Neotoma micropus* by *Lutzomyia anthophora* was developed to predict the threshold vector density below which *L. mexicana* would be eliminated from a population of *N. micropus* within two years. Model results supported the hypothesis that *N. micropus* is a reservoir of *L. mexicana*. *Leishmania mexicana* could be maintained in a focus with an initial annual prevalence of 5.7%, which approximated the lowest non-zero prevalence found in field studies, and a peak annual vector density of only 11 female sand flies/woodrat. At a peak density of 3 flies/woodrat, the prevalance approached zero at the end of two years.

294 THERAPY OF CHAGAS DISEASE: ACTIVITY OF THIO-SEMICARBAZONES. Kinnamon KE*, Poon BT, Hanson WL, and Waits VB. Department of Preventive Medicine & Biometrics, F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA.

Of all the hemoflagellate infections, the one caused by the protozoan parasite *Trypanosoma cruzi* is perhaps the least responsive to chemotherapy. It is estimated that there are 16-18 million cases of the disease, with 90 million individuals at risk. Nifurtimox, benznidazole and a few other compounds have some limited value, especially in very early infections, but the disease remains essentially incurable. Effective, non-toxic drugs are urgently needed. We evaluated a series of more than fifty thiosemicarbazones in a mouse model. Male albino mice (4-10 weeks old) infected with a Brazilian strain of *T. cruzi* were employed. Eleven chemical structures were identified with significant anti-*T. cruzi* activity. One of these was more effective in reducing the 14 day parasitemia than the reference drug, nifurtimox. To our knowledge, no series of members from this chemical class has been previously

evaluated for *in vivo* activity against this disease organism. These structures may represent a nucleus around which chemical analogues can be synthesized to formulate a drug effective against Chagas disease.

295 EFFECT OF VEPAPAMIL AND RELATED COMPOUNDS IN ACUTE MURINE *TRYPANOSOMA CRUZI* INFECTION. Tanowitz HB*, Chen B, Huang H, Wittner M, Weiss LM, and Morris SM. Albert Einstein College of Medicine, Bronx, NY.

The mortality of CD-1 mice infected with *Trypanosoma cruzi* (Brazil strain) is >60%. When treated with the Ca 21 channel blocker, verapamil from day 1 of infection the mortality rate was <10%. Remarkably, verapamil administered for the first 10 days of infection was as effective as continuous therapy. While a 3 day delay in treatment reduced mortality, a 10 day delay did not influence mortality. The reduction in mortality was greater with the D (+) than with the L (-) isomer of verapamil. Propranolol (β-adrenergic blocker), diltiazem (Ca^{2+} channel blocker) and prazosin (α-adrenergic blocker) reduced the mortality but not to the same extent as verapamil. Of all drugs tested, only verapamil and propranolol reversed the infection associated decrease in myocardial β-adrenergic adenylyl cyclase activity (ACA). Western blot analysis 45 days post-infection did not reveal any significant changes in myocardial G-protein subunits to account for any changes in ACA. Our results suggest that verapamil may influence events that occur during the critical early period of infection and suggests the basis of the action of verapamil to ameliorate acute murine cardiomyopathy is multifactorial.

296 NEW S-ADENOSYLMETHIONINE DECARBOXYLASE INHIBITORS ARE TRYPANOCIAL AGENTS. Bacchi CJ*, Brun R, and Croft SL. Haskins Laboratories and Department of Biology, Pace University, New York, NY; Swiss Tropical Institute, Basel, Swutzerland; and Department of Medical Parasitology, and London School of Hygiene and Tropical Medicine, London, U.K.

A series of novel aromatic derivatives, based on the structure of methylglyoxal bis(guanylhydrazone), (MGBG) were examined for trypanocidal activity in human and veterinary trypanosomes of origin in Africa. One agent, CGP 40215, a bicyclic analog of MGBG which resembles the diamidines diminazene (Berenil) and pentamidine, was curative in 19 isolates of *Trypanosoma brucei* subspecies as well as a *Trypanosoma congolense* isolate. These included several *Trypanosoma rhodesiense* clinical isolates resistant to melarsoprol and the diamidines. Curative doses were < 25 mg/kg/day for 3 days in these acute laboratory model infections. In addition, CGP 40215 also cured a model central nervous system infection in combination with the ornithine decarboxylase inhibitor DL-α -difluoromethylornithine (DFMO). Curative combinations were 14 days of oral 2% DFMO plus 5, 10 or 25 mg/kg/day for 7 days given as intraperitoneal injection or mini-osmotic pump. Combinations were most effective if the CGP 40215 was given in the second half of the DFMO regimen. MGBG has modest activity as an inhibitor of trypanosome S-adenosylmethionine decarboxylase (IC_{50} 130 μM), CGP 40215 was the more active inhibitor (IC_{50} 29-62 μM, preparations from four isolates), indicating that its mode of action may involve polyamine biosynthesis.

297 STRUCTURE FUNCTION ANALYSIS: A NEW APPROACH TO TRIFLURALIN ANALOGS YIELDS RESULTS. Kelley C, Callahan HL*, Pereira TR, and Grogl M. US Army Medical Research Unit - Brazil Unit, American Consulate-Rio; and Department of Medicinal Chemistry, WRAIR, Washington, DC.

Microtubule inhibitors have been exploited previously as antihelmintic drugs, in cancer therapy, and as herbicides. The herbicide microtubule inhibitor, trifluralin, has been shown to inhibit the proliferation of *Plasmodium falciparum, Trypanosoma brucei,* and several species of *Leishmania, in vitro.* Trifluralin is also effective *in vivo* (Balb/c mice) as a topical formulation against *L. major* and *L. mexicana.* Other anti-microtubule herbicides, as well as synthetic anti-microtubule compounds, have shown activity *in vitro* against *Leishmania.* Thus, microtubule inhibitors have the potential to be promising lead drug candidates for several tropical diseases, including leishmaniasis, African trypanosomiasis, and malaria. Although the antimicrobial dinitroaniline herbicides show great activity as anti-protozoa compounds, disputed indications of potential carcinogenicity will probably keep trifluralin from being developed for human use. However, researchers have suggested that an impurity/contaminant in trifluralin, not trifluralin itself, may be responsible for the observed *in vitro* activity against *Leishmania.* We have pursued this lead and identified the structure of the active impurity, evaluated its *in vitro* activity in a *Leishmania* promastigote assay, developed a rational structure-activity model, and based on this model, predicted and tested potentially active analogs in a *Leishmania* assay. We have identified one promising compound (Chloralin), and established a model which will be used for future drug design.

298 DESIGN OF A SERO-PREVALENCE STUDY TO DETERMINE ANTIBODIES TO *TRYPANOSOMA CRUZI* IN LATIN-AMERICAN REFUGEES AND IMMIGRANTS TO CANADA. Steele LS*, MacPherson DW,

Gushulak B, Gully P, and Blajchman M. Faculty of Medicine - M.D. Programme, McMaster University, Hamilton, ON, Canada; Parasitology, St.Joseph's Hospital and Pathology, McMaster University, Hamilton, ON, Canada; Quarantine Health Services, Health Protection Branch, Health Canada, Ottawa, ON, Canada; and Canadian Red Cross Society and Haematology & Pathology, McMaster University, Hamilton, ON, Canada.

Our goal was to design a culturally acceptable study which will provide a valid estimate of the sero-prevalence of *Trypanosoma cruzi* in Latin-American refugees and immigrants to Canada. A literature search was undertaken to: a) review the scientific research available on *T. cruzi* parasitemia in Canada and the United States, b) explore the current interaction between the Latin-American community in the study area and the Canadian health care system, and c) identify the health programs which are currently in place to service the Latin-American community in the study area. Collaboration with health care workers within the Latin-American community was sought. The implications of the study for the Latin-American community were identified and suitable methods to undertake the study in a culturally-sensitive manner were formulated. We determined a sample size of 450 will be needed to be 95% confident of a sero-prevalence of 5% (plus or minus 2%). These samples will be tested by immunoflourescence or ELISA. A demographic data sheet was developed to stratify participants according to risk factors for antibodies to *T. cruzi*. Barriers to satisfactory interaction of the Latin-American community with the health care system were identified. Recommendations were formulated to ensure the greatest benefit of the study to the Latin-American community. These recommendations addressed the following four issues: 1) community education 2) information dissemination and informed consent 3) follow-up and management. 4) anonymity and confidentiality. printed in Spanish and in Portuguese, as well as English. 3) A clear management plan will be offered to identified participants who test positive for *T. cruzi* including referral to a tropical disease clinic and long-term follow-up. 4) Participants will be given anonymity unless they choose otherwise. All test results will remain confidential.

299 THE DRUG SENSITIVITY PROFILE OF FREE AMASTIGOTES: DEVELOPMENT OF A NEW MODEL SYSTEM FOR SCREENING DRUGS. Grogl M, Portal AC, and Callahan HL. U.S.A. Medical Research Unit-Brazil, Walter Reed Army Institute of Research.

Recently, there have been increasing reports in the literature of at least partially successful *in vitro* culture of "free" amastigotes. Similarly to a drug screen using promastigotes, a drug screen using free amastigotes should be relatively quick and easy, but should be more representative of the situation *in vivo*. In addition, it should alleviate the problems associated with testing drugs against amastigotes in macrophages. We have established an amastigote drug screen using free amastigotes from an L. mexicana (M379) strain as described previously. A comparison of the IC_{50} drug sensitivity profiles of the promastigote and amastigote stages of M379 against reference antileishmanials shows amastigotes and promastigotes respond equally to 3 out of 5 drugs tested. For the other 2 drugs, the IC_{50}s of the free amastigotes are more similar to values found testing amastigotes in macrophages than are the values found testing promastigotes. As expected, amastigotes were more sensitive than promastigotes to all antimony compounds tested (nearly 4-fold to 280-fold depending on the source). A comparison with achievable serum levels *in vivo* (where known) will also be presented.

300 GOOD MANUFACTURING PRACTICES (GMP) PRODUCTION OF *LEISHMANIA* SKIN TEST ANTIGEN (LSTA): 2. PRODUCTION OF A MICROFLUIDIZED LYSATE (MFL) LSTA. Stiteler JM*, Ballou WR, Eckels KH, Wellde BT, Topper MJ, Rowton ED, and Magill AJ. Division of Communicable Diseases & Immunology, Walter Reed Army Instituteof Research, Washington, DC.

Viscerotropic Leishmaniasis (VTL) resulting from infection by *Leishmania tropica* was described as a new clinical presentation of Leishmaniasis following isolation and characterization of the parasite from U.S. troops returning from Operation Desert Storm (ODS). The prevalence of VTL in ODS veterans is unknown. The USA/DoD decided to pursue the development of a LSTA for use as such a diagnostic screening method to determine exposure of personnel to *L. tropica*. A soluble, lyophilized, Microfluidized lysate (MFL) LSTA was developed and produced in accordance with FDA's guidelines for current GMP within WRAIR's Pilot Bioproduction Facility. Strain WR#1063, which was isolated from a bone marrow aspirate biopsy of a case of VTL was chosen as the type strain and source of the MFL-LSTA. WR#1063 was cloned, characterized, and then expanded and cryopreserved (MSL). One sample of the MSL was then expanded (PSL). Individual cryostocks of the PSL of WR#1063 promastigotes were grown, harvested, washed, and stored (BLP). Various BLP processing experiments and animal testing of these LSTA preparations led to the current MFL-LSTA protocol. In brief, the BLP was thawed, microfluidized, centrifuged, the supernatant sterile filtered, the filtrate adjusted to dose, lyophilized as MFL-LSTA. Following required testing of the MFL-LSTA, an INDA was prepared for review by FDA. FDA's approval of human use will lead to Phase I/Phase II trials of the LSTA.

301 TREATMENT OF CUTANEOUS LEISHMANIASIS WITH LOCALIZED CONTROLLED HEAT (RADIO FREQUENCY) IN 213 PATIENTS IN LA CHONTALPA, TABASCO, MEXICO. Velasco-Castrejon O, Walton BC*, Rivas-Sanchez B, Garcia MF, Lazaro GJ, Hobart O, Roldan S, Floriani-Verdugo J, Munguia-Saldana A, and Berezaluce R. Instituto del Diagnostico y Recursos Epidemiologicas, Mexico, DF Mexico; Consultant, Gettysburg, PA, PanAmerican Health Organization (Retired); Secretaria de Salud del Estado de Tabasco; and Hospital General de Comalcalco, Tabasco, Mexico.

In Mexico cutaneous leishmaniasis (CL) is a growing Public Health problem. Pentavalent antimonial compounds (Sb^V) are inconvenient and expensive. A treatment using localized controlled field-radio frequency (LCF-RF) energy to generate precisely controlled heat beneath the surface of the skin was shown to be as effective as Sb^V in a double blind controlled trial in Guatemala. This study is not a clinical trial, but rather an evaluation of the feasibility of this form of treatment in this endemic area. A total of 213 patients with 3 clinical forms caused by *L. mexicana*, ages from <5 to >51 yr; males 63% females 37%, were treated in La Chontalpa, Tabasco, Mexico. A single portable THERMOSURGERY™ LCF-RF generator, powered by rechargable batteries (ThermoSurgery Technologies, Inc., Phoenix, AZ) was used in a Regional Hospital and Primary Health Care Centers in 5 municipalities to treat previously diagnosed cases. Lesions were first anesthetized with lidocaine HCl and moistened with normal saline solution. A single application that produced 50°C for 30 sec. was used: a second application was given to 11 patients. After 4 weeks, 122 patients were available for evaluation, of which 95% with localized lesions were completely cured (even those with involvement of ear cartilage, which respond poorly to Sb^V) and distinct improvement was seen in diffuse lesions. This modality would be both feasible and advantageous in Mexico.

302 INHIBITION OF *TRYPANOSOMA CRUZI* WITH DISULFIRAM AND SODIUM DIETHYLAMINE-*N*-CARBODITHIOATE *IN VITRO* AND *IN VIVO*. Lane JE*, Ribeiro-Rodrigues R, Carter CE, Suarez CC, Jones MM, Singh PK, and Romanha AJ. Department of Biology, Vanderbilt University, Nashville, TN; Department of Chemistry, Vanderbilt University, Nashville, TN; and Centro de Pesquisas "Rene Rachou" Belo Horizonte, MG, Brazil.

The inhibitory effects of Disulfiram (DSF) and its metabolite Sodium diethylamine-*N*-carbodithioate (DECD) were tested with *T. cruzi in vitro* and *in vivo*. DSF and DECD were compared to Benznidazole (BNZ), the current treatment for the parasite-induced Chagas' disease. *In vitro* assays included evaluation against the epimastigote, trypomastigote, and amastigote forms. DSF, DECD, and BNZ inhibited 82%, 77%, and 76%, respectively, of epimastigotes at 50 ug/ml. DSF and DECD were less effective against cell culture trypomastigotes (48% and 46%, respectively) as BNZ (89%) at 50 ug/ml. Infected mouse 3T3 fibroblasts were treated with DSF, DECD, and BNZ. Treatment resulted in 93%, 92%, and 94% inhibition for DSF, DECD, and BNZ, respectively. *In vivo* assays included treatment with DSF and DECD in blood cultures harboring bloodstream trypomastigotes, and in infected mice 6 hrs post-infection. Gentian violet (GV) and BNZ served as positive controls. GV inhibited 54% of trypomastigotes at 24.4 µM. DSF inhibited 55% of trypomastigotes at 244 µM, whereas 900 µM BNZ was required for 56% inhibition. DECD was not as effective at 244 µM as DSF. Infected mice were treated with 1.92 mM of DSF and DECD 6 hrs post-infection. Untreated mice had a 130% increase in parasitemia; BNZ treated mice had a 92% decrease. DSF treatment showed a 68% increase and DECD a 49% increase in parasitemia. Similar experiments with longer treatment may prove more effective.

303 KNOWLEDGE, ATTITUDES AND PRACTICES (KAP) SURVEY FOR CHAGAS' DISEASE IN AN ENDEMIC AREA OF GUATEMALA. Nix NA*, Hernandez B, Mendoza C, and Klein RE. Universidad de Valle de Guatemala, Guatemala City, Guatemala; Medical Entomology Research and Training Unit/Guatemala; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Chagas' disease is considered a public health problem in the southeastern region of Guatemala. In an on-going multidisciplinary pilot project for the control and surveillance of Chagas' disease, a Knowledge, Attitudes and Practices (KAP) and demographic survey was conducted. The KAP questionnaire focused on the vector, disease and transmission, treatment-seeking and prevention, behaviors, and perceptions of housing and housing improvements. Sixteen percent of households in three communities in the endemic region were surveyed. Sero-epidemiologic data indicate a sero-positivity rate of approximately 11% in these communities. Survey results indicate that none of the residents know of "Chagas' disease". Several interviewees indicated that bites from the vectors can cause disease, but none of the signs and/or symptoms described were associated with Chagas' disease. Triatomines were readily identified by the residents, but are considered more of a nuisance than a disease risk.

304 ROLE OF MALARIA ANTIBODIES IN THE DEVELOPMENT OF HTLV-I IMMUNOREACTIVITY IN INDONESIAN TRANSMIGRANTS. Porter KR*, Anthony RL, Richards A, Sandjaya B, Ignatias H, Hadiputranto H, Wignall FS, and Hayes CG. Department of Infectious Diseases, Naval Medical Research

Institute, Bethesda, Maryland; and Department of Immunology, Naval Medical Research Unit #2, Jakarta, Indonesia.

Plasmodium falciparum antibodies that cross-react with Human T-Lymphotropic Virus Type 1 (HTLV-I) proteins were previously detected in populations living in malaria endemic areas of the Philippines, Indonesia, Brazil and Papua New Guinea. A unique seven amino acid epitope for these antibodies was mapped to the carboxy-terminus of the HTLV-I p19 protein and was found to be similar to a seven amino acid sequence on the malaria blood stage antigen Exp-1. To further study the relationship between malaria antibodies and HTLV-I proteins, we tested pre- and post-migration serum samples from Indonesian volunteers who transmigrated from malaria non-endemic areas of Java to Irian Jaya, where malaria is hyperendemic. The samples were tested for *P. falciparum* antibodies by indirect immunofluorescence assay (IFA) and for HTLV-I immunoreactivity by Western blot and an enzyme immunoassay (EIA). Samples were also tested for immunoreactivity against synthetic peptides containing the HTLV-I p19 epitope that cross-reacts with malaria antibodies. The results showed that HTLV-I EIA and indeterminate Western blot immunoreactivity, as well as immunoreactivity to the p19 cross-reactive epitope, was detected in several serum samples taken from volunteers three to 12 months post-migration. Pre-migration samples showed no significant immunoreactivity. The HTLV-I immunoreactivity paralleled the appearance of anti-*P. falciparum* antibodies. The HTLV-I indeterminate Western blot immunoreactivity could be eliminated or greatly reduced by blocking with malaria antigens. These data provide further evidence that anti-malaria antibodies cross-react with HTLV-I proteins.

305 LYMPHOCYTE SUBSETS, MALARIA INFECTION, AND DISEASE AMONG MEN IN WESTERN KENYA. Mak'obongo M, McElroy PD, Guo WS, Scott LA, Sullivan AD, Orago AS, Oloo AJ, and Weiss WR*. Department of Zoology, Kenyatta University, Nairobi, Kenya; Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan; Vector Borne Disease Control and Research Center, Kenya Medical Research Institute, Kisumu, Kenya; and Malaria Program, Naval Medical Research Insititute, Bethesda, MD.

We wished to know if changes in the frequency of lymphocyte subsets were associated with either malaria parasitemia, or malaria disease. The study site was in Western Kenya, at villages holoendemic for *Plasmodium falciparum*. Seventy-four Luo men aged 15-55 were followed longitudinally for periods of 6 to 12 months. These men were part of a case-control study comparing groups with short and long times to reinfection, which was defined as "susceptiblity". Venous blood was taken at baseline, and periodically when volunteers sought medical care at the health clinic. A malaria thick smear was taken, and peripheral blood lymphocytes were collected by centrifugation over Ficoll. Cells were stained for the following lymphocyte markers using fluorescent conjugated antibodies: CD4, CD8, α-β TCR, γ-δ TCR, and CD56 (NK cells). Clinic records were reviewed, and each visit was coded as one of six clinical categories: Healthy Individual, Parasitemic Asymptomatic, Parasitemic Symptomatic, Other Febrile Illness, Other Non-febrile Illness, or After Recovery followup. A subject specific mixed effect logistic regression model was used to model the relationship between clinical groups and lymphocyte subset frequencies. When comparing parasitemic vs. non-parasitemic visits, after controlling for season and susceptiblity, CD8 and CD56 levels were highly predictive of parasitemia. When comparing symptomatic vs. asymptomatic parasitemias, no lymphocyte parameter was highly predictive, but γ-δ TCR levels tended toward statistical significance. CD8+ T cells and NK cells levels are inversely associated with parasitemia.

306 CHANGES IN RESPONSE AGAINST *PLASMODIUM FALCIPARUM* PEPTIDES OF T AND B LYMPHOCYTES FROM CHILDREN SUBMITTED TO ONE SEASON OF MALARIA TRANSMISSION IN HIGHLANDS OF MADAGASCAR. Rasamoel P*, Ralamboranto L, Ramambanirina L, Ranaivo L, Raharimalala L, Ollivier G, Druilhe P, Laventure S, Jambou R. Laboratoire du paludisme, and Unité d'entomologie, Institut Pasteur de Madagascar, Antananarivo, Madagascar; and Laboratoire de Parasitologie biomédicale, Institut Pasteur, Paris France.

After more then 20 years of disappearance, malaria transmission increased dramatically between 1980 and 1990, in Highlands of Madagascar. However, this transmission never disappeared in some area of these highlands as in Ankazobe. In this small town, where transmission occured from January to April, 750 persons are followed for malaria since 1992, by longitudinal medical survey. Among this population, 70 children (3 to 7 years) were investigated three time during one season of malaria transmission for T and B cells responses against *Plasmodium falciparum* antigens derived peptides (LSA1, LSA3, MSP3 and RESA). We investigated particularly proliferation of T cells, CD4 and CD8 typing of these cells, and isotype modulation of B cells responses against these peptids. These results were discussed according to the "Malaria exposure background" of these children during previous years.

307 LONGTERM ISOTYPE MODULATION OF B LYMPHOCYTE RESPONSES AGAINST *PLASMODIUM FALCIPARUM* PEPTIDES AFTER ARREST OF MALARIA TRANSMISSION IN A VILLAGE OF THE HIGHLANDS. Ralamboranto L*, Rasamoel P, Ramambanirina L, Ranaivo L, Raharimalala L, Ollivier G, Druilhe P, Laventure S, Jambou R. Laboratoire du paludisme, and Unité d'entomologie, Institut Pasteur de Madagascar, BAntananarivo, Madagascar; Laboratoire de Parasitologie biomédicale, Institut Pasteur, Paris France.

After more then 20 years of dIsappearance, malaria transmission increased dramatically in Highlands of Madagascar, between 1980 and 1990. As shown by extensive medical survey of the population and probably related to permethrin housespray and chloroquine supply, this transmission stopped as soon as 1990-1991 in some area of this country. The village of Manarinstoa (near Antananarivo) was the first place where reappearance of Malaria was described. In this village, longitudinal medical and serological survey of population was carried on from 1988 to 1995. Since 1991 no local transmission of Malaria was reported, and plasmodium specific antibody titers decreased slowly among the population. In this study, *Plasmodium* sero-positive people, were investigated for isotype modulation of their B cell responses against *P. falciparum* antigen-derived peptids (LSA1, LSA3, MSP3 and RESA), from 1988 to 1995. These results were presented and discussed according to their epidemiological background.

308 HUMAN IMMUNE RESPONSE TO SYNTHETIC PEPTIDES CONTAINING CTL MOTIVES ON THE *PLASMODIUM VIVAX* CS PROTEIN. Herrera MA, Perlaza BL, Toala A, Ferro B, Reyes ME, Betterns F, Corradin G*, and Herrera S. Institute de Inmunologia del Valle, Universidad del Valle; Institute of Biochemistry, University of Lausanne, Lausanne, Switzerland.

The complete sequence of the CS *Plasmodium vivax* protein was analyzed and a fragment corresponding to positions (31 6-326) on the carboxyl region containing a previously described CTL-binding motive was selected. Four peptides PVI (317-326), PV2 (316-325), PV3 (381-390) and PV4 (374-384) containing the motifs (L/V, F/L, and Y/T) were synthesized and used to study the interaction with putative MHC molecules. PVl and PV2 peptides were incubated with the transgenic mouse T2 cell line expressing HLA-A2 molecules. The expression of this molecules on the cell surface was highly increased as determined by flow cytometry analysis. A positive proliferative response was present in the 30 - 45% of the studied samples. The percentage of responders was higher in the immune donors indicating an increased number of circulating precursor cells. γIFN (γ-IFN) production was measured in the supernatant cultures using an ELISA assay. Peptides PVl to PV3 induced production of variable amounts of γ-IFN in some of the donors. The highest production was induced by PV2 whereas PV4 did not induce any production. Although there was no correlation between cell proliferation and γ-IFN production, it might indicate CTL stimulation. Results suggest that the PVI, PV2 and possibly PV3 peptides bind HLA-A2 and could represent a potential HLA-A2 restricted malaria CTL epitopes. Isolation and further characterization of specific T-cell clones from malaria immune individuals is being intended.

309 CHARACTERIZATION OF SERA FACTORS CAUSING *IN VITRO* INHIBITION OF *PLASMODIUM FALCIPARUM*. Perlaza BL*, de Plata C, Zapata C, Clavijo P, Herrera MA, AND Herrera S. Instituto de Inmunologia del Valle, Universidad del Valle, Cali Colombia; and Department of Medical and Molecular Parasitology, New York University, NY.

Parasites exposed *in vitro* to sera of individuals from malarial areas suffer what has been called "crisis phenomenon" consisting in growth retardation, morphological changes and death. Sera from 29 individuals living in a malaria endemic region (Colombian pacific coast) were evaluated by an inhibition assay for their capacity to retard the growth of *Plasmodium falciparum in vitro*. Parasite was affected by sera from most individuals exhibiting variable levels of inhibition, while parasite strain were found differentially susceptible to this effect. Inhibitory activity was independent of antibody activity. Degree of inhibition was variable depending on the malaria transmission season. Plasma with high inhibitory activity was studied by anion exchange and gel filtration Fast Performance liquid chromatography, to determine the chemical nature of molecules involved. Plasma from a healthy donor living in non-endemic area was studied in parallel. Parasite growth inhibition by plasma and by isolated fractions was determined by metabolic labelling. Results correlated with the appearance of degenerated parasite forms during culture. SDS-PAGE analysis of pooled fractions obtained from the inhibitory plasma presented a single 100 kD band that was not apparent in similar fractions obtained from normal plasma. Five pools from anion exchange chromatography were subsequently fractionated by gel filtration and retested. Two fractions from pool 3, corresponding to about 40 and 100 kD, displayed inhibitory activity. Results suggest that inhibitory activity might result from an additive effect of several molecular species with different molecular weight and net charges.

310 SERA FROM PATIENTS WITH FALCIPARUM MALARIA INDUCE SUBSTANCE P GENE EXPRESSION IN CULTURED HUMAN BRAIN MICROVESSEL ENDOTHELIAL CELLS. Chiwakata CB, Hort G, Hemmer CJ, and Dietrich M*. Department of Medicine, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

In an effort to contribute to the understanding of the pathophysiology of cerebral malaria, we have evaluated the effects of sera obtained from (i) patients suffering from cerebral malaria, (ii) patients suffering from mild malaria and (iii) a healthy donor with no previous history exposure to malaria on the expression of substance P (SP) gene by cultured human brain microvessel endothelial calls (HBMEC) and human umbilical vein endothelial cells (HUVEC). Polymerase chain reaction (PCR), southern blotting, hybridization with an internal probe and densitometry demonstrated that treatment of HBMEC with sera from patients with cerebral malaria caused remarkably increased expression of the SP gene. In HBMEC, SP gene expression was not significantly influenced by serum from a healthy donor. SP was expressed at almost undetectable levels in untreated HBMEC. Treatment of cultured human umbilical vein endothelial cells (HUVEC) with the same sera produced no signal in our hands. The influence of different sera on the expression of SP by HBMEC suggests that SP may be involved in events leading to the development of cerebral malaria.

311 CYTOKINES INDUCED BY PROTECTIVE VACCINATION IN THE *PLASMODIUM YOELII* BLOOD-STAGE MODEL. Patterson PS*, Udhayakumar V, Bosshardt SC, Kidd MR, Xiao L, Hunter RL, and Lal AA. Immunology Branch, DPD, Centers for Disease Control, Atlanta, GA; Department of Pathology, Emory University, Atlanta, GA; and School of Medicine, Vanderbilt University, Nashville, TN.

Whole blood-stage antigen, along with P1005 and detoxified RaLPS in saline, protected 100% of outbred ICR mice against lethal *P. yoelii*challenge, while identical components in a water-in-oil formulation gave only 50% protection. Here, we evaluated whether the protective or semi-protective vaccine formulations differ in their ability to induce cytokine production. The levels of IL-2, IL-4, IL-10, and IFNγ produced by spleen cells following stimulation with +-CD3 or malarial antigens *in vitro* were measured 5 days after primary immunization, 5 days after boost, and days 4 and 6 post-challenge. Although both vaccine formulations induced IL-4 and IL-10 production, protective vaccine formulation induced higher levels of IL-4 than the semi-protective vaccine formulation. Prechallenge levels of IL-2 and IFNγ, were lower in vaccinated groups than in unimmunized controls. Four days after a lethal challenge with *P. yoelii*, animals immunized with the protective formulation displayed a peak in IFNγ production. However, peak IFNγ levels declined rapidly by day 6 with a concomitant increase in IL-4. Spleen cells from partially protected and unimmunized groups displayed little IL-4 response post-challenge. Altogether, these data showed that the protective vaccination induced higher levels of Th2-type cytokine IL-4, which persisted at all time points, whereas partially-protective vaccination induced a transient and lower IL-4 response.

312 GUT IMMUNITY OF BALB/C MICE INFECTED WITH THE LETHAL STRAIN OF *PLASMODIUM CHABAUDI ADAMI*. Dimayuga FO*, Dimayuga ER, and Wei Y. Department of Biological Sciences, Ohio University, Athens, OH; and Edison Biotechnology Institute, Ohio University, Athens, OH.

The mucosal immune system is independent of systemic or peripheral immune system and is regulated in a different fashion but both have access to the lymph system. The circulation allows trafficking of lymphocytes via postcapillary venules. We attempted to determine whether plasmodial infection affects not only lymphocytes in the peripheral but also the mucosal lymphoid organs. The numbers of lymphocyte populations in normal and *Plasmodium chabaudi*-infected BALB/c mice were determined by flow cytometric analysis to compare the effect of parasite infection upon Peyer's patch and spleen lymphocytes using monoclonal antibodies to CD3, CD4, CD8, TCR , TCR , IgG and IgM. Splenic IgG+ cell numbers and flourescence intensity fluctuate with rising parasitemia but Peyers' patch IgG+ cells significantly decrease during ascending and peak parasitemia. Splenic IgM+ cell numbers generally remain the same in infected and noninfected mice but IgM fluorescence intensity is significantly suppressed in the Peyer's patches upon infection. The numbers of CD3+ T cells decrease by 50% in the spleen but significantly increase in the Peyer's patches during ascending and peak parasitemia. The numbers of TCR +, CD4+, and CD8+ T cells remain the same or decrease in the spleen but increase in the Peyer's patches when parasitemia increases or reaches peak level. Numbers of TCR + cells increase by 50% or more for both splenic and Peyer's patch populations during the course of parasite infection. These data demonstrate that lymphocyte regulation differs in the gut and the systemic lymphoid tissues in response to *P. chabaudi*.

313 ENHANCEMENT OF MALARIA IN MICE IMMUNIZED WITH PEPTIDE FORMULATIONS; A MODEL SYSTEM TO STUDY IMMUNOPATHOLOGY OF MALARIA. Jennings VM*, Udhayakumar V, Lal AA, and Hunter RL. Department of Pathology, Emory University, Atlanta, GA; and Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

A/J mice infected with *Plasmodium berghei* malaria typically live for 20-30 days before they die with parasitemias greater than 50%. We found that immunization of these mice with certain peptide formulations caused them to die at 5-10 days after infection with neurologic signs and parasitemias of only 5-10%. Since the pathology of malaria is associated with Th1 cytokines, particularly tumor necrosis factor (TNF) produced in excess or inappropriately, we hypothesize that these immunizations may have sensitized mice to produce deleterious cell mediated responses. Mice were immunized with an antigen that had induced deleterious immune responses (*P. berghei* circumsporozoite T epitope peptide coupled to monopalmitic acid) in four adjuvant formulations in an effort to induce stronger cell mediated immunity. The adjuvant were copolymer P1005 with detoxified RaLPS, copolymer L101, mycobacterial cell wall skeletons and DDA. Immunization with each of these formulations increased the proportion of mice that developed enhanced deleterious immune responses to malaria. These data confirm that the incidence of enhanced malaria can be increased by immunization and provide a new model for studying the immunopathology of the disease.

314 IDENTIFICATION OF T CELL EPITOPES IN CONSERVED AND SEMI-CONSERVED REGIONS OF ELEVEN *PLASMODIUM FALCIPARUM* ANTIGENS IN CONGENIC MICE. Parra ME*, Roberts T, Quakyi IA, Berzofsky JA, Miller LH, Houghten RA, and Taylor DW. Department of Biology, Georgetown University, Washington, DC; Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD; Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda MD; Torrey Pines Institute of Molecular Biology, San Diego, CA.

Since T cells play a central role in immunity to malaria, we sought to identify T cell epitopes in conserved regions of immunologically relevant asexual-stage *Plasmodium falciparum* antigens. Accordingly, in 1990 we compiled all known DNA/protein sequences for asexual-stage *P. falciparum* antigens in all available databases. Sequence alignments were initially made using GAP-BESTFIT and then hand-aligned. Conserved regions were then analyzedusing several predictive T cell motifs including, AMPHI, Rothbard-Taylor, and the H-2 (IA^d and IE^d) motifs of Sette. Based on the results, a total of 24 putative T cell sites and 6 control peptides were selected from the 11 relevant antigens. Reanalysis in 1995 showed that the most of the peptides are from conserved regions. The peptides, about 20 amino acids in length, were synthesized and used to immunize 5 strains of B10 congenic mice. Cells from draining lymph nodes were tested for proliferation upon stimulation with the corresponding peptide and an extract of malarial parasites. The majority of peptides were able to prime T cells in one or more strains of mice. Two peptides, one from ABRA and one from MSP-1, were of particular interest because they stimulated T cells from all strains of mice tested, whereas others were H-2 restricted. Thus, the use of predictive motifs allowed us to identify potentially useful T cell sites in conserved regions of asexual-stage *P. falciparum* antigens.

315 POPULATION EFFECTS OF STAGE-SPECIFIC MALARIA VACCINATION. Burke MA*, Halloran EM, and Lal AA. Rollins School of Public Health, Emory University, Atlanta, GA; and Division of Parasitic Disease, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

Various malaria vaccine candidates will have differing effects on the overall morbidity effects of vaccination program. This will depend on the direct effects in vaccinated individuals, such as reduction in susceptibility to infection with or without sterile immunity, reduction indisease, morbidity and mortality per case, and reduction in duration anddegree of transmissiblility to mosquitoes. Using a dynamic transmission model, we have explored the population effects of vaccine candidates, such as the Spf66 vaccine and the transmission blocking vaccine (TBV) in regions of differing endemicity. A vaccine that moderately reduces the probability of infection by an infective mosquito and reduces parasitemia could result in an overall reduction in morbidity while allowing sufficient transmission to maintain semi-immunity. In regions of low endemicity, a TBV could help reduce morbidity. In regions of moderate endemicity, a TBV could reduce morbidity if the decreased number of new infections were important in determining morbidity. A vaccine that reduces infection or morbidity and not just transmissibility will be better at reducing overall morbidity than a vaccine that only reduces transmitilibility. Results are sensitive to assumptions about duration, strain-specificity, and boosting of immunity, emphasizing the importance of gaining understanding of these phenomena in malaria.

316 T CELL RESPONSES TO *PLASMODIUM FALCIPARUM* LSA- I IN IMMUNE ADULT RESIDENTS OF TH E WOSERA, PAPUA NEW GUINEA. Connelly M, Genton B, King C, Hollingdale M, Boykins R, Alpers M, and Kazura J*. Case Western Reserve University, Cleveland, OH; Papua New Guinea Institute of Medical Research, M aprik, Madang, and Goroka; and Food and Drug Administration, Bethesda, MD.

LSA-1 is a 230 kD *Plasmodium falciparum* antigen expressed by liver-stage schizonts. With the long-term goal of determining its potential as a vaccine candidate, we have initiated studies to determine the antigenicity of the native molecule in residents of the Wosera, Papua New Guinea. Synthetic peptides encoding epitopes in the N-

terminal nonrepeat region (T1: beginning with amino acid 84, LTMSNVKNVSQTNFKSLLRNLGVS) and the non-repeat region of the C-terminus (T3: amino acid 1813, NENLDDLDEGIEKSSEELSEEKI; amino acid 1888, DNEILQIVDELSEDITKYFMKL) were used to stimulated PBMC from "immune" adults. PBMC from control uninfected and unexposed individuals did not proliferate in response to TI, T3, or T5 (1-20 μg/ml). In contrast, 7 of 10 Wosera residents had proliferative responses with stimulation indices (SI) ≥ 2.0. 6 of 10 persons responded to TI (SI range 3.0 - 12.3); 6 of 10 to T3 (SI range 2.6 - 4.7) and 6 of 10 to T5 (SI range 2.2 - 3.9). These studies indicate that T cell responses to LSA-1 are common in this population. Studies are underway to ascertain the LSA-1 specific Th subset responses and their relationship to simple morbidity in children.

317 MALARIA VACCINE TRIALS AGAINST PRE-ERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM* IN CHIMPANZEES: 2) CORRELATION BETWEEN EFFECT ON PARASITEMIA AND T-CELL ACTIVATION AFTER CHALLENGE ELICITED BY A PRE-ERYTHROCYTIC STAGE MALARIA VACCINE BASED ON LSA-3. Luty A*, Millet P, Dubreuil G, LeRoy E, DeBels F, Tartar A, Eling WM, George AJ, and Druilhe P. Centre International de Recherche Médicale de Franceville (CIRMF), Franceville, Gabon; Institut Pasteur, Roux, Paris, France; and University Hospital Nijmogen, Department of Parasitology, Nijmegen, The Netherlands.

We have used synthetic peptides and recombinent proteins corresponding to four different pre-erythrocytic stage antigens of *Plasmodium falciparum* (LSA-1, LSA-3, SALSA & STARP), combined with adjuvant to immunize a group of 5 chimpanzees. We evaluated the immunogenicity of the antigens at the cellular immune level, assessing both proliferative and cytokine responses and we also investigated the phenotypic characteristics of proliferating cells responding to individual peptides by flow cytometric analysis. The immunological assessments were repeated before, during and after a challenge infection with sporozoltes of *P. falciparum* and correlations were sought between the immune responses and putative protection determined through parasitological investigations. Immunization with LSA-1, LSA-3, and SALSA was found to have generated strong and long-lasting antigen-specific proliferative and cytokine responses. All chimpanzees received a challenge time of 120,000 sporozoites of *P. falciparum* During the hepatic phase of the infection, peptide-spedfic proliferative responses were profoundly suppressed In both the LSA-1- & SALSA- immunized animals, contrasting with the marked enhancement of peptide-specific responses in the animal immunized with LSA-3, the latter being the only one to exhibit a degree of protection from infection as defined by parasitological investigations. The profile of peptide-specific cellular responses in this animal was of the Thl type, characterized by the production of IFN-γ and proliferation of CDS+ (cytoxic) T cells as well as natural killer (NK) cells. Memory generation was indicated by the simultaneous expansion of CD45RO+ cells. These results, combined with the parasitological data indicative of a protective immune mechanism induced by LSA-3 in chimpanzees demonstrate that this protein merits further evaluation as a promising candidate for inclusion in a pre-erythrocytic stage vaccine. The date are discussed with reference to recent findings concerning the mechanisms of protective immunity to pre-erythrocytic stages to both human and rodent malarias.

318 *PLASMODIUM VIVAX* INFECTIONS IN CHIMPANZEES FOR SPOROZOITE VACCINE CHALLENGE STUDIES IN MONKEYS. Sullivan JS*, Morris CL, McClure HM, Strobert EA, Richardson BB, Galland GG, Goldman IF, and Collins WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Yerkes Regional Primate research Center, Emory University, Atlanta, GA.

The development and testing of vaccines directed against *Plasmodium vivax* has relied on *Saimiri* and *Aotus* monkeys as the animal test system and on chimpanzees to provide infective gametocytes to produce sporozoites for monkey challenge studies and vaccine development. One sporozoite-induced and 28 blood-induced infections with the Salvador I strain of *P. vivax* were studied in splenectomized chimpanzees. Eighteen primary infections resulted in maximum parasite counts ranging from 1,519 to 81,810/μl (median 29,100/μl). Eleven infections induced in animals previously infected with the homologous or heterologous strains of *P. vivax* had maximum parasite counts ranging from 155 to 14,136/μl (median 1,736/μl). A total of 241 of 268 lots containing 293,175 *Anopheles stephensi, An. gambiae, An. freeborni, An. quadrimaculatus, An. maculatus,* or *An. dirus* mosquitoes were infected by membrane feeding on gametocytes from chimpanzees. Despite much lower levels of parasitemia during secondary parasitemia, 61 of 72 lots of mosquitoes (84.7%) were infected. Sporozoites from *An. stephensi, An. gambiae, An. dirus,* and *An. freeborni* infected with the Salvador I strain of *P. vivax* produced in chimpanzees were used to infect 194 *Saimiri* and 6 *Aotus* monkeys as well as 1 chimpanzee.

319 NATURAL IMMUNE RESPONSES TO THE C-TERMINAL DOMAIN OF *PLASMODIUM FALCIPARUM* MSP-1; CORRELATION OF ANTIBODY RESPONSES WITH PARASTEMIA AND ILLNESS. Branch OH*, Udhayakumar V, Bloland PB, Hightower AW, Oloo AJ, Hawley WA, Nahlen BL, Kaslow DC, and Lal AA. Division of Parasitic Diseases,NCID, CDC, Atlanta, GA; Vector Biology and Control Research Center, Kenya

Medical Research Institute, Kissian, Kenya; Laboratory of Malaria Research, National Institute of Health, Bethesda, MD.

This study was a part of the Asembo Bay Cohort Project (ABCP), being conducted in western Kenya, which is aimed at delineating characteristics of naturally acquired protective immunity to malaria. In this study, we investigated the humoral responses to the 19kDa C-terminal of the merozoite surface protein (MSP-1) of infants for up to one year from the time of birth. The presence and level of antibodies at one month prior to an infant's first infection and at the time of first infection correlated significantly with a decreased incidence of febrile illness (p=0.0015) andan increased likelihood of clearing an untreated infection (p=0.0020). The presence of antibodies also correlated significantly with protection; the average number of infections was 5 fold greater when the infant did not have IgG present then when it did (p=0.0045). We found that maternal antibodies conferred protection against infection, and appeared to provide longer-lasting protection that the infant's IgG. These results suggest that the MSP-1 19kDa fragment is a potential protective target *in vivo*.

320 CHARACTERIZATION OF PROTECTIVE ANTIBODIES GENERATED BY IMMUNIZATION WITH BOTH EGF-LIKE DOMAINS OF MSP-1 FROM *PLASMODIUM YOELII*. Calvo PA*, and Long CA. Program in Molecular and Cell Biology; Department of Microbiology and Immunology, MCP-Hahnemann University, Philadelphia, PA.

The merozoite surface protein-1 (MSP-1) is a leading vaccine candidate against the erythrocytic stages of malarial parasite development. MSP-1 is a large protein which is proteolytically processed prior to merozoite invasion. A 19 kDa, C-terminal fragment, postulated to contain two epidermal growth factor-like (EGF-like) domains, remains associated with the merozoite and is the target of invasion-inhibiting antibodies. We have previously demonstrated that immunization with both EGF-like domains of MSP-1 from *P. yoelii*, administered as a fusion protein with glutathione-*S*-transferase (GST), can generate a protective host immune response that is predominately antibody mediated. In contrast, immunization with the individual EGF-like domains as GST fusion proteins failed to generate antibodies which could recognize native MSP-1and did not elicit protective responses. Fractionation of protective sera has demonstrated that most antibodies are directed against the first of the two EGF-like domains, and these antibodies can recognize the native MSP-1 protein. However, the second EGF-like domain is poorly immunogenic and antibodies specific for this region isolated from protective sera are unable to recognize native MSP-1. We have also identified a population ofantibodies which are specific for epitopes only found in the double EGF-like domain structure and which can also react with the native molecule. These findings suggest that the second EGF-like domain may play a role in stabilization of this disulfide-rich region, and we are testing the hypothesis that epitopes found in the first and double EGF-like domains play a predominant role in protection.

321 AN ALTERNATIVE STRATEGY FOR VACCINATION AGAINST MALARIA: IMMUNIZATION WITH PLASMID DNA ENCODING THE C-TERMINAL REGION OF THE *PLASMODIUM YOELII* MSP-1. Kang Y, Farley PJ, Hedstrom R, Hoffman SL, and Long CA. Department of Microbiology and Immunology, Molecular Biology and Biotechnology Program, MCP-Hahnemann University, Philadelphia, PA; and Malaria Program, Naval Medical Research Institute, Bethesda, MD.

Immunization with plasmid DNA containing sequences encoding relevant antigens can induce immune protection against viral infection in several animal models. Recently, adjuvant-free immunization of mice with plasmid DNA encoding the *P. yoelii* circumsporozoite protein (CSP) was reported to induce protection against challenge infection with sporozoites. However, this novel approach has not been tested in the induction of protective immunity against erythrocytic stages of malaria. The merozoite surface protein-1 (MSP-1) is a leading candidate antigen for a vaccine against blood stages of malaria. Several lines of evidence have suggested that the C-terminal, cysteine-rich region of MSP-1 contains protective B cell epitope(s), including the demonstration that this region fused to glutathione-*S*-transferase (GST) induces protective immunity in mice. We have designed a set of constructs comprising DNA sequences encoding the C-terminal region of *P. yoelii* MSP-1 (designated PyC2) fused to GST. Since proteins expressed extracellularly or intracellularly may undergo different processing routes and consequently be presented in different ways to the host immune system, the same constructs were also fused with the sequence encoding the signal peptide of mouse tenacin. All these constructs have been cloned into the nkCMVintpoly1 vector and are being confirmed by sequencing. By avoiding the complex process of peptide synthesis, expression and purification of recombinant protein, and use of an adjuvant, this strategy may provide an important alternative approach for subunit vaccine development against erythrocytic stages of malaria.

322 IMMUNOGENICITY OF PvMSP1 DNA VACCINES IN BALB/c MICE. del Portillo H*. Departamento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, Brasil

Nucleic acids are becoming an attractive alternative for vaccine development as opposed to synthetic peptides or recombinant proteins. *Plasmodium vivax* is the most widely distributed human malaria parasite and one of the two major species responsible for infections. Immunity in malaria, however, is species specific and thus it is unlikely that an effective *P. falciparum*vaccine will protect against *P. vivax*. Accordingly,efforts should be made to develop a vaccine against this parasite species, *per se*. DNA fragments encoding the first most N-terminal 300 aa and the last most C-terminal 111 aa from the Merozoite Surface Protein (PvMSP1) gene of the Belem strain were cloned into appropriate expression vectors. Balb/c mice were injected i.m. with 200 μg of plasmid dissolved in saline and boosted twice every three weeks. Analysis of the humoral immune response from these animals, both ELISA and Western blots, revealed that these constructs are immunogenic and capable of inducing specific responses against PvMSP1-derived recombinant proteins. Mapping of the epitopes recognized by these antibodies and analysis of the T-cell immune responses is currently under study. Due to the simplicity of this approach, plasmid constructs representing available coding sequences from *P. vivax* should facilitate the development of vaccine candidates for pilot monkey vaccination trials.

323 EFFECTS OF ADJUVANTS ON MURINE IMMUNE RESPONSES TO THE C-TERMINAL DOMAIN OF PV200 OF *PLASMODIUM VIVAX*. Yang C*, Collin WE, Reed RC, Hunter RL, Patterson P, Udhayakumar V, Kaslow DC, and Lal AA. Division of Parasitic Diseases, CDC, Atlanta, GA; Department of Pathology, Emory University, Atlanta, GA; and National Institute of Health, Bethesda, MD.

In this study, we evaluated the effect of Freund's complete adjuvant, alum and different formulation of copolymer P1005 on murine immune responses to the C-terminal 19Kd domain of *Plasmodium vivax* MSP-1 (Pv200). Female ICR mice were immunized with recombinant 19Kd protein of Pv200 with different adjuvants. After three immunizations, all mice except the unimmunized control animals were boosted with 10,000 *P. vivax*blood-stage parasites (Sale I). Antibody isotyping by ELISA revealed that Freund's adjuvant gave the highest IgGl titers at 3 weeks post 3rd immunization. The P1005 + RaLPS in water-in-oil or oil-in-water emulsion groups had IgGl titers similar to or higher than Freund's adjuvant 18 days after the whole parasite boosting. The latter also had higher IgG2a and IgG2b titers after both 3rd immunization and boosting. Antibody titers increased after the whole parasite boosting. When splenocytes were stimulated with the 19Kd fragment *in vitro*, the P1005 + RaLPS in water-in-oil emulsion group had the highest stimulation index among all groups at 4 days post parasite boosting. These results indicate that P1005 + RaLPS may be a good adjuvant to induce antibody responses against blood stage antigens of *P. vivax*.

324 ANALYSIS OF POTENCY OF THE SPf66 MALARIA VACCINE. Ballou WR*, Scheumann D, Kolodny N, Williams JA, and Gordon DM. Department of Immunology, WRAIR, Washington DC.

Immunogenicity data from a number of clinical trials of the malaria vaccine candidate SPf66 have shown significant variability in seroconversion rates not readily explained by differences in the vaccinated populations. We have established a potency assay in BALB/c mice in which the read out is antibody response following intraperitoneal immunization at various dilutions of vaccine. SPf66 can be shown to have a very steep dose response curve in this system which permits comparison among various vaccine formulations. We used this model to demonstrate that a low molecular weight (presumably monomeric) component purified from SPf66 peptide by size exclusion chromatography is essentially nonimmunogenic. This low molecular weight component binds poorly to aluminum hydroxide and makes up a significant proportion of SPf66. Variability in its content among clinical lots may have contributed to the divergent results obtained in human vaccine studies. Whether vaccine potency as measured by this assay in any way correlates with efficacy has not been established, but this system may be useful as a release assay to insure comparability among vaccine lots and to compare differences with new SPf66 formulations.

325 CHARACTERIZATION OF THE PEPTIDE POLYMER SPf66 MALARIA VACCINE. Ballou WR*, Kolodny N, Lees RA, and Hagopian RA. Department of Immunology, WRAIR, Washington DC; and Multiple Peptide Systems, Inc. San Diego, CA.

The malaria vaccine candidate SPf66 is being considered for large scale morbidity and mortality trials by the World Health Organization. If such studies are to be interpretable, it is critical that the product be well characterized, meet standardized release criteria, and perform in a consistent manner. SPf66 is produced by solid phase peptide synthesis at large scale using the "tea-bag" method. The product is synthesized with cysteines at both ends of the peptide which permit the linear peptide to form chains under oxidative conditions. The polymerized product is purified after cleavage from the resin by a simple dialysis or diafiltration procedure. The manufacturing process thus has the potential to produce peptide having substantial lot to lot variability. We have produced several lots of SPf66 under GMP specifically for the purpose of product characterization and establishing standardization criteria.

The methods of analysis include mass spectroscopy, reverse phase high performance liquid chromatography, size exclusion chromatgraphy, polyacrylamide gel electrophoresis, amino acid analysis, sequence analysis, assessment of free thiols, and determination of counter ions. These analyses indicate that SPf66 exists as a mixture of monomeric and multimeric species, but in our hands, the ratios of these species are remarkably consistent from lot to lot. Our data indicate that SPf66 can be reproducibly manufactured and provide a rational basis for developing reference standards for in-process and final product release criteria.

326 IMMUNITY TO MALARIA ELICITED BY HYBRID HEPATITIS B VIRUS SURFACE ANTIGEN PARTICLES CARRYING EPITOPES OF *PLASMODIUM FALCIPARUM*. Chen A*. First Military Medical University, Molecular Biology Institute, Guangzhou, P.R. China

The hepatitis B virus surface antigen was investigated as a carrier moiety for SPf66, a 45 amino-acid peptide having many epitopes of *Plasmodium falciparum*. Before we synthesized and expressed the gene encoding SPf66. In order to express the hybrid HBsAg lipoprotein particles, we inserted SPf66 gene into 31 end of HBsAg gene.The hybrid genes were expressed in yeast. The resulting hybrid HBsAg-SPf66 particles purified from yeast were displayed SPf66 and Hbs antigenicity, however the HBs antigenicity was reduced compared to native- recombinant HBsAg. Immunization of several mouse strains with HBsAg-SPf66 antibodies representing all murine immunoglobulin G isotopes. The possible influence of carrier-specific immunosuppression was examined, and preexisting immunity to HBsAg did not significantly affect the immunogenicity of the SPf66 within HBsAg-SPf66 particles. Similarly, the choice of adjuvant did not significantly alter the immunogenicity of HBsAg-SPf66 hybrid particles. Immunization in complete or incomplete Freund' s adjuvant resulted in equivalent anti-HBsAg and anti-SPf66 humoral responses. Most importantly, the sera of rabbit immunized with HBsAg-SPf66 particles markedly inhibited *in vitro* the growth of *Plasmodium falciparum* compared to the sera of rabbit immunized with SPf66. Therefore HBsAg particles may be a useful vehicle for the export and presentation of foreign peptide sequences.

327 RABBIT ANTI-PFS25 SERA, THAT BLOCKS PARASITE INFECTION IN 6-8 DAY OLD ANOPHELES MOSQUITOES, ONLY SUPPRESSES OOCYST DEVELOPMENT IN YOUNGER MOSQUITOES. Keister DB*, Muratova OV, and Kaslow DC. Malaria Vaccine Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.

The standard membrane feeding assay, in which immune sera and *Plasmodium falciparum* gametocytes derived from *in vitro* culture are fed to starved Anopheles mosquitoes resulting in suppression or blocking of oocyst development, is an accepted measurement of the transmission-blocking activity in immune sera. Although several parameters, such as gametocytemia and temperature, influence the density of oocysts that develop in control mosquitoes, the age of the mosquito (2-8 days old) was not considered to be an important variable. When immune rabbit serum was fed to 2-3 day old mosquitoes, the blocking activity normally seen in older mosquitoes was not observed. Oocysts developed to nearly the extent seen in mosquitoes fed on nonimmune rabbit serum. This phenomenon was not observed when feeding immune mouse or primate sera. When immune mouse sera were combined with an equal volume of immune rabbit sera and fed to mosquitoes, blocking ability was unaffected. These findings indicate that in some but not all circumstances the age of the mosquito used in the standard membrane feeding assay is an important variable.

328 CLONING OF THE FUSED DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE (DHFR-TS) GENE OF *PLASMODIUM FALCIPARUM* IN THE SHUTTLE VECTOR PYES2. Tsai YL*, and Krogstad DJ. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

We and others have attempted to clone the fused dihydrofolate reductase-thymidylate synthase (DHFR-TS) gene of *Plasmodium falciparum* as a single continuous sequence. Our initial efforts to clone the entire coding sequence (plus 5' and 3' untranslated flanking regions [UTFRs]) into plasmid vectors such as pcDNAII and pUC18 were unsuccessful because of rearrangements and deletions within the 5' coding sequence for DHFR. In the DHFR-TS gene plus ~2 kb of UTFR upstream (5') and ~2.3 kb of UTFR downstream (3'). This was accomplished using the shuttle vector pYES2 (which is larger [6 kb] than either pcDNAII or pUC18 [3 kb]), and the TOP10 F'*rec*A1 strain of *Escherichia coli*. We have recently used site-directed mutagenesis to introduce a new restriction site (that does not alter the amino acid sequence) to distinguish exogenous (transfected) from endogenous sequences in transfection experiments. That change (conversion of GGT to GGA at amino acid 166) creates a novel DNA sequence that does not occur naturally, a new *Bam*HI restriction site, does not change the Glycine at position 166, is relatively close to the point mutations knownto affect DHFR resistance (at positions 16, 51, 59, 108 and 164), and is well-separated from the 2.0-2.3 kb UTFRs at the 5' and 3' ends of the construct.

329 IMMUNOGENIC PROPERTIES OF THE N- AND C-TERMINAL REGIONS OF THE *PLASMODIUM VIVAX* MSP1 PROTEIN AFTER INJECTION OF DNA. Levitus GL*, Hoffman SL, and del Portillo HA. Departamento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, Brasil; and Malaria Program, Naval Medical Research Institute, Rockville, MD.

Nucleic acids are becoming an attractive alternative for vaccine development as opposed to synthetic peptides or recombinant proteins. *Plasmodium vivax* is the most widely distributed human malaria parasite and one of the two major species responsible for infections. Immunity in malaria however, is species-specific and thus is unlikely that an effective *P. falciparum* vaccine will protect against *P. vivax*. Accordingly, efforts should be made to develop a vaccine against this parasite species, *per se*. DNA fragments encoding the first most N-terminal 300 aa and the last most C-terminal 111 aa from the Merozoite Surface Protein 1 (PvMSP1) gene of the Belem strain were cloned into appropriate expression vectors. Balb/c mice were injected i.m. with 200 μg of these constructs dissolved in saline and boosted twice every three weeks. Analysis of the humoral immune response from these animals, both by ELISA and Western blot, revealed that these constructs are immunogenic and capable of inducing specific responses against PvMSP1-derived recombinant proteins. Mapping of these antibodies and analysis of the T-cell immune responses is currently under study. Due to the simplicity of this approach, plasmid constructs representing available coding sequences from *P. vivax* should facilitate the development of vaccine candidates for pilot monkey vaccination trials.

330 EST ANALYSIS AND ANTIGEN DISCOVERY FROM *BRUGIA MALAYI* L3 CDNA LIBRARIES. Scott AL*, Raghavan N, Ghosh I, Blaxter M, Lu W, and Williams SA. Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.; and Biology, Imperial College, London; Biological Sciences, Smith College, Northampton, MA.

Progress in the identification of filarial genes encoding antigens that are targets of a protective immune response has been slow due to a variety of parasitological and logistical problems. To overcome one of the major limitations, a lack of representative cDNA libraries from carefully staged infective larvae (L3), we have developed procedures that allow for the construction of cDNA libraries from very limited numbers of *Brugia malayi* L3s. The content of the two L3 libraries, SAW94SL-BmL3 (made in a conventional fashion from 2,000 third stage larvae) and JHU93SL-BmL3 (made using the linked reverse transcriptase-polymerase chain reaction and consisting of spliced leader (SL)-containing transcripts from 1,500 third stage larvae), were characterized by obtaining expressed sequence tags (EST) from randomly selected clones. Over 600 ESTs have been obtained from the two libraries. Comparison of the *B. malayi* L3 EST sequences with the sequences logged in the major public data bases, identified four major classes of parasite genes: (1) about 50% of the clones appear to be unique to the *B. malayi* data set, (2) about 25% show strong similarity to universal housekeeping genes, (3) about 20% appear to be homologues of genes previously identified in other species through functional analyses, (4) about 5% are *B. malayi* homologues of otherwise anonymous *C. elegans* ORFs and cDNAs which may constitute a group of nematode-specific genes. A number of the genes discovered through the EST analysis encode proteins of immediate immunological or drug-discovery interest. We suggest that such extensive, genome-wide approaches to analysis of larval gene expression are both a feasible and a valid strategy for the discovery of target molecules that can be used to control filariae.

331 MOLECULAR CLONING OF AN *ONCHOCERCA VOLVULUS* CYSTEINE PROTEASE ESSENTIAL FOR THE SUCCESSFUL MOLTING OF THIRD-STAGE LARVAE. Lustigman S*, Shah K, Huima T, Hough M, McKerrow JH, and Brotman B. Virology and Parasitology, The Lindsley F. Kimball Research Institute, New York, NY; Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia; Department of Pathology, University of California, San Francisco, CA.

Our previous studies have indicated the presence of a cysteine protease inhibitor, onchocystatin, in the cuticles of *Onchocerca volvulus* larvae that may be important to the development of the parasite. In this study we investigated the possible role of the endogenous onchocystatin and the cysteine protease it regulates in the molting process of L3 to L4. The effect of specific synthetic cysteine protease inhibitors on the survival of L3 and the molting of L3 to L4 was studied *in vitro*. L3 were cultured in the presence of increasing concentrations of different dipeptidyl-FMK substrate analogs that bind irreversibly to the active site of the enzyme. The viability of the larvae was assessed by MTT staining. None of the inhibitors reduced the viability of the larvae, however, all inhibitors reduced 50-100% of L3 molting at concentrations of 10-250 μM. The effect of the inhibitors on molting was critical during the first days in culture. Ultra structure analysis of the larvae that did not molt in the presence of the inhibitors indicated that the larvae had started the molt, but the separation between the cuticles was never complete. In addition, many larvae had an unusual swollen cuticles. A putative target cysteine protease was detected in soluble extracts and in the culture medium of molting larvae. We cloned the enzyme by PCR, the full length cDNA was expressed and an active enzyme was purified. Antibodies raised to the recombinant enzyme localized the protein around the areas where the separation between the cuticles of L3 and L4 occur. The

identification of a cysteine protease as an essential enzyme for molting process of *O. volvulus* L3 and the development of L4 and probably other stages, opens up important new avenues for drug development.

332 A THIOL-SPECIFIC ANTIOXIDANT FROM *BRUGIA MALAYI* L3'S. Ghosh I*, Raghavan N, and Scott AL. Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

To study gene expression during certain larval stages of filarial parasite development, one must overcome the problem of a shortage of parasite material. A feature of transcripts from all of the nematode species tested, is the presence of a conserved 22 nucleotide spliced leader (SL) sequence at the 5' end of a large proportion of mature messages. Using the SL sequence and the poly (A) tail as priming sites, PCR cDNAs were produced and used in the construction of a *Brugia malayi* SL cDNA library from late vector stage larvae (JHU93SL-BmL3). The library was characterized and used in an immunoscreen and in expressed sequence tag analysis. One clone from the expressed sequence tag analysis showed significant similarity to the family of thiol-specific antioxidants (TSA). The full length gene of the putative *B. malayi* TSA was sequenced and found to contain the SL sequence at the 5' end, a poly adenylation signal and the poly (A) tail. The 940 bp clone, designated BmTSA, had an open reading frame of 690 bp encoding for a protein of 230 amino acids (25 kDa). The deduced amino acid sequence of BmTSA was 60% identical to rat TSA and 58% identical to human TSA. BmTSA was found to be transcribed during the L2, L3, L4, adult and microfilaria stages. Antioxidants such as glutathione peroxidase and superoxide dismutase have been isolated from *Brugia*, however there is no report of TSA from nematode parasites. It will be interesting to see if this molecule has any role in the parasites evasion from the hosts immune system.

333 PROTECTIVE IMMUNITY IN ONCHOCERCIASIS: IDENTIFICATION OF NOVEL LARVAL ANTIGENS RECOGNIZED BY SERUM FROM INDIVIDUALS RESISTANT TO INFECTION. McCarthy JS*, Raghavan NK, Scott AL, Lu WH, Williams SA, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Johns Hopkins University, Baltimore MD; Smith College, Northampton MA.

Evidence from epidemiological studies in endemic regions and from experimental animal models suggests the existence of protective immunity in filarial infection. The paucity of parasite material, particularly from the L3 and L4 larval-stage has hampered identification of antigens which may be vaccine candidates. To identify such antigens, cDNA expression libraries were constructed from larvae of *Onchocerca volvulus* (OV) and from the related filarial parasite *Brugia malayi* (BM) using novel strategies that use small parasite numbers. These were screened using a serum pool from 10 individuals identified as being potentially immune to OV infection. Of the 12 unique recombinants identified in the BM libraries, 7 were found exclusively in the L3 stage and 2 in the L4, with 3 present in both. An additional 11 unique recombinants have been identified in the OV L3 library, 7 of which have homology to previously described proteins while 4 are unique. Homology to known proteins include previously identified filarial proteins, structural proteins and enzymes from other organisms and *C. elegans* gene products of unknown function. While preliminary counter-screening of these recombinant antigens suggests that many are recognized by pools from infected subjects, and therefore likely to be common antigens recognized by all individuals exposed to the parasite, 3 appear to be preferentially recognized by the immune serum pool. As recombinants uniquely recognized by individuals immune to infection are of particular interest, the characterization of the pattern of recognition by individual sera from both infected and immune groups is underway.

334 A POTENTIALLY PROTECTIVE *ONCHOCERCA VOLVULUS* ANTIGEN IS A NEURONAL PROTEIN. Erttmann KD*, BÅttner DW, and Gallin MY. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany.

In order to identify antigens of *Onchocerca volvulus* involved in protection we screened a cDNA expression library of adult *O. volvulus* with sera from putatively immune individuals. One full-length clone of 2043 bp (E1) encoding a protein of 462 amino acids (aa) was further analyzed. The predicted aa sequence indicates that this protein is most closely relatedto a human brain protein. No homology to known proteins of parasitic helminths was found. The recombinant non-fusion protein was used to generate antibodies in rabbits. By immunohistology the protein was localized in *O. volvulus* adults, microfilariae and infective larvae. In adult worms a distinct staining pattern of neuronal structures such as neuronal cell bodies, the nerve ring as well as the extracellular clefts was observed. The results identify a novel *O. volvulus* protein associated with the nervous system and suggest that neuronal proteins may represent important targets for protective immunity against *O. volvulus in vivo*.

335 A *DIROFILARIA IMMITIS* L3 CDNA CLONE WITH HOMOLOGY TO A VESPID VENOM ALLERGEN ANTIGEN 5. Tripp CA*, Mika-Grieve M, Frank GR, Rushlow MB, and Grieve RB. Paravax, Inc., Fort Collins, CO.

Vaccine development against parasitic helminth infections is complicated by the parasite life cycle which includes a variety of life forms, each presenting different targets, and challenges for immunization. In addition, the administration of whole killed parasites or parasite-derived antigens can produce an antibody response which, although quantitatively significant, is not sufficient to protect the animal against infection. It has been shown that dogs were protected from challenge infection with *Dirofilaria immitis* larvae by administration of chemically abbreviated larval infections and that the protective effect was passively transferred with the immune dog sera. Immunoblots of native *Dirofilaria* larval proteins have been analyzed to identify molecules uniquely recognized by the immune dog sera. In addition, third and fourth stage larval cDNA expression libraries were made to target potential vaccine candidate molecules. A full length clone (DiVA) was isolated from the 48 hour third stage (L3) larval cDNA library. The clone is 833 nucleotides and the deduced amino acid sequence shares about 25% homology to venom allergen antigen 5 described in Hymenopteran insects. DiVA comprises a mature polypeptide of 205 amino acids preceded by a 16 amino acid putative signal peptide. Characterization of the life stage specificity, size of the native protein and cellular localization are in progress.

336 CLONING AND CHARACTERIZATION OF ECDYSONE RECEPTOR AND *ULTRASPIRICLE* HOMOLOGS FROM A FILARIAL PARASITE, *DIROFILARIA IMMITIS*. Shea C*, Richer J, Hough DM, and Maina CV. Molecular Parasitology Group, New England Biolabs, Inc., Beverly, MA.

Dirofilaria immitis, the causative agent of dog heartworm disease, is a model system for the study of filarial parasites. Filariasis afflicts over 300 million people worldwide, causing serious and debilitating disease. Knowledge of the molecular mechanisms involved in parasite development will facilitate the design of therapeutic agents. Nematodes such as*D. immitis* undergo a series of larval molts before becoming sexually mature adults. The steroid hormone ecdysone is present in *D. immitis* and it is capable of stimulating molting in the parasite *in vitro*. This suggests that ecdysone has a role in regulating molting and possibly other developmental processes in filaria. In order to understand these processes, we have identified a *D. immitis* ecdysone receptor (EcR) homolog, dinhr-3, by PCR using degenerate primers derived from the sequence of the DNA binding domain of the Drosophila *EcR*. A dinhr-3 cDNA was also isolated. Compared with the Drosophila *EcR*, dinhr-3 shows 79% amino acid identity in the EcR DNA binding domain and 59% amino acid identity in the hormone binding domain. In Drosophila, EcR heterodimerizes with *ultraspiricle (usp)*, an RXR family member. A putative *usp* homolog from *D. immitis*, *dinhr-4* has also been identified. In addition we have isolated 2 cDNAs from *D. immitis* and identified 6 genes from *C. elegans* which show significant sequence similarity with members of the nuclear hormone receptor (NHR) superfamily. Further molecular characterization of *dinhr-3*, *dinhr-4* and the other NHR superfamily members is currently underway.

337 PRACTICAL AND THEORETICAL IMPLICATIONS OF A REPEATED DNA SEQUENCE OF *WUCHERERIA BANCROFTI*. Hamburger J*, Abassi I, Ramzy R, Gad A, Anjilli C, Mbogo C, Agure R, Ochola J, Githure J, and Williams SA. Hebrew University, Jerusalem, Israel; Ain Shams University, Cairo, Egypt; Kenya Medical Research Institute, Kenya; and Smith College, Northampton, MA.

For facilitating the determination of the prevalence of infection by *Wuchereria bancrofti* (*Wb*) in both mosquito vectors and patients we have developed DNA-based identification procedures. We cloned a *Wb* repeated DNA sequece (*Wb19*) and demonstrated that it is species-specific and that PCR with primers derived from it can detect one infected mosquito within a large mosquito batch. We also developed a simple method for preparing DNA from infected mosquitos by treatment with NaOH followed by neutralization, heat treatment, and alcohol precipitation. In humans, peripheral *Wb* microfilaremia is generally nocturnal, and in cases with clinical symptoms may be absent altogether. Thus, diagnosis by detecting peripheral microfilaremia may be problematic. Since part of the clearance of the microfilariae is likely to occur through the lungs, we attempted to identify filarial DNA in pulmonary exudates. Preliminary results suggest that this approach may prove useful for a non-invasive, daytime detection of parasites even in cases where peripheral microfilariemia is absent. The cloned repeated sequence (*Wb19*) is dispersed. It demonstrates GC and AT rich regions and its analysis suggests the presence of Scaffold Associated Repeats (SARs) or Matrix Associated Repeats (MARs) which are known to bind to the nuclear matrix (scaffolding) proteins of eukaryotes. Since SARs are often close to promoters and cohabit with upstream regulatory sequences the study of flanking sequences is expected to yield interesting information on the *Wb* genome.

338 FURTHER CHARACTERIZATION OF THE FILARICIDE UMF-078, A BENZIMIDAZOLE CARBAMATE, AGAINST LYMPHATIC INFECTIONS OF *BRUGIA PAHANGI* IN DOGS. Dzimianski MT*, McCall JW, Supakorndej P, and Jun JJ. Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, GA.

The preclinical development of the benzimidazole carbamate UMF-078 by the World Health Organization was continued in a trial that evaluated 1) selected oral (PO) and intramuscular (IM) dosages of the drug against filariae, 2) the effect of fasting prior to PO administration, and 3) the effect of splitting the dose of IM administered drug into 2 sites. Forty microfilaremic beagles infected 144 days earlier with 200 infective larvae (100 in each hind paw) were selected and randomly allocated to 10 groupsof 4 dogs each. Dogs in 4 groups were given UMF-078 PO postprandially for 3 days as follows: 1) 100 mg/kg/day, 2) 50 mg/kg bid, 3) 25 mg/kg bid, and 4) 12.5 mg/kg bid. Dogs in 1 group were fasted before PO treatment at 25mg/kg bid x 3 days. Dogs in 3 groups were given deep IM injections of the drug into 1 site in the dorsal lumbar muscles as follows: 1) 50 mg/kg single dose, 2) 25 mg/kg single dose, and 3) 25 mg/kg/day x 2 days. Dogs in one group were given a single dose of 25 mg/kg that was equally divided and injected into 2 sites in the dorsal lumbar muscles. The remaining group of dogs served as a control. The dogs were bled for microfilarial counts prior to treatment and on days 7, 14, 28, 42, 56, 84, 112, 140, and 168 after treatment started. All of the dogs were necropsied between168 and 170 days after treatment began. The number of adult *B. pahangi* recovered from the control dogs ranged from 2 to 48 with anaverage of 20.5 worms per dog. A dosage of 50 mg/kg bid x 3 days given PO was 100% effective against macrofilariae of *B. pahangi*. Moderate reductions (71-94%) in the number of macrofilariae occurred in all of the other dogs treated with UMF-078 except for dogs that received a single IM dose at 25 mg/kg given in 1 site where the drug was ineffective. Macrofilaricidal efficacy of the drug appeared to be enhanced by fasting prior to PO dosing and by splitting the IM administered dose into 2 sites. The only dosage of UMF-078 that was microfilaricidal was 50 mg/kg bid x 3days given PO (95% reduction in counts from day 14 through day 168).

339 MOSQUITO CELLS, GENES, AND TRANSFORMATION SYSTEMS. Fallon AM*. Department of Entomology, University of Minnesota, St. Paul, MN.

Using cell lines from the mosquitoes *Aedes albopictus* and *Aedes aegypti*, we have developed transformation systems and have characterized a variety of genes that provide tools for genetic disruption of disease transmission in vector mosquitoes. The biological framework for our research is the reproductive physiology initiated in the female mosquito by blood-feeding. Mosquito vectors acquire a pathogen in an initial blood meal, and transmit the pathogen in subsequent feedings. Disruption of this cycle, such that those mosquitoes bearing pathogens fail to take a second blood meal, provides a means of interrupting disease transmission. In transformed *Drosophila*, antisense expression of a ribosomal protein gene has already been shown to disrupt reproduction, resulting in a small-egg, female-sterile phenotype. We have characterized ribosomal protein and rRNA genes from *Ae. aegypti* and *Ae. albopictus*, and have used standard molecular techniques to define promoter elements that regulate expression of these genes. Parallel development of gene transfer technologies for mosquito cells, using a selectable mosquito dihydrofolate reductase gene that confers methotrexate resistance, facilitated analysis of core promoter regions and the involvement of introns in the regulation of the rpL8 gene. Ribosomal protein genes provide useful, constitutive promoters for transcription of recombinant gene products by RNA polymerase II. Similarly, the rRNA promoter elements are recognized by RNA polymerase I. We anticipate that these cloned genes and their promoters will be generally useful in basic and applied studies leading to transgenic manipulation of vector mosquitoes.

340 GUT-SPECIFIC TRANSCRIPTIONAL REGULATORY ELEMENTS ARE CONSERVED BETWEEN BLACK FLIES AND *DROSOPHILA*. Xiong B, and Jacobs-Lorena M*. Case Western Reserve University, School of Medicine, Department of Genetics, Cleveland, OH.

Germ line transformation of disease vectors, including mosquitoes, is presently an area of intense research. Introduction into disease vectors of genes that hinder vectorial capacity is a possible strategy for disease control. The midgut of blood-sucking insects plays a central role in the development of parasites within their insect hosts. Therefore, the ability to promote the secretion of a transgenic product into the gut lumen constitutes a potentially important measure for disease control. However, promoters to drive such transgenes are not yet available. Previously, we isolated from the black fly *Simulium vittatum* a gut-specific gene encoding a digestive carboxypeptidase. To investigate whether promoter elements that direct gut-specific expression might be conserved in evolution, we have placed the putative promoter in front of a ß-glucuronidase (GUS) reporter and transformed the construct into *Drosophila*. We find that the hybrid gene is expressed strongly and with the correct tissue specificity in the transgenic flies. This gut-specific black fly carboxypeptidase promoter provides a valuable tool for the study of disease vectors.

341 CHARACTERIZATION OF PHENOLOXIDASE IN HEMOLYMPH AND MIDGUT OF ADULT *ANOPHELES STEPHENSI* MOSQUITOES. Sidjanski S*, Mathews GV, and Vanderberg JP. Department of Medical & Molecular Parasitology, New York University School of Medicine, New York, NY.

Some *Anopheles* mosquitoes may respond to invading malaria parasites with a rejection mechanism consisting of melanization of ookinetes that have entered the mosquito midgut epithelium. Melanization requires two key enzyme activities: monophenoloxidase activity (MPO), which hydroxylates a substrate such as tyrosine, and diphenoloxidase activity (DPO), which oxidizes this product to a quinone and leads to melanin formation. Whether melanization within the midgut epithelium is due to endogenous midgut enzymes or exogenous enzymes that have moved into the midgut epithelium from the hemolymph is not clear. We have attempted to separate and characterize phenoloxidase enzymes from midgut and hemolymph of *An. stephensi* by means of non-denaturing gel electrophoresis and zymography. The results show that the enzymes from the two mosquito sites have distinctively different electrophoretic characteristics. Phenoloxidase from two tisses are bifunctional enzymes. It should be possible to apply this approach to malaria-susceptible vs. malaria-refractory strains of *An. gambiae*. Future studies on phenoloxidase-mediated rejection of ookinetes may have more physiological significance if done with enzymes from the midgut epithelium rather than from the hemolymph.

342 BIOCHEMICAL PATHWAY OF CHORION HARDENING IN *AEDES AEGYPTI*. Li JY*, Hodgeman BA, and Christensen BM. Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI.

Newly oviposited mosquito eggs are white and soft, but the egg chorion becomes black and rigid in <4 hr after oviposition. Most chorion proteins are solubilized if the newly oviposited eggs are treated with SDS/urea in neutral buffer. In contrast, the black chorion is totally insoluble in SDS/urea. Because of these dramatic changes, the hardening reactions of mosquito chorion were considered to be phenol oxidase-mediated melanization reactions. Results from our recent studies, using SDS-PAGE with tropolone and H_2O_2 staining or dopa staining, revealed the presence of both peroxidase and phenol oxidase in solubilized chorion protein (SCP). Rapid accumulation of dityrosine was observed when the SCP was mixed with tyrosine in the presence of H_2O_2. Acid hydrolysis (6 M HCl and 110°C) of black egg chorion also resulted in the detection of dityrosine in the hydrolysate, which provides convincing evidence for protein crosslinking through tyrosine residues among the chorion proteins. Little dityrosine was produced in a SCP/tyrosine reaction mixture in the absence of H_2O_2, but incorporation of NADPH into the SCP/tyrosine reaction mixture resulted in a greater accumulation of dityrosine during incubation. Therefore, H_2O_2 required for peroxidase catalyzed protein crosslinking reactions *in vivo* likely is supplied by NAD(P)H-dependent reactions, in which oxygen is reduced to H_2O_2 at the expense of NAD(P)H. These data, in conjunction with our previous experimental results, indicate that the hardening process of the mosquito egg chorion includes both phenol oxidase catalyzed melanization reactions and peroxidase mediated chorion protein crosslinking through tyrosine residues. Because a hardened egg chorion is critical for egg survival, understanding the specific biochemical mechanisms involved this aspect of mosquito reproduction could provide new avenues for control through the disruption of these processes.

343 DOPA DECARBOXYLASE EXPRESSION AND REGULATION: EVAULATION WITH RESPECT TO DIVERSE BIOLOGICAL FUNCTION IN *AEDES AEGYPTI*. Ferdig MT*, Li JY, Severson DW, and Christensen BM. Department of AHABS, University of Wisconsin-Madison, Madison, WI.

Insects utilize dopa decarboxylase (DDC) for egg development, cuticle tanning, neurotransmission and defense against pathogens. Because these events, essential to an insect's maintenance of self, all require transcription of the same gene, but in unique temporal and spatial ways, we have directed our focus towards understanding DDC expression and regulation. In mosquitoes, egg development and oviposition are delayed in females undergoing a melanotic defense response against parasites. This effect is due, at least partly, to the overlapping requirements for the catecholamine pathway substrates and products. DDC enzyme activity is elevated in the hemolymph upon ingestion of a *Brugia malayi*-infected bloodmeal in *Armigeres subalbatus*. The levels also are increased in the ovaries of *Aedes aegypti* mosquitoes during egg development induced by ingestion of a bloodmeal, and the increased activity is a result of transcription of the DDC gene in the ovaries. Through the cloning and sequencing of the DDC gene, and in the context of known mechanisms of DDC regulation and expression in Drosophila, we have used analyses of tissue and timing of expression, and of 5' promoter sequence to identify specific DDC regulatory features that direct transcription according to the specific biological event requiring that activity.

344 IDENTIFICATION AND TEMPORAL EXPRESSION OF THREE ANTIBACTERIAL DEFENSINS PRODUCED BY *AEDES AEGYPTI* IN RESPONSE TO BACTERIAL CHALLENGE. Lowenberger CA*, Ferdig MT, Bulet P,

Hetru C, Hoffmann JA, and Christensen BM. AHABS, University of Wisconsin, Madison WI; and IBMC, Strasbourg, France.

The elucidation of mechanisms that determine the compatibility/incompatibility between vectors and the parasites they transmit has been the focus of much recent research. Mosquitoes respond to bacterial challenge or injury by the rapid production of potent bactericidal and bacteriostatic compounds that have a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. We have identified three novel defensins that differ by only 1-2 amino acids, and are produced in extremely high levels (>100 μg/g insect). Primers designed using this amino acid sequence data were used in standard PCR techniques to amplify and clone defensins from *Aedes aegypti* genomic DNA. We have used these clones to generate probes for use in Northern analysis of mosquito RNA at different times after bacterial inoculation. Defensin mRNA transcripts are largely confined to the fat body where expression occurs 15-30 minutes after inoculation, and defensin levels in the hemolymph peak 24 hours after inoculation. The rapid production of defensins and other, yet unidentified, peptides undoubtedly plays a role in mosquito defenses against prokaryotic organisms. We are currently assessing the potential role defensins play in the defense response of several strains and species of mosquitoes to eukaryotic organisms such as *Plasmodium* sp. and filarial worms.

345 EXPRESSION OF CECROPIN A. AN ANTI-TRYPANOSOMAL PEPTIDE, IN ENDOSYMBIOTIC BACTERIA OF *RHODNIUS PROLIXUS*. Durvasula RV*, Aksoy A, Beard CB, and Richards FF. Department of Internal Medicine, Yale University School of Medicine, New Haven, CT; Centers for Disease Control, Atlanta, GA.

The strategy of expressing foreign genes in arthropod vectors to limit spread of parasitic diseases has been applied to the system responsible for Chagas' Disease: the vector, *Rhodnius prolixus*, the parasite, *Trypanosoma cruzi*, and *Rhodococcus rhodnii*, a streptomycete endosymbiont of *R. prolixus*. A streptomycete-derived shuttle plasmid, pRr 4.1/22, containing the origin of replication of *E. Coli* and *R. rhodnii*, a thiostrepton resistance gene, and a gene encoding for mature cecropin A was constructed. Cecropin A is a 38 amino acid peptide with cidal activity against gram negative organisms and *T. cruzi*, but little activity against *R. rhodnii*. Wild type *R. rhodnii* isolated from the Colombia strain of *R. prolixus* were transformed with the shuttle plasmid and grown under thiostrepton selection. *In vitro* expression of cecropin A by the transformants was demonstrated using Western Blot. Cell-free protein extracts of the transformants exhibited cidal activity against *E. coli* and inhibitory activity on *T. cruzi*. Similar effects were not observed with extracts of wild-type bacteria. Newly emerged aposymbiotic *R. prolixus* which were fed transformants with their first blood meal successfully reached the adult stage. Assays of the fecal contents of these insects, performed at monthly intervals, confirmed the *in vivo* production of cecropin A. Effects of the gene product on transmission of *T. cruzi* will be presented.

346 AMPLIFICATION OF *PLASMODIUM FALCIPARUM* DNA FROM SPOROZOITE-INFECTED ANOPHELINES. Toure YT*, Koita O, Doumbo O, Cogswell FB, Kaslow DB, Keister DB, Beier JC, and Krogstad DJ. National School of Medicine and Pharmacy, Bamako, Mali; National Institutes of Health, Bethesda, MD; and Tulane University, Covington and New Orleans, LA.

Our previous studies used PCR to amplify polymorphic sequences in the haploid genome of asexual (blood stage) parasites to examine the poly-clonality of natural *P. falciparum* infection. The purpose of these studies was to adapt this technique to sporozoites in the mosquito. Because only 1-7% of anophelines are infected with *P. falciparum* in Mali, the initial problem was how to preserve the head/thorax (with the salivary glands) while screening the abdomen for infection using an ELISA for CSP. In these preliminary studies, we found that fresh mosquitoes kept on moist filter paper for 4 hours (to permit ELISA testing for CSP) can be dissected readily to isolate the salivary glands. Although freezing made the dissection much more difficult, we used frozen *Anopheles freeborni* mosquitoes infected with the NF54 (3D7) strain of *P. falciparum* to amplify the polymorphic region of Block 2 in MSP-1. After homogenization with a micro-pestle, extraction with Chelex-100, and amplification, gel electrophoresis revealed a common 264 bp K1-type MSP-1 fragment from both salivary gland sporozoites and asexual stage parasites. Using this strategy, it should now be possible to follow the parasite through its life cycle under conditions of natural transmission using PCR markers for polymorphic regions of the parasite genome.

347 INEFFICIENT SPOROGONIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN THE VECTOR *ANOPHELES GAMBIAE* IN MALI, WEST AFRICA. Vernick KD*, and Toure YT. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; and Ecole Nationale de Medecine et de Pharmacie, Bamako, Mali.

We have developed a quantitative assay for the presence and number of *Plasmodium falciparum* parasites in mosquitoes based on detection of sporozoite-specific 18S ribosomal RNA molecules using competitive reverse transcriptase-polymerase chain reaction (RT-PCR). The assay is species-specific and is sensitive to less than 0.1

sporozoite. We used the RT-PCR assay to examine the efficiency of sporogonic development of *P. falciparum* in *A. gambiae* which were infected by feeding on volunteer gametocyte carriers in Mali. We observed a block to complete sporogony in a high frequency of mosquitoes in which parasite development appeared to be arrested at a point after the establishment of a midgut infection but before sporozoites invaded the salivary glands. This inefficiency of parasite development in a natural transmission system may represent the operation of a previously undescribed naturally-occurring refractory mechanism in *A. gambiae*.

348 REGULAR PRODUCTION OF INFECTIVE MALARIA SPOROZOITE IN COLONIZED *ANOPHELES ALBIMANUS* MOSQUITOES. Hurtado S, Salas ML, Romero JF, Ortiz H, Herrera S, and Herrera MA*. Instituto de Inmunologia del Valle, Universidad del Valle, Cali, Colombia.

The availability of human malaria sporozoites is crucial for the study of the biology of this parasite stage as well as for the analysis of the immune response induced both by natural exposure to the parasite or by experimental immunization. The goal of the present study was to develop the complete life cycle of both *Plasmodium falciparum* and *P. vivax* under laboratory conditions, as well as to establish a regular production of infective sporozoites. For this purpose three *Anopheles albimanus* strains previously colonized were exposed to either blood from malaria infected patients or to experimentally infected *Aotus* monkeys. Asexual stages of the *P. vivax* VCC-2 isolate from Colombia and of the *P. falciparum* Santa Lucia isolate from El Salvador were used to infect *Aotus* monkeys. The *An. albimanus* Tecojate and Cartagena strains showed to be susceptible to *P. vivax* infection and produced infective sporozoites whereas the *An. albimanus* Buenaventura strain demonstrated to be susceptible to infection by *P. falciparum*. Mosquitoes fed on human blood containing *P. vivax* gametocytes induced better infections than those exposed to monkey blood. *P. vivax* was better transmitted than *P. falciparum*, using human samples. *Aotus* monkeys intravenously injected with *P. vivax* sporozoites allowed as to mantain the whole parasite cycle. The possibility of reproducibly transmitting *P. vivax* parasites through a system comprising human-mosquito-monkey, indicate the great potential of this model as a parasite source for both biological studies and to test the efficacy of malaria vaccine candidates.

349 EVIDENCE FOR Fe(IV)=O IN THE MOLECULAR MECHANISM OF ACTION OF THE TRIOXANE ANTIMALARIAL ARTEMISININ. Posner GH*, Cumming JN, Ploypradith P, and Oh CH. Department of Chemistry, The Johns Hopkins University, Baltimore, MD, USA; and Department of Chemistry, Inje University, Kimhae, Korea.

We have recently shown that carbon-centered radicals are key intermediates in the molecular mechanism of action of the trioxane antimalarial artemisinin. Now we report further evidence about this molecular mechanism of antimalarial action showing that the carbon-centered radical intermediate leads to a strongly alkylating epoxide intermediate and to a high-valent iron-oxo intermediate; three reactions characteristic of such iron species (*i.e.*; rearrangement of hexamethylDewar-benzene into hexamethylbenzene, oxidation of tetralin into hydroxytetralin, and oxygen atom transfer oxidation of methyl phenyl sulfide into methyl phenyl sulfoxide) constitute the first evidence of such iron species being formed during ferrous ion activation of artemisinin. This is the first report that such high-valent iron-oxo species can be formed from an endoperoxide (dialkyl peroxide) rather than, as usual, from hydrogen peroxide, an organic hydroperoxide, or molecular oxygen. The implications of these new mechanistic findings will be discussed in the context of designing better chemotherapeutic trioxanes in the worldwide fight against malaria.

350 MECHANISM OF CARDIOTOXICITY OF HALOFANTRINE. Wesche DL*, Chen Y, Wang W, Schuster BG, and Woosley RL. Division of Clinical Pharmacology, Department of Pharmacology, Georgetown University Medical Center, Washington, DC; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Halofantrine HCl is a phenanthrenemethanol which has been shown to be associated with electrocardiographic prolongation of QT interval. Several cases of multiform ventricular tachycardia (torsades de pointes) have been reported. The arrhythmogenic potential and the effect of halofantrine or its metabolite on repolarization have not been systematically examined. We have investigated the effects of (±)-halofantrine and *N*-desbutylhalofantrine on cardiac repolarization in the isolated perfused cat heart. In addition, we compared the effects of (±)-halofantrine, (+)-halofantrine, and (-)-halofantrine on the delayed rectifier potassium channel (I_K) in isolated feline ventricular myocytes using the whole cell patch clamp technique. Results indicate that racemic halofantrine (10 μM) prolongs cardiac repolarization in the isolated perfused cat heart, whereas *N*-desbutylhalofantrine has minimal effect on QT interval at the same concentration. Racemic halofantrine blocks the delayed rectifier potassium channel in isolated feline myocytes, indicating that this drug is similar to Class III antiarrhythmics and quinidine, all of which cause torsades de pointes. In the same preparation, (±)-halofantrine and (+)-halofantrine at a concentration of 10 μM had

an approximately two-fold greater propensity ($p < 0.05$) to block I_K than did (-)-halofantrine at the same concentration, indicating that halofantrine's cardiotoxic effect is stereoselective. These results indicate that halofantrine and not its metabolite is responsible for QT prolongation and torsades de pointes. We conclude that dosage regimens which lead to high plasma concentrations of halofantrine and/or (+)-halofantrine should be avoided. Further studies are indicated to determine conditions in which the stereoselective metabolism of halofantrine to *N*-desbutylhalofantrine may be restricted.

351 A *PLASMODIUM FALCIPARUM* BIOASSAY FOR THE MEASUREMENT OF TOTAL ANTIMALARIAL ACTIVITY OF HALOFANTRINE AND ITS METABOLITE(S) IN PLASMA. Taamasri P, Kyle DE*, Schuster BG, and Wesche DL. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Phramongkutklao College of Medicine, Bangkok, Thailand.

Halofantrine is a phenanthrenemethanol drug that is approved for the treatment of multi-drug resistant malaria and is being evaluated at WRAIR for use as a suppressive prophylactic agent. In support of the development process for the prophylactic indication, we have developed a *Plasmodium falciparum* bioassay for the measurement of the total antimalarial activity of halofantrine and its metabolite(s) in human plasma. The assay is a modification of the semi-automated microdilution drug susceptibility test and is similar to a recently developed bioassay for artemisinin derivatives. Briefly, the plasma from individuals given halofantrine is treated with Sepharose-Protein A conjugate to remove antibody that may inhibit growth of the parasite. The plasma is then added to duplicate wells of a microtiter plate and serially diluted (2-fold) with control plasma. In each experiment a standard curve is produced from a series of plasma samples that are spiked with known concentrations of halofantrine or desbutylhalofantrine (the principal human metabolite). Synchronous cultures of the W-2 *P. falciparum* clone (>85% rings) are added to the microtiter plate and incubated for 24 hrs. before ^{3}H-hypoxanthine is added; all plates are harvested at 42 hrs. Data are analyzed in a non-linear regression model and antimalarial activity in unknown samples is determined from equivalent activity due to halofantrine in the standard curve for that experiment. In a typical standard curve, the coefficient of variation was <10% for a range of concentrations from 43 - 1388 ng/ml of halofantrine. This validated bioassay is now being used to analyze plasma samples from a Phase I clinical study to determine the feasibility of weekly dosing for prophylaxis with halofantrine and to determine pharmacokinetic-pharmacodynamic relationships.

352 PHARMACOKINETIC (PK) AND PHARMACODYNAMIC (PD) OF A SINGLE DOSE OF ARTEMETHER (AM) BY THREE ROUTES OF ADMINISTRATION IN HEALTHY CAUCASIAN VOLUNTEERS. Teja-Isavadharm P*, White NJ, Brewer TG, Peggins JO, Nosten F, and Kyle DE. Department of Immunology & Parasitology, Armed Forces Research Institute of Medical Sciences, Bangkok,Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; and Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford.

The pharmacokinetics of a single dose of artemether (5 mg/kg) following oral (P.O.), intra-muscular (I.M.) and intra-rectal (I.R.) routes were compared in a cross-over study in 6 healthy Caucasian volunteers. Simultaneous measurement of artemether (AM) and its metabolite dihydroartemisinin (DHART) in plasma, were performed by high-performance liquid chromatography with electrochemical detection. Plasma antimalarial activity against *Plasmodium falciparum* was measured by bioassay *in vitro* in duplicate, and expressed as DHART equivalents. The pharmacokinetic parameter estimates were derived using non-compartmental analysis of fitted concentration-time and effect-time curves. The pharmacokinetic parameter estimates for all 3 routes of administration are shown below (Tables). The concentration-antimalarial effect relationship for P.O. andI.M. routes after AM normalized to DHART equivalents revealed clockwise hysteresis loops, while the levels for the I.R. route were too low to obtain useful information. When the loops were collasped by assuming linear models, the slopes of the best fitted lines were 0.8580, 0.6764 and 0.6668 with the r2 of 0.8286, 0.6422 and 0.5677 for P.O., I.M. and I.R. routes respectively. These data show that artemether is rapidly absorbed and eliminated, and extensively metabolized to DHART after P.O. administration compared to both I.M. and I.R. administration. The AUC(012) value of artemether antimalarial effect following I.R. administration was slightly less than that of I.M. administration (92%); and, both values were less than half of the value following P.O. administration (41% and45% respectively). Conclusions: 1) for artemether, simultaneous drug/metabolite pharmacokinetics and effect kinetic (pharmacodynamics) analyses are now possible; 2) these data reveal striking differences in single dose antimalarial effect kinetics related to route of administration; 3) the rectal route of administration deserves further study as a potential alternative to parental artemether treatment in the rural tropics.

353 THE EFFECTS OF ARTEMISININ DERIVATIVES ON MALARIA TRANSMISSABILITY. Price R*, Nosten F, Luxemburger T, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University Bangkok, Thailand.

Between 1990 and 1991, 1310 patients were enrolled into 13 different antimalarial drug studies at the SMRU and completed their course of treatment. Antimalarial drugs studied included mefloquine, halofantrine, quinine and the qinghaosu derivatives artesunate and artemether. Gametocytes were detected in 195 (6%) of patients on admission with no significant difference between treatment groups. Subsequent gametocyte carriage was assessed by person week gametocytes (number of weeks in which blood slides were positive divided by the total number of weeks followed up). The gametocyte carrier rates were positively correlated with the time taken to clear the asexual parasites from the blood (X^2 for trend $p < 0.0001$). Further those patients who went on to recrudesce by day 28 were at greater risk for developing a patent gametocytaemia during follow up (RR-4.6[95% CI 3.3-6.5]). Following mefloquine treatment gametocyte carriage was considerably higher than following treatment with an artemisinin derivative. The relative risks (RR) for gametocyte carriage was 7.1 [95% CI 4.0-12.7], ($p<0.001$). Gametocyte carriage following mefloquine or halofantrine treatment was similar. Although gametocyte carriage was closely related to treatment efficacy this did not account for all of the differences between the antimalarial drugs. Patients who were succesfully treated by day 28 still had a significantly less risk of gametocyte carriage following treatment with artemisinin than following mefloquine treatments ($p<0.0001$, RR 6.5[95% CI3-14.2]) or halofantrine (p 0.001,RR 4.7[95% CI 1.9- 11.9]).

354 ARTEMETHER EFFICACY IN THE CEREBRAL MALARIA MODEL: *PLASMODIUM COATNEYI* IN THE RHESUS MONKEY. Todd GD*, Morris CL, Sullivan JJ, Aikawa M, and Collins WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention. Atlanta, GA; and Institute of Medical Research, Tokai University. Boseidai, Isehara, Japan.

Plasmodium coatneyi in the rhesus monkey exhibits important facets of cerebral malaria such that has become an effective model system. Artemether and other artemisinin derivatives are known to have a rapid efficacy against *P. falciparum* in humans. Using this cerebral malaria model, we closely examined the time course, extent and molecular aspects of artemether efficacy against *P. coatneyi*. Four splenectomized rhesus monkeys were inoculated with 1.37×10^6 parasites on day 0. Parasitemia was followed by examination of Giemsa-stained blood films. At day 10, parasite counts ranged from 200,000 to 536,000 per µl. A 3-day treatment regimen with artemether (3.2 mg/kg injected intramuscularly) was initiated with twice daily blood films and collections for drug level assessment. Parasite counts for the 4 monkeys decreased at 24 hours by 67.7%; between 24 and 48 hours, parasite counts decreased by 93.4%. By 72 hours, parasites were no longer detected in 2 monkeys; all animals were free of parasites by 96 hours. Artemether at the dosage given was found to be extremely rapid and effective in decreasing parasitemia due to *P. coatneyi* in these rhesus monkeys. On day 14, animals were euthanized and tissue samples from heart, lung, kidney, pancreas, eleum, skin and major brain areas were obtained for light and electron microscopy and drug deposition. Artemether levels and microscopic results will be presented.

355 AZITHROMYCIN COMPARED TO DOXYCYCLINE FOR MALARIA CHEMOPROPHYLAXIS IN WESTERN KENYA. Andersen SL*, Oloo AJ, Gordon DM, Klotz FW, Aleman GM, Ogutu BO, Koech DK, Berman JD, and Shanks GD. US Army Medical Research Unit-Kenya; Kenya Medical Research Institute, Nairobi, Kenya; and Walter Reed Army Institute of Research, Washington, DC.

There are few chemoprophylactic drugs for use when traveling to endemic areas of chloroquine-resistant malaria. Doxycycline has been used for this purpose, but it requires daily administration and cannot be given to young children and pregnant women. Azithromycin, an azalide antibiotic, has been shown to prevent malaria parasitemia in human challenge trials when 250 mg daily is given and then continued for four weeks following infective mosquito bites. We will report preliminary results from a placebo-controlled, randomized, double-blinded field trial currently in progress in a semi-immune population in an area of intense malaria transmission in Western Kenya. Four different regimens are being tested: azithromycin 250 mg daily, azithromycin 1000 mg weekly, doxycycline 100 mg daily, placebo. 250 adult Kenyan volunteers of either sex were enrolled. Volunteers were given a 7 day course of quinine and doxycycline in order to clear pre-existing parasitemia. Medication is to be continued for 10 weeks. Thick films will be examined each week for *Plasmodium* parasitemia. At the time of abstract preparation, the randomization code had not been broken. The implications of the use of azithromycin as a malaria preventive measure will be discussed.

356 SAFETY, TOLERANCE, PHARMACOKINETICS AND PRELIMINARY ANTIMALARIAL EFFICACY OF WR 238605 IN MAN. Brueckner RP*, Coster T, Wesche DL, Shmuklarsky M, Lasseter KC, and Schuster BG. Division of Experimental Therapeutics, WRAIR, Washington, DC; Medical Division, USAMRIID, Ft Detrick, Frederick, MD; and Clinical Pharmacology Associates, Miami, FL.

WR 238605 succinate is an 8-aminoquinoline drug, currently under development for prophylaxis and treatment of malaria. Animal studies have demonstrated that it has greater efficacy and lower toxicity compared with primaquine. To date, WR 238605 has been administered to 70 humans, in single oral doses up to 600 mg (base). It has been well tolerated, with mild headache and gastrointestinal disturbances as possible side-effects. Linear kinetics have been demonstrated at the doses studied. WR 238605 is slowly absorbed and metabolized, with a tmax of 12 hours and an elimination half-life of two weeks. In a pilot double-blind, placebo-controlled study, a single 600 mg oral dose of WR 238605 was administered to four individuals one day before inoculation with *P. falciparum* sporozoite infected mosquitos. The two placebo subjects developed parasitemia with clinical symptoms on day 10. Three subjects who received WR 238605 were completely protected, and one subject developed parasitemia and became symptomatic on day 30. This one subject who developed malaria after receiving WR 238605 had peak drug blood levels of 244 ng/ml; in contrast, the three who were protected had levels of 417- 489 ng/ml. Although additional studies are needed, these data suggest that WR 238605 has potential as a long-acting prophylactic drug that could be administered on a weekly basis.

357 USE OF ANTISENSE OLIGODEOXYNUCLEOTIDES AGAINST *PLASMODIUM FALCIPARUM*. Barker Jr. RH*, Metelev V, Rapaport E, Agrawal S, and Zamecnik PC. Worcester Foundation for Experimental Biology, Shrewsbury, MA; and Hybridon, Inc., Worcester MA.

Previous studies showed that antisense oligodeoxynucleotides (AS-ODNs) are selectively taken up by *Plasmodium falciparum*-infected red blood cells, can inhibit parasite growth *in vitro*, and are active against drug-resistant strains. The present studies sought to further examine the sensitivity and sequence specificity of ODNS, possible use of alternative gene targets, and possible alternative chemical structure of the ODNS. Parasites from the W2, W2mef, HB3, and D6 strains were maintained by *in vitro* culture and were examined for sensitivity to different concentrations of ODNS. Effect of ODNs was assessed both by microscopic examination of cultures after 48 hr, and by hypoxanthine incorporation. Results showed that using phosphorothioate (PS) ODNs at concentrations of 1 μM or greater, all ODNs inhibited parasite growth by 90%, regardless of nucleotide sequence. However, at lower concentrations, some AS-ODNs directed against dihydrofolate reductase had an LD_{50} of less than 0.05 μM, whereas the ODNs containing mismatches, the complementary "sense" strand sequence, or sequences directed against genes from HIV were minimally inhibitory. Similar results were obtained with some, but not all, AS-ODNs directed against a variety of other gene targets. Experiments were also done examining the effect of chemical modification of the ODN structure. ODNs containing 3'and 5' PS-linked nucleotides, but whose central portion was phosphodiester (PO) linked showed less sequence-independent inhibition at high concentrations. Similarly, use of 2'-O-methyl linkages also yielded similar efficacy at low concentrations, but reduced sequence-independent inhibition at high concentrations. Antisense ODNs therefore hold promise both as potential chemotherapeutic agents against malaria, and also for examining questions concerning gene function.

358 THE RIBOSOMAL RNAS OF *PLASMODIUM FALCIPARUM* AS A DRUG TARGET. McConkey GA*, Rogers MJ, Li J, and McCutchan TF. Growth and Development Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.

The cytoplasmic ribosomal RNAs of *Plasmodium* are unique in showing developmentally regulated expression of the genes. We have recently characterized the complete 18S-5.8S-28S gene sets of *P. falciparum*, and show that, as was found for the 18S genes, a distinct set of 5.8S-28S genes are expressed in either the blood-stage (asexual- or A-type) or mosquito stage (sporozoite- or S-type). We have drawn a predicted secondary structure for the cytoplasmic rRNAs which allows comparison with the secondary structures for the rRNAs expressed from the mitochondrial-like 6 kb genome and from the 35 kb plastid-like genome. From an analysis of these structures, we can correlate the site of action of a number of antibiotics with its rRNA target. We have tested some of these eukaryote- and prokaryote-specific antibiotics for inhibition of growth and for inhibition of protein synthesis in *in vitro* cultures of *P. falciparum*. A number of antibiotics of the hexose cytosine class and of the chloramphenicol type, which are inhibitors of peptidyl transferase activity, were found to be effective in sub-μM concentrations. In addition, inhibitors of the GTPase activity inherent in the ribosome were also found to be effective. These data are promising as they provide a means of studying the structure and function of the *Plasmodium* ribosome as the target may be investigated by its interaction with these drugs. In particular, this suggests specific targeting of the rRNAs of *P. falciparum* as a future goal for drug development, and as a selectable marker for stable transfection by resistance to these drugs.

359 SEROPREVALENCE OF HEPATITIS B AMONG SCHOOL-AGED CHILDREN IN THE STANN CREEK DISTRICK OF BELIZE, CENTRAL AMERICA. Chamberlin J*, Bryan JP, Jones DL, Reyes L, and Hakre S. Department of Preventive Medicine and Biometrics, Uniformed Services Univ of the Health Sciences, Bethesda, MD; and Epidemiology Research Center, Ministry of Health, Belize City, Belize.

Adults in Stann Creek District, Belize have a high prevalence of hepatitis B virus (HBV) infection, but the age at onset of these infections is unclear. We conducted a seroprevalence study of hepatitis B markers among school-aged children in that district to provide information for planning a hepatitis B vaccine program. After informed parental consent, 587 students, aged 4-22 (mean 13 years), from five schools were tested for antibody to hepatitis B core antigen (anti-HBc) and hepatitis B surface antigen (HBsAg). The overall prevalence of hepatitis markers was high: 43.3% had anti-HBc and 7.7% had HBsAg. Anti-HBc was more common in males than females (52% vs. 36.5%; $p<.05$). There was also marked variation between ethnic groups. At the two rural primary schools attended mainly by Mayan and Mestizo children, >65% of children ≤ 8 years had anti-HBc, with no increase with age. In contrast, at the urban primary school attended mainly by Garifuna and Creole children, only 9% of children ≤ 8 years had anti-HBc, and seropositivity increased with age ($p<.05$, by chi-square test for trend). Anti-HBc was found in 42% and 36% of students at the two high schools. In conclusion, among school-aged children in Stann Creek District, the prevalence of HBV infection varies by location and ethnicity. Because most children in the rural areas,are exposed to hepatitis B before entering school, immunization against HBV should be integrated into the routine infant immunization program.

	Mayan (n=133)	Mestizo (n=92)	Garifuna (n=173)	Creole (n=141)	Other (n=48)
Anti-HBc +	76%	50%	37%	25%	19%
HBsAg +	9%	11%	9%	4%	2%

360 PREVALENCE OF HEPATITIS C INFECTION AMONG PERUVIANS. Sanchez JL*, Sjogren MH, Watts DM, Chauca G, Callahan J, Hinostroza S, Ramos-Garcia S, Carrillo-Carrillo L, Cardenas R, Cabezas C, Rodriguez G, and Estacio-Rojas C. U.S. Army Medical Research Unit-Brazil, Rio de Janeiro, Brazil; Walter Reed Army Medical Center, Washington, DC; U.S. Navy Medical Research Institute Detachment-Lima, Lima, Peru; Clinica Santa Isabel, San Borja, Lima, Peru; and Hospital Edgardo Rebagliatti M.

A sero-survey of blood donors and high-risk groups was undertaken in April-May 1984 in 902 Peruvians to estimate prevalence of infection with hepatitis C virus (HCV). A retrospective comparison was made with 1389 serum samples from high risk groups collected in 1986-93. Demographic and epidemiologic information was collected on both groups using a standardized interview form. To be considered for testing, serum samples had to be obtained from pre-determined patient risk groups including blood donors (BD), hemodialysis (HD), hemophilia (HE), acute hepatitis (AH), chronic hepatitis (CH) and non-IV drug users (DU). All samples were screened by second generation enzyme-linked immunosorbent assay (EIA). Repeatedly positive EIA results were required for the sample to be considered HCV positive. Recombinant immunoblot assay (RIBA) confirmation was performed on a sample of 82 EIA-positive sera. Age and sex distribution for both collections of sera were found to be similar. Overall, 16% of both groups were found to be HCV positive. HCV prevalence rates were found to be higher in the 1994 cohorts of HD (57%), HE (100%), and CH (44%) patients as compared to comparable groups in 1986-93. By comparison, the anti-HCV rates for BD and DU groups were found to be similar (0.7 to 1.7%) as well as those for AH patients (3 to 4%). These observations indicate that hepatitis C is a significant health problem for the Peruvian population, particularly in hemodialysis, hemophilia and chronic hepatitis patients. The anti-HCV prevalence observed in blood donors (0.7% to 1.4%) is similar to that reported in the US and other countries.

361 EPIDEMIOLOGICAL EVIDENCE FOR FAMILIAL TRANSMISSION OF HEPATITIS C VIRUS IN RURAL EGYPT. Kamel MA, Miller FD*, Baraket R, Ghaffer Y, and Troonen H. School of Public Health, University of Hawaii, Honolulu, HI; Faculty of Medicine, Ain Shams University, Cairo, Egypt; Abbott GmbH Diagnostika, Wiesbaden, Germany; High Institute of Public Health, Alexandria University, Alexandria, Egypt; and Faculty of Medicine, Cairo University, Cairo, Egypt.

Recent reports from Egypt indicate an unusually high level of hepatitis C virus (HCV) endemicity. In a population-based epidemiologic study of an entire hamlet located in the northern Nile Delta, we investigated the relationship of anti-HCV antibody positivity between family members. Enzyme immunoassay (HCV EIA 2.0, Abbott) was used to determine anti-HCV IgG (anti-HCV), and was validated by supplemental tests and MATRIX™ (Abbott). Anti-HCV core IgM (anti-HCV IgM) was detected by Abbott HCV IgM EIA; HCV RNA cDNA amplification was completed on a random subsample of positive sera. Liver disease was detected by ultrasonographic examination using standardized conditions. A total of 1,259 sera were collected from a population of 1,850 villagers included residents of all ages, from all households. Overall, 15.9% (SE ± 1.0) of hamlet residents were confirmed to be seropositive, increasing to 45.1% in those 35 to 39 years old without significant gender differences. The odds ratios of anti- HCV positivity for women married to anti-HCV positive men was 2.3 (95% CI 1.1-4.8) when controlling for age and martial status. Restricting analysis to anti-HCV positive men with hepatomegaly, the odds ratio of anti-HCV positivity in their wives was 15.7 (95% CI 2.1-683). Including only those men who had hepatomegaly and were anti-HCV IgM

positive, the odds ratio of anti-HCV positivity in their wives increased to 18.0 (95% CI 1.6-861). No other significant associations were found between other family members. This study reaffirms the high rate of endemic HCV transmission in Egypt, and suggest that village women married to anti-HCV positive men, especially those with hepatomegaly and evidence of active infection (i.e. anti-HCV IgM positive), have an increased likelihood of HCV infection.

362 CROSS-SECTIONAL PROFILE OF EPIDEMIC/SPORADIC HEPATITIS E VIRUS TRANSMISSION WEST KALIMANTAN, BORNEO, 1994. Putri MP*, Lubis I, Jarot K, Orndorff G, Punjabi NH, Tan R, Wignall SF, Graham RR, and Corwin AL. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; National Institute of Health Research, Jakarta, Indonesia; and Dinas Kesehatan, Kabupaten Sintang, West Kalimantan, Indonesia.

Hepatitis E virus (HEV) was first identified in Indonesia during a 1987 outbreak from West Kalimantan, involving 5000 persons. A second outbreak was detected in.West Kalimantan in 1991. A cross- sectional survey was conducted to geographically profile HEV prevalence in a jungle riverine area that was one focus of epidemic hepatitis E during 1987. Additionally, a contiguous, although distinct population, with no identifiable historical exposure to epidemic HEV, was surveyed further downstream for comparation. The study communities were all situated along a 150 kilometer stretch of the same river. Sera (n=885) were assayed (EIA) for IgG anti-HEV and IgM anti-HAV markers. Overall IgG anti-HEV prevalence in both outbreak and control areas was 40%. Ninety percent of the population had IgG anti-HAV by age 9. Prevalence of IgG anti-HEV in the outbreak area (50%) was significantly higher than the control area (23%) ($p<0.0001$). In the outbreak area, prevalence of IgG anti-HEV increased significantly with age ($p<0.01$). No rise in age-specific prevalence was noted from the control area. Antibody prevalence (53%) in the population aged > 7 years from the outbreak area (alive during the actual 1987 outbreak) was significantly ($p<0.01$) greater than among children aged <7 years (born after the outbreak) (15%). In contrast, antibody prevalence in the control population did not differ between the >7 (23%) and <7 (20%) age groups. There was no difference in antibody prevalence between males (40%) and females (41%) in both outbreak and control areas. Other risk factors will be presented. This study shows persistence of IgG anti-HEV acquired 7 years earlier in 1987 and evidence of continuing transmission of HEV in children since 1987.

363 A WATER-BORNE OUTBREAK OF HEPATITIS E VIRUS TRANSMISSION IN THE SOUTHWEST OF VIETNAM. Corwin AL*, Ha BK, Clayson ET, Pham KS, Vo TT, Vu TY, Cao TT, Vaughn D, Merven J, Richie TL, Wignall FS, and Hyams KC. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; Pasteur Institute, Ho Chi Minh City, Republic of Vietnam; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Abbott Laboratories (Singapore), PTE Ltd; and U.S. Naval Medical Research Institute , Bethesda, MD.

A water -borne hepatitis outbreak affecting primarily adults occurred in the southwest of Vietnam, along the Cambodian border in June and July, 1994. One month after the outbreak, sera and epidemiologic information were collected from 150 subjects comprised of 50 patient cases, 50 healthy controls, and 50 geographical controls. The prevalence of IgG to HEV was significantly ($p<0.001$) higher (76%) among cases than among the matched (38%) and geographical (38%) control populations. IgM to HEV was detected by EIA and western blot in 16% of sera collected from patients one month after the outbreak. Likewise, HEV RNA was detected with PCR in 6% of sera collected from patients one month after the outbreak. HEV RNA was not detected in sera collected from controls. These results indicate that HEV was the etiological agent responsible for the outbreak. Children were underrepresented among cases (selection based on hepatitis-related signs and symptoms). River water served as the principal source for drinking, and bathing among most (96%) of the case and control study populations. Boiling of drinking water was negatively associated ($P<0.05$) with infection (IgG antiHEV). Unusually heavy rainfall likely contributed to conditions that favored the outbreak. This is the first recognized outbreak of epidemic hepatis E in Indo-China (Vietnam, Cambodia and Laos).

364 ACUTE HEPATITIS IN HANOI, VIETNAM, 1993-1995. Dao DD*, Tran CD, Hoang TN, Clayson E, Vaughn D, Kanti L, Widjaja S, Punjabi NH, Graham RR, Wignall FS, Hyams KC, and Corwin AL. Institute of Clinical Research in Tropical Medicine, Hanoi, Vietnam; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; and Naval Medical Research Institute, Bethesda MD.

A study of acute hepatitis was conducted in Hanoi, Vietnam, from January 1993 through February 1995. One hundred and eighty- eight sera from clinical hepatitis cases (with signs and symptoms compatible with acute hepatitis) were screened by enzyme-linked immuno absorbent assay (EIA) for IgM anti-HAV, IgM anti-HBc, anti-HCV, IgG anti-HEV and IgM anti-HEV. Additionally, 186 sera from patient controls matched by age, sex, and month, with no recent history of hepatitis disease, were also tested for comparative purposes. There was serological

evidence of recent HAV (29%) and HBC (24%) infections in 53% of cases (two mixed infections), compared with 2% of controls. Hepatitis C virus infections were detected among 10% of case (in the absence of IgM anti-HAV or IgM anti-HBc) and 1% of control sera. There was no significant difference in the proportion of IgG anti-HEV positives between cases (in the absence of IgM anti-HAV or IgM anti-HBc) and controls: 17% and 14% respectively. IgM anti-HEV and PCR results are pending. Younger case subjects aged <20 years were more likely ($p<0.01$) to have recent HAV infections (56%) than those >20 years (21%). In contrast, a higher percentage of case adults had IgM anti-HBc, anti-HCV and IgG anti-HEV (in the absence of recent HAV or HBV infections) markers compared to children. The male: female ratio among cases was 2.46: 1. However, the proportion of females with IgM anti-HAV (33%) was higher than for males (27%). Similarly, a larger proportion of females (37%) had IgM anti-HBc markers than did males (18%) ($p<0.01$). No seasonal trends in hepatitis admissions were detected, or association between water-borne spread infections (HAV and HEV) and the warmer months. Residential distribution of hepatitis patients was from throughout Hanoi and surrounding areas, with no identifiable geographical clustering, regardless of serological marker.

365 MIXED VIRUS INFECTIONS IN PATIENTS WITH HEPATITIS E. Binn LN*, Asher LV, Longer CF, Martinez E, Miele T, Ticehurst J, Hoke C, and Innis BL. The Walter Reed Army Institute of Research, Department of Virus Diseases, Washington DC; and Johns Hopkins University School of Medicine, Department of Pathology, Baltimore, MD.

Hepatitis E often occurs after contamination of water supply systems and it is not surprising that multiple bacterial and viral diseases may also occur. Therefore, to select suitable preparations for virus propagation *in vitro* or for inoculation of monkeys, candidate preparations containing hepatitis E virus (HEV) were inoculated into cell cultures to screen for other agents. 10 candidate human fecal specimens containing HEV were tested in primary human embryonic kidney (HEK), Buffalo green monkey kidney (BGM), human diploid lung (MRC-5), and rhabdomyosarcoma (RD) cells. After 1 passage, cytopathic agents were recovered in HEK and/or MRC-5 cells from 2 of 10 specimens. Electron microscopic examination of the infected cell cultures tentatively identified these agents as picornaviruses. Neutralization tests identified one of these as echovirus type 7. The other isolate was not typable by the Lim and Benyesh-Melnick anti-enterovirus serum pools. In other studies to propagate HEV in hepatoma G-2 cells, cytopathic effects were evident during the second passage of 1 of 4 fecal specimens containing HEV. Electron microscopic examination of this infected culture revealed the presence of an adenovirus in the nuclei of the cells. Using the polymerase chain reaction, HEV was not detected in the above cell cultures after 3 serial passages. These findings emphasize the need to test candidate HEV inocula for other transmissible agents

366 VIROLOGIC AND IMMUNOLOGIC EVENTS DURING ACUTE HEPATITIS E. Clayson ET*, Myint KS, Snitbhan R, Vaughn DW, Innis BL, Chan L, Cheung P, and Shrestha MP. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC; Genelabs Diagnostics, Singapore; Infectious Disease Hospital, Kathmandu, Nepal.

The virologic and immunologic events during infection with the hepatitis E virus (HEV) are poorly understood. To better characterize HEV infections, viremia, fecal shedding, and antibody responses were examined in 67 patients with acute markers for hepatitis E and who were admitted to the Infectious Disease Hospital in Kathmandu, Nepal during August to December 1993. A single stool and multiple sera specimens from each patient were examined with the polymerase chain reaction (PCR) to detect HEV RNA. Sera were also examined for the presence of antibodies to HEV with enzyme-linked immunosorbent assay (ELISA). Viremia, fecal shedding, IgM, and IgG to HEV were detected in 93, 70, 79, and 87% of the patients respectively. Viremia and/or fecal shedding were detected in 14 patients from whom antibodies to HEV were not detected. Viremia lasted for at least two weeks in nearly all individuals and at least 39 days in one individual. Our results suggest that viremia is a common occurrence in patients infected with HEV.

367 BACULOVIRUS EXPRESSION OF THE HEPATITIS E VIRUS (HEV) GENE SEGMENT CONTAINING THE FULL LENGTH COMBINED OPEN READING FRAME (ORF) 2 AND 3. Zhang HY*, Burrous J, Zhao BT, Caudill JD, Gandre HC, Putnak R, Warren R, and Longer CF. Dept of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

Separately expressed portions of the HEV genome ORF 2 and ORF 3 are immunoreactive. Our purpose was to express the entire ORF 2 and 3 as one gene segment and to assess the immunoreactivity of the products. Segments of ORF 2 and 3 were amplified from cynomolgus macaque bile containing HEV Mexico 86 (Telixtac 14 feces, 2nd pass). The segments were ligated to produce a 2106 bp cDNA fragment containing the entire ORF 2 and 3. The fragment was inserted into the pCR II cloning vector and transformed into E. coli competent cells. Endonuclease

cleavage and partial sequencing confirmed the expected nucleotide products. The cloned cDNA was ligated into the PVL 1393 baculovirus transfer vector. This plasmid was then amplified in E. coli HB 101 competent cells. Recombinant baculovirus was produced by cotransfecting SF9 cells with the recombinant transfer vector PVL 1393 and BaculoGold. SF9 cells infected with the recombinant baculovirus were positive in an indirect immunofluorescence assay using monkey or human serum containing anti-HEV and FITC-conjugated goat anti-human IgG. SF9 cells infected with wild-type baculovirus showed no antibody binding. Western blot was performed using human and monkey anti-HEV containing serum and rabbit polyclonal anti-ORF 3 serum (Genelabs Technologies, Inc). At least 4 distinct bands were visible, of which 2 were the weight expected for the ORF 2 and 3 proteins. Further analysis of these proteins is ongoing. These simultaneously expressed immunoreactive products should represent all the antigens coded by both ORF 2 and 3 and may be useful in diagnostic tests or as vaccine candidates.

368 CELL SURFACE GLYCOSAMINOGLYCANS ARE NOT REQUIRED FOR *PLASMODIUM BERGHEI* SPOROZOITE INVASION *IN VITRO*. Frevert U*, Sinnis P, and Nussenzweig V. Department of Molecular and Medical Parasitology, New York University Medical Center, New York, NY; and Department of Pathology, New York University Medical Center, New York, NY.

The malaria circumsporozoite protein (CS) with its conserved region II-plus binds to glycosaminoglycan (GAG) chains from heparan sulfate proteoglycans (HSPG) on the basolateral cell surface of hepatocytes, and is rapidly cleared from the blood circulation by the liver. These findings suggest that sporozoite homing to the liver is mediated by a specific CS /HSPG interaction. Here we have asked whether the presence of GAGs on the plasma membrane of target cells is required for sporozoite invasion *in vitro*. Two types of target cells were used: HepG2 cells, which are permissive for *Plasmodium berghei* sporozoite development into mature exoerythrocytic forms (EEF), and CHO cells, in which the intracellular development of the parasites is arrested early after invasion. Invasion of mutant CHO cells expressing undersulfated GAG chains (CHO pgsE), or no GAG chains (CHO pgsA), was only inhibited 41-50% or 24-33%, respectively, in comparison to invasion of wild type CHO cells. In agreement with these findings, treatment of HepG2 cells with chlorate, which inhibits the sulfation of GAG chains, reduced CS binding and EEF development by a maximum of 39%. Furthermore, cleavage of the HepG2 surface membrane GAG chains with heparinase or heparitinase had little inhibitory effect on the development of *P. berghei* EEF, although the GAG lyase treatments removed over 80% of CS binding sites from the cell surface.

369 *PLASMODIUM BERGHEI* SPOROZOITES RELEASE CS PROTEIN INTO THE HOST CELL CYTOPLASM. HÅgel FU*, Pradel G, and Frevert U. Department of Molecular and Medical Parasitology, New York University Medical Center, New York, NY.

To examine the role of the circumsporozoite (CS) protein during the intrahepatocytic development of malaria parasites, we used two cell types for invasion by Plasmodium berghei sporozoites: 1. HepG2 cells, which support the development to extrahepatocytic (EE) forms *in vitro*, and 2. CHO cells, in which the parasites are arrested at the sporozoite stage shortly after invasion. CS protein was shed from the parasite surface into the cytoplasm of both cell types. This intracellular CS protein was detected already while the parasite was still attached to the host cell surface. In contrast to soluble recombinant CS protein, which was taken up by HepG2 cells and introduced into the endocytotic pathway, parasite-derived CS protein did not colocalize with endosomal or lysosomal markers by confocal microscopy. Rather, we found the released CS protein in association with the endoplasmic reticulum at all stages of the intrahepatocytic development from the sporozoite to the EE form stage. In HepG2 cells, the parasite-released CS protein reached a peak concentration at six hours after invasion and was present for up to 24 hours. Afterwards, CS protein was only present in association with the developing EE forms. CHO cells, however, contained cytoplasmic CS protein in high amounts for at least 36 hours. The significance of the parasite-released CS protein is discussed.

370 INCREASED EXPRESSION OF ICAM-1 AND VCAM-1 IN THE BRAINS OF MICE INFECTED WITH *PLASMODIUM YOELII* 17XL A MODEL OF CEREBRAL MALARIA IN MICE. Das B, Berman JW, Buchwald D, Nagel RL, and Shear HL*. Division Hematology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; and Department Pathology, Albert Einstein College of Medicine, Bronx NY.

Recent studies from our laboratory have examined *Plasmodium yoelii* 17XL infection in mice as a model for cerebral malaria (CM). We found that the post-capillary venules of the brains of these mice were occluded with infected erythrocytes, in the absence of inflammation. In *ex vivo* studies using the rat mesocecum preparation and the cremaster muscle of infected mice, we found that infected erythrocytes adhered to the venular endothelium and that this resulted in decreased blood flow and increased shear stress as described previously. In this study, SW female mice infectedwith $1x10^5$ PRBC of *P. yoelii* 17XL were sacrificed after 9 days and brains removed and fixed.

Sections, 40-60 μm, were cut using a Vibratome and immunostained with rat Mabs against mouse ICAM-1, VCAM-1 or an irrelevant rat Mab of the same isotype. We observed a marked increase in ICAM-1 expression in the capillaries and small vessels in both the cortex and cerebellum of infected compared to uninfected mice. We also observed an increase in VCAM-1 expression in the postcapillary venules of the brains of infected animals but less so in the capillaries. These results suggest that VCAM-1 expression could be related to red cell adhesion since it shares the same anatomical distribution as adhesion. Thus, this model of CM has potential for analyzing the roles of these adhesion molecules in the sequestration of infected erythrocytes.

371 STUCTURE-FUNCTION OF THE PfEMP1 DOMAIN MEDIATING THE BINDING OF *PLASMODIUM FALCIPARUM* PARASITIZED ERYTHROCYTES TO CD36. Baruch DI*, Singh HB, Ma XC, Bi X, Pasloske BL, and Howard RJ. Affymax Research Institute, Santa Clara, CA.

Of the four species of human malaria, *Plasmodium falciparum* is the most lethal. Sequestration of mature stage parasitized erythrocytes (PE), results from PE adherence to microvascular endothelial cells in diverse organs and is associated with the pathology of *P. falciparum*. CD36, thrombospondin and ICAM-1 are the major host cell receptors for adherence of PE. The capacity of the parasite to express variant antigens on the surface of PE contributes to the special virulence of *P. falciparum*. PfEMP1, a malarial variant protein expressed on the surface of PE is also associated with adherence of these cells to microvascular endothelium. Thus, PfEMP1 plays a central role in the biology and the pathology of *P. falciparum*. We investigated the binding of different domains of PfEMP1 to CD36 and TSP. One of the domains tested, derived from the sequence of the Malayan Camp PfEMP1 gene, specifically binds to CD36. Antibodies to this domain block adherence of PE to CD36 in a strain specific manner. However, the recombinant protein blocked adherence of PE from several *P. falciparum* strains to CD36. The structure-function of the CD36 binding domain was investigated by generating a series of deletion mutants. Several regions important for the function or structure of the domain were identified. The characterization of a domain, derived from the sequence of PfEMP1, that binds to CD36 supports the concept that PfEMP1 is a *P. falciparum* cytoadherence receptor. This CD36 binding domain is a prime target for a malaria vaccine and for the development of novel therapeutics to block and reverse PE adherence.

372 SWITCHES IN EXPRESSION OF *PLASMODIUM FALCIPARUM VAR* GENES CORRELATE WITH CHANGES IN ANTIGENIC AND CYTOADHERENT PHENOTYPES OF INFECTED ERYTHROCYTES. Smith JD*, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson DS, Pinches R, Newbold CI, and Miller LH. Laboratory of Parasitic Diseases, National Institute of Health, Bethesda, MD; and Molecular Parasitology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

Plasmodium falciparum expresses on the host erythrocyte surface clonally variant antigens and ligands which mediate adherence to endothelial receptors. Both are central to pathogenesis, since they allow chronicity of infection and lead to concentration of infected erythrocytes in cerebral vessels. Recently, Su et al. identified several novel gene sequences that possessed homology with the binding region of malarial erythrocyte binding proteins. These genes hybridized with many bands in Southern analysis, suggesting that they belonged to a large family of genes and have been named *var* genes (Su et al.). *Var* genes have many properties that might be expected of the variant antigen and cytoadherence ligands: they are polymorphic, contain large open reading frames, and possess multiple DBL domains known to have diverse binding specificities in erythrocyte binding proteins. Exploiting homologies between DBL domains, we used degenerate oligonucleotide primers to study *var* message expression in a *P. falciparum* clonal tree whose members had been characterized as to their antigenic and cytoadherent characteristics. We found that expression of distinct variant antigens at the surface of infected erythrocytes correlates with expression of distinct *var* genes. Moreover, expression of a specific *var* gene correlated with binding to ICAM-1. Thus, our results are consistent with the involvement of *var* genes in antigenic variation and binding to endothelium. These findings permit studies addressing the mechanism of antigenic switching, the relationship between antigenic and adhesive phenotypes, and the role of the variant repertoire in evading the immune response as well as inducing protection.

373 GENES ENCODING VARIANT ANTIGENS EXPRESSED ON THE SURFACE OF *PLASMODIUM KNOWLESI*-SCHIZONT-INFECTED ERYTHROCYTES. Al-Khedery B*, Barnwell JW, Galinski MR. New York University School of Medicine, Department Medical and Molecular Parasitology, New York, NY.

Antigenic variation in *Plasmodium* was first described in the simian malaria *P. knowlesi* and the proteins found to vary were designated the Schizont Infected Cell Agglutination (SICA) antigens. These antigens are high MW proteins of parasite origin that become inserted into the infected erythrocyte membrane, can be surface-labeled with ^{125}I, and are extractable only in strong ionic detergents. Knowledge of these proteins lead to the identification of an analogue in *P. falciparum*, known as Erythrocyte Membrane Protein-1 (PfEMP-1), which is believed to participate in

cytoadherence. Since this phenomenon does not occur in *P. knowlesi*, or other malaria species such as *P. vivax*, the variant proteins likely have another critical purpose that has not yet been identified. To investigate the function(s) of these proteins and the molecular mechanisms of variation we chose to characterize the variant genes and proteins of the stable Pk1(A+) and Pk1(B+)1+ clones of *P. knowlesi*, which express known variant types. An antiserum, prepared by immunizing rabbits withthe Pk1(B+)1+ antigen, was used to screen Pk1(B+)1+ cDNA expression libraries. Several reactive cDNA clones are being characterized that recognize a large multigene family on genomic Southern blots and a transcript of the predicted size (7kb), as well as a smaller transcript of about 2.5 kb, from Pk1(B+)1+ RNA. Further analyses will be presented.

374 CONSERVATION OF STRUCTURE AND FUNCTION OF THE ERYTHROCYTE BINDING DOMAIN OF *PLASMODIUM FALCIPARUM* EBA-175. Liang H*, and Sim BK. EntreMed, Rockville, MD.

EBA-175 of *Plasmodium falciparum* binds to and mediates the invasion of erythrocytes. We have identified the domain of EBA-175 which binds to its erythrocyte receptor glycophorin A to be a 616 amino acid sequence (aa 145-760) called region II which contains a cysteine-rich motif. Both sialic acids and the peptide backbone of glycophorin A are required for binding. We have now obtained the gene sequence encoding region II from 16 strains of *P. falciparum* from throughout the world. Sequence analysis shows complete conservation of cysteine residues indicating that the tertiary structure of region II is probably required for function. At the amino acid level there is tight conservation for sequences between cysteines with the exception of 5 to 13 specific residues scattered throughout region II. To determine if these few changes limited to specific residues play a role in the binding of region II to erythrocytes, we expressed region II of all 16 strains on the surface of separate Cos-7 cells as chimeric proteins using the secretory signal and transmembrane segments of herpes simplex virus glycoprotein D, and determined if erythrocytes would bind to these transfected Cos-7 cells. Region II of all 16 strains bound erythrocytes. Furthermore, the sialic acid binding specificity was conserved for all 16 region IIs, since none bound to neuraminidase-treated erythrocytes. These results further emphasize the importance of assessing EBA-175 region II as a candidate for receptor blockade and vaccines.

375 POST-TRANSLATIONAL MODIFICATION OF THE *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTE MEMBRANE CORRELATES WITH EXPRESSION OF MESA. Magowan CC*, Coppel RL, Esperanza RG, and Narla M. Lawrence Berkeley Laboratory, Berkeley CA; and Monash University, Department of Microbiology, Clayton, Victoria, Australia.

Modifications of the host erythrocyte membrane during *Plasmodium falciparum* infection include the association of parasite encoded MESA (Mature Parasite Infected Erythrocyte Surface Antigen, or PfEMP-2) with erythrocyte skeletal protein 4.1. We recently demonstrated that survival of MESA+ parasites was disrupted in erythrocytes deficient in protein 4.1, whereas MESA- parasites successfully maintained the intraerythrocytic cycle in these abnormal cells. Using normal erythrocytes, we have now confirmed previous reports that protein 4.1 is more heavily phosphorylated in infected compared with uninfected erythrocytes, and that the apparent size of protein 4.1, determined by electrophoretic mobility, is increased in erythrocytes infected with cytoadherent parasite lines. Although Western blots of Tx-100 insoluble extracts of trophozoite infected cytoadherent erythrocytes, probed with antibodies to protein 4.1, revealed no change in the size of this erythrocyte skeletal protein, we identified an increase in the apparent size of protein 4.1 by SDS-PAGE of ^{32}P biosynthetically labeled cytoadherent parasites. Interestingly, there was no detectable phosphorylation of protein 4.1 in erythrocytes infected with a MESA parasite strain. These results suggest it is a subpopulation of protein 4.1, perhaps bound to MESA, that is phosphorylated in infected erythrocytes. Thus, it may be expression of MESA, not simply parasitization, which results in post-translational modification of erythrocyte skeletal protein 4.1.

376 IDENTIFICATION AND CLONING OF ERYTHROCYTE BINDING PROTEIN GENE HOMOLOGUES IN THE RODENT MALARIA SPECIES *PLASMODIUM YOELII* AND *PLASMODIUM BERGHEI*. Kappe SH*, Curley GP, Dalton JP, and and Adams JH. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN; and School of Biological Sciences, Dublin City University, Glasnevin, Dublin, Republic of Ireland.

Erythrocyte invasion by malaria parasites requires the molecular interactions of the merozoites with erythrocyte surface receptors. A well-conserved, functionally important family of erythrocyte binding proteins of *Plasmodium* are expressed in the merozoite micronemes (MP-1) and play an integral role in the invasion process. In the human malaria parasites *P. vivax* and *P. falciparum* the MP-1 includes the Duffy binding protein and the EBA-175, respectively. The MP-1 family is characterized by a similar gene structure and conserved sequence motifs in the functionally important cysteine-rich domains. Because the study of the MP-1 is hindered by limited availability of parasite-derived proteins and the use of primates *in vivo*, we set about to identify the MP-1 homologues in rodent malaria parasites. The homologous MP-1 genes of *P. yoelii* and *P. berghei* were identified by PCR amplification of

the gene fragment coding for the carboxyl Cys-rich region. Oligonucleotide probes based on this region identified a single copy of the MP-1 gene in each species by Southern blot hybridization and these probes were used to isolate genomic clones. Based on similarity of conserved deduced amino acid sequence and genomic structure, the cloned *P. yoelii* and *P. berghei* gene fragments are identified as part of MP-1 gene family. Comparison with other MP-1 genes establishes three groups: vivax (*P. vivax, P. knowlesi*); falciparum (*P. falciparum*); and berghei (*P. berghei, P. yoelii*). Identification of the MP-1 in rodent malaria parasites will provide an *in vivo* laboratory model to facilitate studies of the role of these proteins in merozoite invasion of erythrocytes.

377 PCR CHARACTERIZATION OF *PLASMODIUM VIVAX* ISOLATES FROM COLOMBIA, SOUTH AMERICA. Alger J*, Acosta MC, Saravia NG, and Krogstad DJ. Tulane School of Public Health and Tropical Medicine, New Orleans, LA; and Fundacion CIDEIM, Cali, Colombia.

Our previous studies have shown that primers designed to amplify the polymorphic region between Interspecies Conserved Blocks 5 and 6 in MSP-1, and the repeat region of CSP in *Plasmodium vivax* distinguish *P. cynomolgi* strains that are identical by other criteria, and that these markers are conserved *in vivo* (during relapse in the *P. cynomolgi*/rhesus monkey model of human *P. vivax* infection). These studies used the same primers and conditions to examine the diversity of natural *P. vivax* infections, and thus to examine their ability to distinguish between human reinfection (due to different clones) and relapse (due to the same clone). Studies performed during the past year in *P. vivax*- endemic areas of Tumaco, Buenaventura, Caqueta and other regions of Colombia revealed 19 MSP-1 polymorphisms (298-453 bp) and 16 CSP polymorphisms (571-720 bp). Although these genes sort independently at meiosis because they are on different chromosomes, several linkages (non-random associations) were observed in Tumaco. All 3 infections with 394 bp MSP-1 fragments had 716 bp CSP fragments; 5 of 7 infections with 408 bpMSP-1 fragments had 681 bp CSP fragments; and 4 of 4 441 bp MSP-1 fragments had 720 bp CSP fragments. These results suggest that this strategy should be able to distinguish between reinfection and relapse, and that it may also permit one to follow individual parasite clones in the community.

378 A CLONE OF AN ANTIGEN CONTAINING EPITOPES THAT ARE COMMON TO *PLASMODIUM FALCIPARUM* AND *PLASMODIUM YOELII*. Kironde FA*, Ma H, Ray P, Sahoo N, Patra LK, Paliwal S, and Singh B. International Centre for Genetic Engineering and Biotechnolology, New Delhi, India; and Vector Control Research Centre, Medical Complex, Pondicherry, India.

Interspecies conserved antigens of *Plasmodium* may be useful in the development of widely applicable diagnostics and prophylactic compounds, given the extensive diversity of malarial parasite proteins. In order to identify new useful conserved malarial antigens, we immunoscreened *P. falciparum* DNA expression libraries with anti-*P. yoelii* sera and identified a positive recombinant clone, pc3.2, which when subcloned in the vector pGEX, expressed a 17-kDa polypeptide fused to the vector-encoded 28-kDa glutathione-S-transferase. The cloned DNA insert hybrized to a 4.0 kbp fragment of mung-bean nuclease-digested *P. falciparum* genomic DNA and did not hybridize to human DNA. Analysis of the partial sequence of pc3.2 insert DNA showed an open reading frame encoding a novel *P. falciparum* protein. Rabbit antibodies raised against the pc3.2-expressed fusion product clearly recognized a polypeptide in lysates of *P. falciparum* cells. The recombinant fusion product was also recognized by sera from malaria endemic areas, confirming that pc3.2 encodes at least one interspecies conserved epitope. We have shown previously that immunoglobulin G antibodies of BALB/c mice convalescent from *P. yoelii* infection bind at least 15 polypeptides of *P. falciparum* and inhibit *P. falciparum* growth. These observations suggest that one or more analogous antigens shared between the two species induce protective immunity. The native protein, structure and immunological properties of pc3.2 will be discussed.

379 STABLE TRANSFORMATION OF *PLASMODIUM FALCIPARUM* WITHIN HUMAN ERYTHROCYTES: REPLICATING EPISOMES AND HOMOLOGOUS INTEGRATION. Wu Y*, and Wellems TE. Laboratory of Parasitic Diseases, N.I.A.I.D./N.I.H., Bethesda, MD.

The stable transformation of *Plasmodium falciparum* cultivated within human erythrocytes will facilitate analysis of molecular processes in this pathogen. Toward this goal we have developed constructs containing mutant dihydrofolate reductase-thymidylate synthase (*dhfr-ts*) genes that confer resistance to pyrimethamine, an antimalarial drug. Transformants were obtained following transfection of parasites with these constructs and selection with pyrimethamine. Presence of transfected *dhfr-ts* genes was confirmed by Southern analyses of restricted genomic DNA. The transformants contained unrearranged episomes as demonstrated by plasmid rescue experiments. Episomes extracted from transformants were resistant to Dpn I, indicating the episomes are replicated within parasites because this enzyme cleaves GATC sequences only when adenine is methylated by Dam methylase that is absent from eukaryotes. PCR using primers designed to detect homologous recombination events amplified fragments with expected sizes and restriction patterns. Integration events were also detected by Southern analyses of

restricted genomic DNA and pulse-field electrophoresis chromosome separations. These results formally demonstrate the role of *dhfr-ts* mutants in pyrimethamine resistance and provide a foundation for genetically manipulating *P. falciparum.*

380 A NOVEL PROTECTIVE 67-KDA INTERSPECIES-CONSERVED INTEGRAL MEMBRANE ANTIGEN OF *PLASMODIUM FALCIPARUM.* Ma H*, Ray P, Paliwal S, Patra KK, Sahoo N, Singh B, and Kironde FA. International Centre for Genetic Engineering and Biotechnolology, New Delhi, India; and Vector Control Research Centre, Medical Complex, Pondicherry, India.

Extensive diversity associated with most of the known proteins of *Plasmodium* underlies the importance of antigens which are shared between different species of malaria parasites and which may, thus, contain antigenic epitopes that have been conserved for common biological roles. Interspecies conserved epitopes have potential to form the basis for development of diagnostics, vaccines or curative drugs. Recently, we demonstrated that serum antibody immunoglobulin G of mice convalescent from *P. yoelii* infection cross-reacts with at least 15 *P. falciparum* polypeptides and inhibits the growth of the parasite *in vitro.* We have immunoscreened a lambda gt11-*P. falciparum* DNA library with anti-*P. yoelii* sera and identified a recombinant expression clone, pcL800, which encodes a *P. falciparum* interspecies conserved polypeptide of an apparent mol wt = 32 kDa. The clone was also recognized by antisera raised against a triton 114-phase separated preparation of *P. falciparum* total integral membrane proteins. Rabbit antisera against the pcL800-expressed fusion polypeptide inhibited parasite invasion of erythrocytes and recognized a 67-kDa and an 85-kDa polypeptide in lysates of *P. falciparum* and *P. yoelii* cells, respectively. Studies with syncronized *P. falciparum* cultures showed that the native 67-kDa protein is predominantly found in schizont stages. The cloned parasite DNA hybridized both to a 1.8-kbp fragment of mung-bean digested *P. falciparum* genomic DNA and to a 4-kbp fragment of Dra I-digested *P. yoelii* DNA. Sequence analysis of pcL800 insert DNA indicated that the cloned *P. falciparum* antigen is novel and has not been described before. Total primary sequence and potential importance of the novel antigen will be discussed.

381 IDENTIFICATION OF PUTATIVE GENE EXPRESSION CONTROL ELEMENTS IN OOKINETTE SURFACE PROTEIN PGS28 OF *PLASMODIUM GALLINACEUM.* Mbacham WF*, Budge E, Golightly LM, Ruel K, Sullivan TJ, and Wirth DF. Department of Tropical Public Health, Harvard School of Tropical Health, Boston, MA.

Specific antigens are expressed in *Plasmodium* parasites as they develop through the different stages in their hosts. These antigens have stage specific properties that might confer pathogenicity or play a role for the survival of the parasite. Stage specific expression is therefore important in malaria, yet little is known about it. Gene expression control elements usually occur in the untranslated region that flank coding regions of eukaryotic genes at the 5' (initiation) and the 3'ends (termination). To understand mechanisms of gene expression, an ookinette stage specific gene that encodes a surface protein Pgs28 in *P. gallinaceum,* was used in constructing a chimera in which the firefly luciferase gene was inserted 60 amino acids from the start of the Pgs28 gene. Nested deletions of the 5' flanking sequence were performed and the deletion constructs were transfected into zygotes and luciferase activity assayed in developing ookinettes after 24 hours. Because transfection is such an inefficient process, a full length similar construct with another reporter gene, β-glucuronidase (GUS), was co-transfected to determine the amount of DNA that went into the cell. Luciferase activity measured in relative light units were normalized against GUS. Activity decreased with increased sequential deletions up to 400bp from the ATG start site of the Pgs28 gene. Deletions beyond this point showed no detectable activity in this transient system. 1200 bp of the 5'UTR have been sequenced and shown to contain 2 TATA-like boxes at -368bp and -443bp; 2 start sites for mRNA at -333 and -408 and a series of 5bp and 27 bp repeats clusterred within -142 and -239. Distances were measured from the ATG of Pgs28 pending identification of the true mRNA initiation site. Ongoing experiments will confirm their relevance in stage specific or basal control of gene expression. Comparison with Pfs25, a similar homologous protein in *P. falciparum* could provide leads for the rational design of drugs or identification of mechanisms for the further development of transmission blocking intervention measures that will suppress infectivity of the mosquitoes by the parasites.

383 CRITERIA FOR PRIMARY RESERVOIR ADAPTED TO WILD HOSTS OF LEISHMANIASES IN THE NEW WORLD. Van Wynsberghe NR*, Canto-Lara SB, Andrade-Narvaez FJ, and Itza-Ortiz MF. Department of Immunology, Center of Regional Research (CIR), University of Yucatan (UADY), Merida, Yucatan, Mexico.

Leishmaniases are mostly enzoonotic diseases. The mammalian species in which a parasite population is maintained is the reservoir of the disease. In 1984, the World Health Organization enumerated the criteria to incriminate a primary reservoir. However, those criteria cannot be applied as such to New World reservoirs of Leishmaniases because of the high biodiversity of the area. The result of a two-year study of hosts of *Leishmania (L.) mexicana* in the State of Campeche, Mexico, is used as a model to precise WHO's criteria for primary reservoir. A

capture-recapture method was used to document the ecological and behavioural requirements for a primary reservoir. Following WHO's criteria, *Oryzomys melatonis* is the prime reservoir, however this rice-rat was found in very small number during only one year. *Ototylomys phyllotis* and *Peromyscus yucatanicus*, two probable reservoirs, were not very abundant but the population was relatively constant and sedentary. Monthly cultures of aspirate of naturally infected captive-mice showed that the infection was long-lived.The rate of infection of free-living rodents was found during the months of high transmission. A mathematical formula was created to ajust this rate of infection to the estimated population for each species. With those results, WHO's criteria for a wild primary reservoir were precised and adapted to leishmaniases in the high biodiversity of the Neotropics.

384 IS CUTANEOUS LEISHMANIASIS DUE TO *LEISMANIA TROPICA* A ZOONOSIS IN NORTHERN JORDAN? Kamhawi SA*, Abdel-Hafez SK, Al-Daher HA, and Qadoumi MZ.

A stable focus of cutaneous leishmaniasis caused by *Leishmania tropica* was recently identified from Bani Kinana District in northern Jordan. Sixty six cases were identified from seven villages in the district between February 1994 and February 1995. The majority (65.5%) of active cases were living in houses located at the outskirts of villages facing rural wadis in which wild animals such as foxes, jackals and hyraxes are abundant. A leishmanin survey of a random sample of 626 inhabitants of two of the district villages showed a 23.3% positivity rate. When families of active cases living on the outskirts were leishmanin tested, the positivity rate increased to 40.8%. The dispersed distribution of cases within and between villages, the seemingly increased risk of inhabitants living on the outskirts of villages indicate that the disease in this area is a zoonosis. Entomological investigations of the area revealed the presence of 10 *Phlebotomus* species. *P. syriacus* (64,3%) and *P. papatasi* (16.0%) were the most abundant followed by *P. mascitti* (8.0%) and P. tobbi (7.5%). *P. sergenti*, the classical vector of *L. tropica* was rare (0.2%) In both peridomestic and rural habitats. Bloodmeal analysis of 227 *Phlebotomus* specimens using capture EI,ISA reveal.ed that *P. syriacus* and *P. papatasi* females were opportunistic feeders. Of 112 specimens of *P. syriacus*, 71.4% contained sheep blood, 25.9% contained human blood and the rest contained mixed bloodmeals. *P. papatasi* was more anthropophagic with 74.0% containing human blood, 8.0% sheep blood and the rest contained mixed bloodmeals. *P. mascitti* was zoophagic with 91.4% of 58 females containing sheep blood. At present, the search for the vector and possible reservoir of *L. tropica* in this focus is underway.

385 DETECTION OF *LEISHMANIA* INFECTED RESERVOIR HOSTS USING THE POLYMERASE CHAIN REACTION. Carrion R*, Melby PC, McHugh CP, Alvarez RA, and Kerr SF. Department of Biology, Incarnate Word College, San Antonio, TX; and The University of Texas Health Science Center, San Antonio, TX; Armstrong Laboratory, Brooks Air Force Base, TX.

The efficacy of the polymerase chain reaction (PCR) for detection of *Leishmania sp.* in field-isolated reservoir hosts has not been investigated. This study compares the value of a PCR-based detection method against the currently utilized in vitro culture technique in *Leishmania sp.* field studies. Tissue samples from the ears of *Neotoma micropus* (incriminated as a reservoir of *Leishmania mexicana* in Texas), *Sigmodon hispidus*, and *Peromyscus leucopus* were studied by PCR using primers which amplify a 120 bp sequence of the conserved region of the *Leishmania* kDNA minicircle. Positive and negative controls were included in each assay. The PCR products were subsequently analyzed using agarose gel electrophoresis. Studies using non-infected rat tissue and *in vitro* cultured *L. mexicana* promastigotes confirmed that the test was specific and sensitive to the equivalence of one parasite. Results from the test were compared to *in vitro* culture results from the same tissue sample. Of the 166 samples tested, *in vitro* culture detected 2 positive samples. PCR detected the same two samples positive by culture with an additional 7 tissue samples positive by PCR alone. This evidence suggests that PCR is more sensitive than *in vitro* culture and confirms the prevalence of infected reservoir hosts in south Texas. In addition, this PCR-based detection method provides a means for rapid detection by cutting detection time from several days for *in vitro* culture to several hours for PCR.

386 PREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION IN A SOUTHEASTERN REGION OF GUATEMALA. Powell MR*, Nix NA, Hernandez B, Arana FE, Ramirez L, and Greer GJ. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Medical Entomology Research and Training Unit (MERTU/G), USEMB/HHS/MERTU, Guatemala City, Guatemala.

Chagas' disease is wide spread in much of Central and South America. As a prelude to a National Control Program in Guatemala to reduce transmission of *Trypanosoma cruzi* by decreasing vector density in domicilliary and peridomicilliary areas, a pilot study has been initiated in 3 villages in the Department of Chiquimula. Villages are rural with populations of approximately 300-1200. A representative study population, based on housing construction, was chosen. Of 365 individuals examined to date, 10.2% are positive for anti-*T. cruzi* antibodies by ELISA. Sex distribution in the infected and uninfected populations are virtually identical with slightly more

females than males. There is a significant trend for an increase in prevalence of infection with age (p=0.00053). For example, 11 of 197 individuals less than 20 years of age are seropositive (5.6%), but 33 of 164 of those over 20 years of age (11.6%) showed evidence of infection (p=0.0024 by Chi Square analysis). None of the 51 children under the age of 5 tested were sero-positive. This group should therefore provide an effective sentinel population for the longterm evaluation of the effectiveness of efforts to reduce vector borne transmission.

387 SEROPREVALENCE OF *TRYPANOSOMA CRUZI*-ANTIBODIES IN A SUBPOPULATION OF AMERICAN RED CROSS BLOOD DONORS. Leiby DA*, Read EJ, Lenes BA, Pan AA, Stumpf RJ, and Dodd RY. Transmissible Diseases Department, American Red Cross, Rockville, MD; Southern California Region, American Red Cross, Los Angeles, CA; South Florida Region, American Red Cross, Miami, FL; and Abbott Laboratories, Abbott Park, IL.

During the past 25 years millions of immigrants have entered the U.S. from *Trypanosoma cruzi*-endemic countries. It is estimated that over 100,000 of these individuals may be infected with *T. cruzi*. To assess the likelihood of transfusional transmission of *T. cruzi*, we conducted a large scale sero-epidemiological study. All blood donors in our Los Angeles and Miami blood centers were asked a question concerning birth or time spent in *T. cruzi*-endemic regions. Donors responding "yes" and a cohort of controls were tested for *T. cruzi*-antibodies by EIA (Abbott). Samples repeatedly reactive by EIA were confirmed by RIPA. Confirmed *T. cruzi*-seroreactive donors were entered into case-control and lookback investigations. Of the 196,832 Los Angeles and 17,043 Miami donors queried regarding risk, 14,480 (7.4%) and 1,868 (11%), respectively, responded "yes," with 20 of these donors confirmed by RIPA (Los Angeles,17; Miami, 3); their only common risk factor was birth, including extended residence in an endemic country. None of 8 recipients of prior donations has demonstrated *T. cruzi*-transmission by transfusion. Thus, nearly 1 in 5,700 Miami, and 1 in 11,600 Los Angeles blood donors were seropositive for *T. cruzi*-antibodies. Despite the absence of demonstrated *T. cruzi*-transmission by transfusion, the finding of seroreactive donors suggests that a significant public health problem may exist.

388 NEW FINDINGS AND SIMPLIFIED CLINICAL STAGING OF AFRICAN TRYPANOSOMIASIS. McGovern TW*, Williams W, Fitzpatrick JE, Cetron MS, Hepburn BC, and Gentry RH. Dermatology Service, Fitzsimons Army Medical Center, Aurora, CO; Infectious Disease Service, Fitzsimons Army Medical Center, Aurora, CO; Division of Parasitic Diseases, National Center for Infectious Diseases, Atlanta, GA; and Department of Family Practice, US Air Force Academy, CO.

As air travel increases, Western physicians must know the clinical manifestations and diagnostic tests for African Trypanosomiasis (AT) so that prompt diagnosis and cure may follow. We report a Caucasian American man who acquired Rhodesian AT with CNS involvement while hunting in Tanzania. He sought medical care in a United States hospital two months after exposure and recovered following treatment with suramin and melarsoprol/dimercaprol. We report results of the first biopsies of a trypanid (trypanosomal exanthem) which included mild lymphocytic spongiosis overlying a superficial perivascular lymphocytic infiltrate with neutrophils and leukocytociasia. No trypanosomes were found after staining and reviewing all the tissue from two punch biopsies. Touch preparations of the trypanid punch biopsies revealed trypanosomes. We recommend a simplified reclassification of the stages of AT based on the syphilis model. Rare color photographs of trypanids will be shown. We present the first biopsy and touch preparation results of trypanids and add touch preparations as a new diagnostic procedure. We reclassify AT with primary chancriform, secondary hemolymphatic, and tertiary central nervous system (CNS) stages that should improve the understanding of AT's complex natural history.

389 DEVELOPMENT OF AN *IN VITRO LEISHMANIA* TEMPERATURE SENSITIVITY MODEL: PRELIMINARY STUDIES WITH STRAINS WITH ABERRANT TROPISM *IN VIVO*. Callahan HL, Portal IF, and Grogl M. U.S.A. Medical Research Unit-Brazil, Walter Reed Army Institute of Research.

Dermotropic and viscerotropic *Leishmania* species show different phenotypic responses to temperature *in vitro*. Visceralizing *Leishmania* strains (*L. donovani*) replicate equally well as amastigotes in macrophages at 35, 37 and 39°C, while cutaneous strains (*L. major, L. tropica, L. mexicana*) replicate best at 35°C. Temperature sensitivity appears to be intrinsic to the parasite (not mediated by the macrophage) since isolated amastigotes of *L. mexicana* also die when incubated at temperatures above 34°C as described previously. Thus, temperature was suggested as at least one of the potential factors influencing the tropism of *Leishmania* species in the human host, since skin has been shown to have a lower temperature than the viscera. Since we are interested in functional complementation studies to determine at the molecular level the factors controlling *Leishmania* tropism, and promastigotes are far more easy to manipulate *in vitro* than amastigotes in macrophages, we have developed a promastigote temperature model. Promastigote strains from 8 different species of *Leishmania* (over 40 strains in all), have been tested for their ability to multiply at 3 different temperatures (30°C, 32°C and 34°C) as compared with growth at the

control temperature (25°C) over a 48-72 hour period. The data shows a clear correlation between the temperature sensitivity of parasites tested in the promastigote model and parasites tested in the amastigote model. Interestingly, parasite strains which have an unusual tropism in the human host also show an unexpected temperature sensitivity in our model.

390 REINVENTING ANTILEISHMANIALS: MODERN ANTILEISHMANIALS FROM TRADITIONAL HERBAL THERAPY. Jackson JE*, Okunji CO, Tally JD, Iwu MM, Hanson WL, Waits VB, Nolan LL, and Schuster BG. Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria; College of Veterinary Medicine, University of Georgia, Athens, GA; and Division of Public Health, University of Massachusetts, Amherst, MA.

Preferred chemotherapy for leishmaniasis remains the 50-year-old injectable pentavalent antimonials (SbV) which have cardio- and nephrotoxicity. Secondary drugs, amphotericin B and pentamidine, have a 10-fold higher toxicity death rate and are considered too toxic for routine use. No chemoprophylaxsis for leishmaniasis exists. Treatment failure, attributed to parasite drug resistance, is reported from most leishmanial endemic regions (80 countries). Growing evidence, based on reactivation of "SbV-cured" disease, prompted some investigators to postulate that SbV are only leishmaniastatic with apparent cure dependent on drug suppression concommitant with patient immunologic mechanisms. Protozoan diseases, including leishmaniasis, offer little incentive for commercial drug development. Thus, the readily available, cheaper, oral, and less toxic traditional medicines frequently prevail in developing countries. Pictures of common leishmanial disease presentation were used to query traditional healers for herbal therapies. Plants identified in consensus by several healers were selected using a series of *in vitro* "screens" with increasing leishmanial specificity. Using this selection strategy, together with the microradiorespirometry, RAM bioassay guided isolation, we have identified 38 highly active fractions (70-99% *in vitro* suppression) from 18 different plants, and 10 moderately active fractions (>/=50% suppression) from 10 additional plants. Identification of four purified compounds and partial chemical characterization of several more, has been accomplished. Chemical data are consistent with the idea that each of these herbal antileishmanial leads belongs to a novel chemical drug class. Drug data from simple cutaneous (nonmetastatic)- and visceral hamster models, chemical structure, *in vitro* human cell toxicity (therapeutic index), and typical isolation strategy will be given for representative compounds including: AD, GG, PN, DM, and NI.

391 PARASITICIDAL MECHANISMS IN MACROPHAGES INFECTED BY *LEISHMANIA DONOVANI* FOLLOWING TREATMENT *IN VITRO* WITH LAK CELLS AND/OR PENTOSTAM. Eslami Z*, and Tanner CE. Institute of Parasitology, McGill University, Quebec, Canada.

LAK cells and Pentostam have parasiticidal activity *in vitro* in C57BL/6 mouse peritoneal macrophages infected by *Leishmania donovani*. LAK cells are most effective in reducing the infection when used in the non-proliferative phase of the parasite's intermacrophage life cycle,whereas Pentostam is most effective in the proliferative phase of the infection. Immunotherapy plus drug treatment reduced the infection to levels significantly below those produced by either treatment alone. It was of interest to determine if these treatments exert their effect by stimulating the infected macrophages to produce the parasiticidal nitrogen oxide response. Macrophages infected by *L. donovani* do not produce nitrogen oxide, as determined by the RNI method. Activation of infected macrophages with LAK cells *in vitro* is very effective in stimulating this anti-parasite mechanism. Treatment with Pentostam does not, however, activate the production of nitrogen oxide by infected cells. Treatment with LAK cells during either the non-proliferative or the proliferative phases of the infection induced the production of equal quantities of nitrogen oxide, suggesting that this antiparasite mechanism may be activated by different signals from IL-2-stimulated splenocytes. Pentostamis probably directly toxic to the parasite, whereas LAK cells act indirectly to stimulate parasiticidal mechanisms in the infected macrophages.

392 INTERFERON-γ AND INTERLEUKIN-5 PRODUCTION FOLLOWING CHEMOTHERAPY OF MURINE VISCERAL LEISHMANIASIS WITH THE 8-AMINOQUINOLINE, WR6026. Shin SS*, and Hanson WL. Department of Parasitology, College of Veterinary Medicine, The College of Georgia, Athens, GA.

The existence of antimony-resistant strains of *Leishmania* and frequent unresponsiveness to conventional antimonial drugs especially among immunodeficient patients have raised a demand for new drugs for use against the parasite as well as for a better understanding of the relationship between chemotherapeutic agents and the immune system of the host. The author investigated inferferon-γ (IFN-γ) and interleukin-5 (IL-5) production in response to infection of *Leishmania (Leishmania) donovani* and to the treatment of *L. (L.) donovani*-infected mice with Glucantime® and the 8-aminoquinoline WR6026, a new leishmanial drug. Infection with C57BL/6 mice with *L. (L.) donovani* for 17 days resulted in an increase in the production of IFN-γ and IL-5 by the spleen cells of infected

mice compared to that of uninfected controls. When numbers of liver amastigotes were decreased 56.1% by treatment with Glucantime®, no change in IFN-γ was observed as compared to that of infected controls while a slight decrease in IL-5 production was noted. Elimination of 99.5% of *L. (L.) donovani* amastigotes by treatment of the infected mice with WR6026 elicited a further increase in IFN-γ, but a decrease in IL-5 production by spleen cells. The results suggest that a new drug, WR6026, delivered its antileishmanial activity in close association with the differential activation of the immune system in that the activity of the TH1-like response was enhanced following the treatment, while that of TH2-like response was inhibited.

393 ALLOPURINOL IN THE TREATMENT OF CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS: A SUMMARY OF CLINICAL STUDIES. Marr JJ, Modabber F, Martinez S, and Llanos-Cuentas. Ribozyme Pharmaceuticals, Boulder, Colorado; World Health Organization, Geneva, Switzerland; University of Popayan, Popayan, Colombia; Alexander von Humboldt Institute, Lima, Peru; and the Latin American Investigative Group.

There is considerable experimental evidence *in vitro* to document the antileishmanial activity of allopurinol. There also is a long clinical experience which demonstrates its low toxicity in humans. It has the additional advantage of being available as an inexpensive generic compound. Until recently, however, there has not been adequate clinical data to demonstrate the appropriate role of this agent in the management of leishmaniasis. In 1989 the Latin American Investigative Group was formed, under the auspices of the World Health Organization, to conduct clinical trials with allopurinol in combination with pentavalent antimonials in cutaneous and mucocutaneous leishmaniasis. Data from the center in Colombia demonstrate efficacy of allopurinol in cutaneous disease using this combination. The cure rate for pentostam was 30% (10/34) and that for the combination was 66% (23/36). The data from Peru show a lack of efficacy in mucocutaneous disease. In this trial there was a 10% cure in severe disease (2/22) with pentostam alone and 0/11 with the combination; in moderate disease pentostam alone cured 21/30 (75%) and the combination 14/29 (64%). In addition, there are two other published clinical trials which document the efficacy of allopurinol in cutaneous leishmaniasis. A previously reported trial from Iran showed 24/25 cures (96%) with allopurinol alone in chronic cutaneous disease which had not responded to several earlier courses of chemotherapy. A published study from Colombia using allopurinol and glucantime in combination as well as allopurinol alone showed a cure rate of 36% (12/33) for glucantime alone; a rate of 74% (26/35) for the combination; and 80% (20/25) for allopurinol alone. A group in the latter study which refused any therapy had 0/17 cures. Allopurinol, although not effective in mucocutaneous disease, has clinical efficacy in both New World and Old World cutaneous leishmaniasis.

394 APPLICATION OF IMMUNODIAGNOSIS ASSAYS IN FIELD STUDIES: I. DETECTION OF ANTIBODIES TO MAMA AND HAMA IN HUMAN SCHISTOSOMIASIS STUDY SURVEY. Osman AM*, Abdelfatah M, Abdelmoneim E, Al-Sherbiny MM, Galal N, and Tsang VC. Immunology Division, Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt; Egyptian Reference Diagnostic Center, VACSERA, Cairo, Egypt; Division of Parasitic Diseases, Centers of Disease Control, Atlanta, GA, USA; Schistosomiasis Research Project, Ministry of Health, Cairo, Egypt.

Diagnosis of schistosomiasis in endemic areas depends mainly on microscopic detection of eggs in stool or urine. In an initial cross-sectional survey, serum, urine and stool samples were collected from 450 person representing 10% of Bahbeet village population in Egypt, an area well-known to be endemic solely for *Schistosoma haematobium.* Stool samples were examined by the Kato-Katz slide and MIF methods while eggs in urine were detected by Nuclopore filtration method. Parasitlogically, the prevalence of *S. haematobium* was 18%. Our approach to routine reference diagnosis of schistosomiasis in this area was based on screening all collected sera with FAST-ELISA and to confirm and speciate with EITB utilizing microsomal antigens from *S. mansoni* (MAMA) and *S. haematobium* (HAMA). The FAST-ELISA and confirmatory EITB, both derive their sensitivity and specificity from microsomal antigens of adult worms. The assay sensitivity and specificity were 96% and 99%, respectively. Additionaly, we were able to detect a substantial percentage (19%) of the participants possessing antibodies to species-specific band (gp 30), thus indicating acurrent or previous infection with *S. mansoni.* Therefore, it should be emphasized that patient management based solely on the presence of ova is overly conservative and may result in patients with low egg counts being undiagnosed, untreated and able to continue transmitting the disease.

395 FIELD APPLICABLE METHOD FOR DETECTION OF ANTIBODIES TO *SCHISTOSOMA* SPECIES AND GENUS SPECIFIC ANTIGENS USING DIPSTICKS. Al-Sherbiny MM*. Egyptian Reference Diagnostic Center, VACSERA, Cairo, Egypt.

Immunodignosis of *Schistosoma* infection depends mainly on either detection of antibodies specific to schistosome antigens or the presence of circulating antigens in patients serum or urine. The microsomal fractions known as *S.*

mansoni adult worm microsomal antigens (MAMA) and *S. haematobium* adult worm microsomal antigens (HAMA) have been shown to contain GP30 and GP23, respectively, which are considered as species and genus specific glycoproteins. Preparation of such fractions from crude adult worm extract needs expensive equipments, sophisticated technical expertise and a lot of manpower. Our target was to purify GP30 and GP23 using an easier and cheaper alternative utilizing a simple electrophoresis device, 491-prep cell (BioRad). Purified glycoproteins were then tested for specificity and cross-reactivity with different heterologous patient sera. Isolated glycoprotein bands were then coated onto a nitrocellulose membrane, supported with an inert prespex matrix and cut into strips. The overall time of the test was about 15 min to detect specific antibodies in patient serum or whole blood. Each strip included positive and negative reference controls. The new dipstick assay is field-applicable as it combines the simplicity of FAST-ELISA and the capability of speciation of two schistosomes on the same strip. The sensitivity and specificity of the new assay formate matched those of the FAST-ELISA and EITB tests.

396 DETECTION OF CIRCULATING ANTIGENS IN PATIENTS WITH ACTIVE *SCHISTOSOMA HAEMATOBIUM* INFECTION. Hassan MM*, Medhat A, Shata MT, and Strand M. Parasitology Department, Zagazig Faculty of Medicine; Tropical Medicine Department, Assuit Faculty of Medicine; Microbiology Department, Assuit Faculty of Medicine, Egypt; and Pharmacology and Molecular Sciences, The Johns Hopkins University, School of Medicine, Baltimore, MD.

An capture ELISA using monoclonal antibody was carried out to detect circulating schistosomal antigens in the sera of 35 actively infected *Schistosoma haematobium* patients. 20 patients were received a single oral dose of PZQ with 60 mg/kg and 15 patients were received 40 mg/kg. All the patients were followed up frequently at 1, 3 and 6 months after treatment. A significant correlation was found between the levels of circulating antigens and the egg count ($r= 0.6$, $P<0.01$). The mean level of antigens was significantly decreased after PZQ treatment. Moreover, the mean level of circulating antigen was significantly lower in those receiving 60 mg/kg PZQ. Although the mean level of the antigens was significantly decreased 6 months after treatment, 16 patients were still had antigenaemia. Reinfection was suspected in 6 cases associated with a rise in the antigen level after the initial drop, while failure of treatment was considered in 10 patients who had a decreasing antigen level during the follow up without reaching the cut-off value. The children below 14 years were found to be significantly slower in clearing antigens from circulation than elder group. A significant correlation was found between circulating antigen levels and the disease morbidity when monitored by ultrasonography. ELISA appeared to be sensitive in diagnosis of active infection, measuring the intensity of infection and detecting reinfection.

397 CORRELATIONS BETWEEN ANTIBODY RESPONSES, CELL MEDIATED IMMUNITY,AND CIRCULATING ANODIC ANTIGEN LEVELS IN A RURAL COMMUNITY IN ZIMBABWE. Ndhlovu PD*, Cadman H, Chidimu M, Vennervald B, Christensen NO, Chandiwana SK, Gundersen SG, and Deelder AM. Blair Research Laboratory, Zimbabwe; University of Zimbabwe, Harare Zimbabwe; Danish Bilharziasis Laboratory, Charlottenlund, Denmark; Department of Infectious Diseases and Department of Microbiology, Ullevaal Center for International Medicine, Research Forum, Ullevaal University Hospital, Oslo, Norway; and Laboratory of Parasitology, Medical Faculty, University of Leiden, Leiden, The Netherlands.

The present paper contains an analysis of any possible correlations or other interactions between humoral (antibody responses) and cellular (Granuloma Index), immunological reactivity and circulating anodic antigens (CAA) as a reflection of adult worm burdens in an *Schistosoma haematobium* endemic rural community in Zimbabwe. The Granuloma Index showed no significant correlations with any of the antibodies. A very weak negative correlation was seen between the Granuloma Index and CAA levels. A positive correlation existed between IgG4 to SEA and CAA levels. However, the correlation between IgG4 to SEA and CAA disappeared following adjustment for the effect of age. The IgA responses to SWA showed an inverse relationship with CAA. However age was a confounding factor. A negative correlation was evident between IgE responses to both SEA and SWA and levels of CAA. A positive correlation existed between IgM antibody responses to both SEA and SWA and CAA levels. The results confirm the association of IgM and IgG4 with susceptibility to *S. haematobium* infection by probably blocking immune effector mechanisms and the association of IgE and IgA to resistance to human *S. haematobium* infection. The study furthermore clearly demonstrates that concurrent observations on antibody responses and (CAA) levels represent a valuable tool for the interpretation of events related to age acquired resistance in human *S. haematobium* infection.

398 PRAZIQUANTEL RESISTANCE TO *SCHISTOSOMA MANSONI* IN EGYPT. Ismail MM*, Metwally AA, Farghaly AM, Benntt J, Coleman R, and Tao LF. Parasitology Department, Zagazig Faculty of Medicine, Zagazig, Egypt; Theodor Bilharz Institute, Cairo, Egypt; Pharmacology Department, Michigan State University, East Lansing, MI.; and Center for Tropical Diseases, University of Massachusetts, Lowell, MA.

These studies were carried out in different villages in lower Egypt. Those who were found infected with *Schistosoma mansoni* were treated with Praziquantel (PZQ) (40 mg/kg). Second similar dose after 6-8 weeks was given to those who were still infected. Those who remained infected, were given a higher third dose (60 mg/kg) after 6-8 weeks. Pharmacokinetics profiles of PZQ in patients passing viable *S. mansoni* ova after each treatment were compared to those cured after the first dose. Faecal samples were taken from patients before treatment and from patients not cured after two or three doses. Isolates were taken and passed through snails and albino mice. Animals were treated with different doses of PZQ and sacrificed 3 weeks after treatment. Drug efficacy was estimated in comparison to control groups of mice (infected untreated). The results revealed that 3 isolates showed significant low susceptibility to PZQ as compared to the control. These 3 isolates represent patients not cured after 2 doses of PZQ from 2 villages. Another isolates from patients not cured after 3 doses from other villages are now under investigations.

399 EVALUATION OF SCHOOL BASED CONTROL PROGRAMS ON *SCHISTOSOMA HAEMATOBIUM* AND *S. MANSONI* PREVALENCE AND INTENSITY OF INFECTION IN EGYPT. Husein MH, Talaat M*, El-Sayed MK, El-Badawi A, and Evans D. Theodor Bilharz Research Institute, Cairo, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Zagazig University, Zagazig, Egypt; Special Program for Reserach and Training, WHO, Geneva; and Faculty of Medicine, Cairo University, Cairo, Egypt.

There has been considerable interest in targeting schistosomiasis control programs at school-aged children. School age children frequently have the highest prevalence and intensity of infection in the community and are accessible. The efficy of school-based programs critically depends on the level of school enrollment and attendance. In Egypt, school-based schistosomiasis treatment programs target school children for screening and treatment twice yearly and have been operating for more than 70 years. In this study we aimed to (1) estimate school enrollment in areas of Upper and Lower Egypt; (2) compare the prevalence and intensity of infection among enrolled and non-enrolled children, and (3) estimate the proportion of infected children likely to be missed by the school-based control programs. Stool specimens were examined for *S. mansoni* by the Kato method, and urine specimens were examined for *S. haematobium* by nuclepore filtration. A total of 30,309 school aged children between the ages of 6-15 years, from nine governorates in Upper and Lower Egypt, were included in analysis. Enrolled and non-enrolled males in Upper Egypt had *S. haematobium* prevalence of 13.3% and 23% respectively ($p<0.01$), and females had a prevalence of 5% and 14% respectively ($p<0.01$). In Lower Egypt, enrolled and non-enrolled males had *S. mansoni* prevalence of 42.3% and 47.4%, respectively ($p<0.05$). *S. mansoni* prevalence for enrolled and non-enrolled females were 36.2%, and 41.9%, respectively ($p<0.05$). Despite relatively high enrollment rates (80% in Lower Egypt and 55% in Upper Egypt) in some governorates approximately half of infected school aged children could not be reached by school-based services due to non-attendance. Over 80% of infected girls in some areas (Assiut and Fayoum) did not receive treatment. This study shows that school based delivery has a positive impact on lowering schistosomiasis prevalence, but may miss many infected children.

400 CHANGING PATTERNS OF *SCHISTOSOMA MANSONI* AFTER SELECTIVE POPULATION CHEMOTHERAPY WITH PRAZIQUANTEL IN THE NILE DELTA, EGYPT. Barakat R*, Farghaly A, El Masry AG, ElSayed MK, Husein MH, and Miller FD. High Institute of Public Health, Alexandria University, Alexandria, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Cairo University, Cairo, Egypt; and School of Public Health, University of Hawaii, HI.

We report on the largest to date selective population-based chemotherapy control program on *Schistosoma mansoni* prevalence and intensity of infection to date in the Nile Delta, Egypt. The target population was the entire rural area of the northern Nile Delta governorate Kafr El Sheikh, from which a large probability sample was drawn (n = 18,777). The sample included 44 villages and hamlets (ezba). Baseline prevalence was determined by the examination of individual stool specimens by two Kato slides after which infected persons were treated [praziquantel (40 mg/kg)]and reexamined one year later. Those found infected in the second round were treated and examined again the following year. The prevalence and geometric mean egg count (GMEC) declined across all ages in each follow up [baseline prevalence: 39.3% (SE±3.3); first follow-up prevalence: 28.4% (SE±2.6), and second follow-up prevalence: 22.4% (SE±2.3), and baseline GMEC: 72.9 (SE±7.3); first follow-up GMEC: 52.5 (SE±4.5); second follow-up GMEC: 41.9 (SE±2.4)]. Reduction in prevalence varied considerably by village and ezba, and was strongly related to the proportion of the village or ezba population infected and treated ($r^2= 0.29$, $p < 0.001$). Selective population chemotherapy had a profound impact on prevalence and GMEC in this area of the Nile Delta. The later observation provides a rationale for the maximum application of chemotherapy in the endemic Nile Delta communities.

401 THE IMPACT OF CONTROL MEASURES ON URINARY SCHISTOSOMIASIS IN SCHOOL CHILDREN IN NORTHERN CAMEROON: A UNIQUE OPPORTUNITY FOR CONTROLLED OBSERVATIONS. Bausch

DG, and Cline BL. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

As part of a program to integrate schistosomiasis control into the primary health care system in northern Cameroon, an unexpected opportunity was recognized to undertake a controlled evaluation of the impact of interventions. Inadvertently, a large part of Mindjil, one of four"assessment villages," had been essentially excluded from the program,creating a unique "natural control." The prevalence of infection with *Schistosoma hematobium* in school-aged children was 7% in the areas where the control program was implemented, and 71% in the excluded areas($p<.0002$). High intensity infection was 1% and 26% in the two areas,respectively ($p<.0002$). *S. hematobium* infections was associated with enrollment in the school where no interventions took place ($p<.005$).Subjects in intervention areas demonstrated greater knowledge about the transmission of schistosomiasis than those in the control area. This study documented and quantified program impact in a controlled manner not usually possible in field studies and also illustrated how unrecognized intracultural diversity in target populations may effect disease control programs in communities.

402 SEROLOGIC PREVALENCE FOR SCHISTOSOMIASIS IN PUERTO RICO (1995) -- FIRST REPORT OF THE BILHARZIA COMMISSION, PR. Tsang VC*, Hillyer GV, Noh JC, Vivas-Gonzales BE, Ahn LH, Pilcher JB, Hightower AW, Deseda C, Feliciano de and Melicio C. Divison of Parasitic Diseases, NCID, Centers for Disease Control & Prevention, Atlanta, GA; Pathology Department, University of Puerto Rico School of Medicine, San Juan, PR; Office of the Secretary, Department of Health, Commonwealth of Puerto Rico, San Juan, PR.

A systematic, island-wide survey for schistosomiasis has not been conducted for 40 years. A thorough survey of Boqueron de Las Piedras, a small community, showed 40% prevalence in 1974. Persistent reports of clinical cases indicate that this disease is still present on the island. Concern for the public health of residents and visitors to the island prompted the formation of the Bilharzia Commission and the systematic survey reported herein. Over 2900 random plasma samples were obtained from the Red-Cross in March and April, 1995. Sex, resident districts, and age of the donors were recorded. The samples represented healthy donors from all but 4 of 79 districts in PR. No samples were available from Maricao, or the 3 out-islands, Mona, Vieques, and Culebra. Male donors out-numbered females by almost 2: 1, ages ranged from 9 - 76 with the majority between 19 - 51. As of abstract submission time, 1741 samples were assayed by FAST-ELISA with microsomal antigens of *Schistosoma mansoni.* All FAST+ samples were confirmed by immunoblot (EITB). Based on 1741 samples tested, 12.5% were FAST+, and 8.5% were confirmed by EITB. More males were EITB+ (11.2%) than females (2.9%). If we exclude those districts with less than 6 samples, the prevalence of EITB+, ranged from 0% - 42%, with the highest prevalence rates (8.6 - 42.9%) concentrated in 24 districts which accounted for 81% of all seropositive samples. There are 2 clusters of high prevalence districts centering around Utuado (42%) and Las Piedras (35.7%), respectively. The previously surveyed area of Boqueron is located in the Las Piedras district. Systematic sero-surveys of children in high prevalence districts and Boqueron residents are planned for the near future.

403 GEOGRAPHIC INFORMATION SYSTEMS FOR CONTROL OF SCHISTOSOMIASIS IN BAHIA, BRAZIL. Bavia M*, Hale L, and Malone JB. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA; and College of Engineering, Louisiana State University, Baton Rouge, LA.

A major concern for the National Schistosomiasis Program in Brazil is the spread of the disease to new foci in newly resettled areas. GIS has been proposed as a tool for improved control program management. Using Intergraph Modular GIS Environmental System Nucleus (MGE/SX) software, a GIS for the State of Bahia was constructed to include digitized maps of soil type, hydrology, topology, climate (maximum, minimum and mean temperature, precipitation, dry periods), vegetation, political boundaries (municipalities), prevalence of the disease and snail distribution. Environmental features were interactively analyzed with infection prevalence, snail distribution and population density to identify patterns that correlate with risk of schistosomiasis. Preliminary analysis suggests that high schistosomiasis prevalence occurs in areas where both *Biomphalaria glabrata* and *B. straminia* are present and in environments with a predominance of coastal and caatinga vegetation, medium latosole-mediterranean soils, maximum annual temperatures of 36-40C, minimum temperatures of 12-16°C, mean monthly precipitation of 40-45 mm and dry periods classified as subtropical or less than 5 dry months annually.

404 LONG-TERM AND ANNUAL COMMUNITY CHANGES IN HEPATIC FIBROSIS WITH ANTI-SCHISTOSOMAL TREATMENT IN NORTHEASTERN LEYTE, THE PHILIPPINES. McGarvey ST*, Olveda RM, Wiest PM, Aligui G, and Olds GR. International Health Institute and Department of Medicine, Brown University, Providence, RI; The Research Institute for Tropical Medicine, Alabang, Muntinlupa, Philippines; and Department of Medicine, MetroHealth Medical Center, Cleveland, OH.

Hepatic fibrosis due to schistosomaiasis was described in 1,688 residents of Leyte, Philippines. Hepatic fibrosis was determined by ultrasonography and classified as absent, or grades 1, 2 or 3. The sample was divided into an untreated group, from villages with sporadic praziquantel treatment before 1989, and a treated group, from a village which had intensive yearly case finding and treatment for 9 years, 1981-1989. Fibrosis prevalence was described for the two groups over 3 years, 1989-1992, when annual case-finding and treatment occurred in both groups. In 1989 the untreated group had significantly more ($p<0.0001$) severe fibrosis, grades 2 and 3, than the treated group, especially in those age >20 years, 45% vs. 24%, but also in those age<20 years, 9% vs. 1%, (both $p<0.0001$). By 1992 there was no difference in the proportion of all individuals with any fibrosis in the two groups, both 17%. In the younger individuals there was a difference in the proportion of any fibrosis 7% vs. 5% ($p<0.02$), but no difference in severe fibrosis, both groups 1%. In the older individuals there was no difference in proportion of fibrosis, both groups 29%, or severe fibrosis. The results indicate that the community prevalence of fibrosis, particularly severe fibrosis, decreases with annual case-finding and treatment. In the younger individuals annual case-finding and treatment appears to almost eliminate severe fibrosis.

405 HOW OFTEN SHOULD PRAZIQUANTEL (PZQ) THERAPY BE ADMINISTERED TO PATIENTS OF SCHISTOSOMIASIS MANSONI TO REDUCE INFECTION RATE AND LIVER MORBIDITY? Ali Homeida MM*. Faculty of Medicine, University of Khartoum, Khartoum, Sudan.

The present study determined if a single mass chemotherapy with PZQ had an effect on infection rate and liver morbidity 8 years after its application. Moreover, it compared this approach to another treatment approach which combined a single mass chemotherapy with yearly treatment of symptomatic patients. Two villages similar in location and ethnic origin of the population were chosen for this study. The first village received mass chemotherapy treatment in 1979 and thereafter yearly treatment of symptomatic patients only. Village 11 received mass chemotherapy only once in 1987 and nothing thereafter. Prevalence and intensity of infection was studied by examining the stools using Kato-Katz method and liver morbidity was studied by ultrasonography. The results showed significant decrease in the prevalence and intensity of infection in both village after mass chemotherapy over the subsequent eight years (1979 - 1987 for Village I, 1986 - 1994 for Village II). The decrease in prevalence and intensity of the infection was greater in village I (where treatment continued). However, using regression analysis of these two lines the difference was not statistically significant. The subsequent eight years of treatment of symptomatic patients in village I did not produce further reduction of prevalence of infection. Children 10-20 years of age responded to treatment in both villages better than adults. Analysis of the prevalence of Symmers' was restricted to the population aged 10 years and more, since most of the children below 10 years would not have been born when the first round of treatment was applied and consequently would not have benefited from the mass chemotherapy. The results clearly demonstrated a reduction of 18.3% in the prevalence of Symmers' in the village which was treated once and a reduction of 44.4% in the village where single treatment was followed by early treatment of symptomatic patients. In Village I where we had the opportunity of continuing the approach of treatment from 1987 - 1994, there was a further reduction in the prevalence of Symmers' of 33% by 1994. Further analysis of the prevalence of Symmers' in the two villages before and after treatment shows clearly that the decrease in prevalence of Symmers' fibrosis is more noticeable in children aged 10-20 years.

406 IDENTIFICATION OF REGULATORY ELEMENTS WITHIN THE UPSTREAM SEQUENCE OF THE *GIARDIA LAMBLIA* GLUTAMATE DEHYDROGENASE GENE BY TRANSIENT TRANSFECTION. Yee J*, and Nash TE. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

We present the first report of a gene transfer system for the protozoan parasite *Giardia lamblia*. This organism is responsible for many cases of diarrhea worldwide and is considered to be one of the most primitive eukaryotes. Expression of a heterologous gene was detected in this parasite after electroporation with appropriate DNA constructs. We constructed a series of transfection plasmids using flanking sequences of the *Giardia* glutamate dehydrogenase (GDH) gene to drive expression of the firefly luciferase reporter gene. The optimal construct consisted of a GDH/luciferase fusion gene in which the first 18 codons of the GDH gene immediately precede the luciferase gene; this fusion gene is flanked by the upstream and downstream sequences of the GDH gene. Electroporation of this construct into *Giardia* yielded luciferase activity that was 3000- to 50,000-fold above background. Removal of either the GDH 5' or 3' flanking sequences from this construct resulted in significantly reduced luciferase activity, and removal of both flanking sequences reduced luciferase activity to background levels. Luciferase activity was proportional to the amount of DNA electroporated and was maximal at 6 h after electroporation. Regulatory elements within the upstream sequence of the GDH gene were localized by examining the effects of serial deletions of this region on the expression of the luciferase gene.

407 IMMUNE RESPONSES OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS INFECTED WITH *GIARDIA LAMBLIA*. Soliman M, Taghi-Kilani R*, Hegazi MM, and Belosevic M. Departments of Biological Sciences and Immunology, University of Alberta, Edmonton, Canada; and Department of Medicine, Mansoura School of Medicine, Mansoura, Egypt.

The infections with *Giardia lamblia* contribute significantly to the outbreaks of diarrhea throughout the World. In this study, we examined the humoral immune response against *Giardia lamblia* in symptomatic and asymptomatic Egyptian children with confirmed giardiasis. Serological tests have been previously used to determine the prevalence of the infection in humans. The patients were divided into the symptomatic and asymptomatic groups using the following criteria: presence of only *G. lamblia* cysts in the feces, diarrhea, vomiting and/or nausea, loss of appetite, abdominal pain, abdominal distention, loss of weight and malabsorption. The anti-parasite immune response was measured using IFA, ELISA and immunoblotting. The IFA results indicated that the percentage of asymptomatic patients with relatively low titer (< 1:500) was 34.7% and that 29.6% of syptomatic individuals had antiparasite antibody titer s (> 1:8000). The ELISA was conducted to assess the total Ig, IgM, IgA, total IgG and IgG isotypes (IgGl, IgG2, IgG3 and IgG4) of the infected individuals. Significant differences were observed in the following: anti-parasite IgM ($P < 0.001$), IgA ($P < 0.001$ and IgGl ($P < 0.002$). Interestingly, antigen recognition by anti-parasite IgA, IgM, IgGl and IgG3 of symptomatic and asymptomatic individuals revealed minor differences in the responses of the two groups.

408 STUDIES ON LIPID UPTAKE BY *GIARDIA* USING FLUORESCENT LIPID ANALOGS. Stevens TL*, Allison ML, Ellzey JT, Das S. Department of Biological Sciences, University of Texas at El Paso, El Paso, TX.

Since previous studies of lipid metabolism in *Giardia lamblia* have revealed little or no de novo synthesis of cellular phospholipids or sterols, we asked whether host lipids are taken up by *Giardia*. *G. lamblia* trophozoites are exposed to high concentrations of saturated and unsaturated fatty acids in the small intestine during their colonization. The use of Bodipy- and NBD- labeled lipid analogs and epifluorescent photomicroscopy has allowed for the visualization of lipid incorporation. Uptake of various lipids by trophozoites were shown to be dependent on lipid architecture and fluorescent probe. We found that phosphatidylcholine and sphingomyelin were incorporated only into the plasma membrane, whereas palmitic acid (16:0) and dodecanoic acid (12:0) were incorporated into the plasma and nuclear membranes as well as in cytoplasm of trophozoites. NBD-labeled cholesterol was rapidly incorporated into the plasma membrane. On the other hand, head-group labeled phosphatidylethanolamine and short acyl chain labeled phosphatidylcholine and phosphatidylinositol were not incorporated into *Giardia*. These studies show that *G. lamblia* trophozoites are capable of selective uptake and utilization of exogenous lipids.

409 RESISTANCE OF NATIVE TSA 417, A *GIARDIA LAMBLIA* VARIANT SURFACE PROTEIN, TO PROTEOLYSIS AND REDUCTION. Reiner DS*, Aley SB, and Gillin FD. Department of Pathology, University of California at San Diego, Medical Center, San Diego, CA.

The outer surface of *Giardia lamblia* is the parasite's front line of defense against host small intestinal proteases. The cysteine rich variable surface proteins (VSPs) which completely cover the trophozoite are a major component of this surface protection. In previous work we demonstrated that TSA 417, the first complete VSP to be characterized at a molecular level, was resistant to degradation by intestinal proteases. This resistance was due largely to extensive intra-chain disulfide crosslinks. Two-thirds of the 84 cysteine residues in TSA 417 were in a tetrapeptide motif (CXXC) in which the intervening amino acids were frequently polar. This amino acid arrangement can lead to reactive sulfhydryls, which are readily reduced and oxidized under physiological conditions. Reduction of disulfides in VSPs could make the parasite surface more susceptible to proteolysis. We have probed the structure and function of native TSA 417 with proteases and with the reducing agent, DTT. However, using a sensitive gel shift assay, we could not detect any free sulfhydryls in TSA 417, even after incubating live cells with 20 mM DTT for 40 min in the presence or absence of 5 mM EDTA, suggesting a very compact protein conformation in which virtually all of the cysteine residues are buried and inaccessible to solvent. In addition, Western blots show ~43 and 30 kDa proteolytic fragments that are increased by limited digestion of intact cells with the intestinal proteinases trypsin and chymotrypsin or partial digestion with proteinase K. These data suggest that native TSA 417, either on the cell surface or in Triton X-114 extracts, has only one domain that is readily susceptible to proteolytic cleavage. This may represent a "hinge" region where the protein is less tightly folded and more exposed to the environment. Interestingly, it was possible to strip TSA 417 from the surface of trophozoites by brief treatment of cells with high levels of Proteinase K. Inclusion of up to 10 mM DTT had no effect on proteolysis. Trophozoites remain viable throughout this treatment and rapidly regenerate TSA 417, allowing us to investigate functions of VSP and factors which affect its regeneration. Our finding of such an extremely resistant and compact structure supports the hypothesis that VSPs have a key role in protecting *Giardia* from environmental assaults.

410 IDENTIFICATION OF A SARCOPLASMA-ENDOLASMIC RETICULUM CALCIUM ATPASE GENE IN *TRICHOMONAS VAGINALIS*. Li C*, Moate ME, Lushbaugh WB, Finley RW, and Meade JC. Division of Infectious Diseases, Department of Medicine, University of Mississippi Medical Center, Jackson, MS; and Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

Trichomonas vaginalis, a sexually transmitted parasitic protozoan has previously been shown to possess several vanadate sensitive ATPase activities (P-type), including one sensitive to stimulation by calcium ions. Oligonucleotide primers based on conserved regions present in P-type ATPases were used in polymerase chain reaction (PCR) to amplify DNA sequences for these *T. vaginalis* ATPase genes. Three distinct PCR products were obtained, ranging in size from 800-1050 bp. The sequence of the 1050 bp PCR band showed homology to the family of calcium translocating ATPases. The complete sequence for this *Trichomonas* gene was determined from genomic lambda library clones isolated by using the 1050 bp PCR product as probe. The gene contains a 2985 nt open reading frame (ORF) that predicts a 995 aa protein with a molecular mass of 109,448 daltons. The genomic sequence is present in a single copy and lacks introns. The *T. vaginalis* gene appears to be an organellar calcium pump, most closely related to the sarcoplasma-endoplasmic reticulum calcium pumps (SERCA) from rabbit, *Plasmodium falciparum* and*Trypanosoma brucei* (35-39% identity, 49-53% homology). Homology with the *Leishmania* proton pump, fungal plasma membrane calcium and hydrogen ATPases, sodium potassium pumps and other P-type ATPases was significantly lower.

411 GENETIC VARIATION IN *GIARDIA DUODENALIS*. Meloni BP*, Lymbery AJ, and Thompson RC. WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, Institute for Molecular Genetics and Animal Disease, School of Veterinary Studies, Murdoch University, Western Australia.

In this study the nature and extent of genetic variation in *Giardia* was investigated to obtain fundamental knowledge on its population structure and zoonotic potential. Ninety-seven isolates of *Giardia*, from throughout Australia and overseas, were obtained from humans, cats, cattle, sheep, dogs, goat, beaver and rats and examined using enzyme electrophoresis. Enzyme electrophoretic characterisation of these isolates revealed extensive genetic variation with the identification of 47 different zymodemes. The 47 zymodemes could be divided into three main groups: one comprising isolates from humans and a sheep from Western Australia; a second group containing isolates with a worldwide geographical distribution from humans, cats, dogs, sheep, cattle and beaver; and a third group comprising genetically diverse isolates from humans, rats, cat, dog and goat from Australia and overseas.

412 GIARDIASIS AMONG SHANTY-TOWN CHILDREN OF MOROCCO: EVALUATION OF HEALTH AND NUTRITIONAL STATUS. El Kadioui F*, Guessous-Idrissi N, Soubhi H, and Dehbi F. Laboratoire de Parasitologie-Mycologie, Hôpital Ibn Rochd Casablanca, Morocco; Service de Médecine Sociale, Faculté de Médecine, et de Pharmacie, Casablanca, Morocco; and Service de Pédiatrie IV, Hôpital d'Enfants, Casablanca, Morocco.

Giardiasis is a common gastrointestinal disease throughout the world. In Morocco, *Giardia intestinalis* is one of the most prevalent intestinal parasite in children mainly associated with diarrhea. Between November 1991 and November 1993, a prospective study was carried out on a sample from a shanty-town of Casablanca in order to determine the prevalence of Giardiasis and evaluate their effect on children health and nutritional status. Though, 350 children were clinically examined, blood sampled for albumin and hemoglobin rate and stool sampled looking for intestinal parasites with a mean of 2.6 stool exams per child. The preliminary results show that 141 (40%) were *Giardia* infested (single infection in 20 cases and multiple infection in 121 cases), while 59 (17%) were non parasitized. These results show the high prevalence of *Giardia* in such population. The effect on their health and nutritional status will be presented by comparing infected vs non-infected children, as well as single infected vs multiinfected patients. This will allow us to discuss therapy and control strategies to suggest in such communities.

413 ENTERIC PARASITE PREVALENCE AND INTER-PARASITE ASSOCIATIONS, HAMILTON, ONTARIO. MacPherson DW*, McQueen RA, MacQueen W, Soo and Lum Y. Regional Parasitology Lab, St. Joseph's Hospital, McMaster University, Hamilton, Ontariao, Canada.

The objective of this study was to examine the frequency of enteric parasite findings and to determine any inter-parasite associations. A prospective recording of all individual patient's positive enteric parasitology results was analyzed for the period April 1991 to March 1995. A frequency table was created and examination of pairings of parasites, beyond chance, was done (Pearson correlation coefficient). Repeat samples were excluded. 2920 positive patient specimens were analyzed. Frequencies (%) of positive findings: *Blastocystis hominis* (70), *Dientamoeba fragilis* (19.3), *Giardia lamblia* (10.5), *Endolimax nana* (10.4), *Cryptosporidium* sp. (4.5), *Entamoeba histolytica* (2.2),

all others (<10). Any helminth (4.6). Pathogenic parasites (excluding B. hominis) were found in over 30% of all positive specimens. Overall positivity rate was about 22%. No inter-parasite associations were found. Notably, no correlation between *D. fragilis* and *Enterobius vermicularis* or *B. hominis* was found.

414 PARTIAL DESCRIPTION OF THE LIFE CYCLE OF *CYCLOSPORA* AND HUMORAL RESPONSE IN ACUTE INFECTIONS. Ortega YR*, Miyagui J, Watanabe J, Kanagusuku P, Gilman RH, and Sterling CR. Veterinary Sciences Department, University of Arizona, Tucson, AZ; Policlinico Peruano-Japones, Lima, Peru; and Johns Hopkins University, Baltimore, MD.

Cyclospora cayetanensis, a newly recognized coccidian parasite, closely resembles *Cryptosporidium parvum* with respect to many diagnostic and clinical features of infection. To better define the complete life cycle of this parasite, intestinal biopsies were obtained from nine consenting individuals (aged 21-71) who were excreting immature *Cyclospora* oocysts as determined by microscopic observation and modified acid fast staining. Proximal jejunal biopsy samples were formalin fixed, paraffin embedded, serial sectioned, and H & E stained. Histologic examination showed a moderate to severe jejunal inflammation, microvillar blunting, and developing intracellular parasites. Meront stages having four and eight merozoites were observed as were macrogametocytes. Parasites resembling coccidia were not observed in biopsies obtained from five control patients. Serum responses from infected patients were examined by ELISA and western blot analysis against purified and sonicated *Cyclospora* antigens and demonstrated high IgM responsiveness and reactivity to 30 and 46 kD antigens. Similar responses were also observed among other patients with *Cyclospora* or *Cryptosporidium* infections. These parasites, therefore, share antigenic and morphologic characteristics which must be distinguished by clinicians since the former is treatable using cotrimoxazole.

415 AN OUTBREAK OF CRYPTOSPORIDIOSIS ASSOCIATED WITH WELL WATER, WASHINGTON. Dworkin MS*, Goldman D, Herwaldt BL, and Kobayashi J. Division of Field Epidemiology, Epidemic Intelligence Service, Centers for Disease Control and Prevention, Atlanta, GA; Communicable Disease Epidemiology, Washington State Department of Health, Seattle, WA; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Preventive Medicine Residency Program, U. S. Army Medical Corps, Tacoma, WA

In September 1994, we investigated an outbreak of cryptosporidiosis associated with unchlorinated well water. We distributed surveys to the 91 households (227 persons) whose water is supplied by two deep wells. Case patients became ill from Jan. 1 through Oct. 1; confirmed cases had stool specimens containing *Cryptosporidium* parvum oocysts and probable cases had diarrhea that lasted >5 days. We conducted an environmental investigation and tested the well water for fecal coliforms, Giardia lamblia, and *Cryptosporidium*. Sixty-two households (68%) with 169 residents responded to the survey. We identified 15 confirmed and 71 probable cases (attack rate=51%). Sixteen case-patients (19%) were ill for >30 days. Persons who had drunk unboiled well water were more likely to be case-patients than persons who had drunk boiled or other water (RR 1.8, 95% CI 0.9-3.8). Treated wastewater was seen dripping along the outer casing of the older well, built in 1908. *Cryptosporidium* oocysts were found in well water and in treated wastewater. This outbreak investigation, the first in which *Cryptosporidium* oocysts were recovered from well water, demonstrates that even underground water systems are vulnerable to contamination.

416 DIRECT IMMUNIZATION OF SHEEP WITH DNA ENCODING A *CRYPTOSPORIDIUM PARVUM* ANTIGEN ELICITS PARASITE-SPECIFIC SERUM AND COLOSTRUM ANTIBODIES. Jenkins MC*, Kerr D, Fayer R, and Wall R. Parasite Immunobiology Laboratory, ARS, USDA, Beltsville, MD; AltraBio, Inc., Arden Hills, MN; and Gene Evaluation and Mapping Laboratory, ARS, USDA, Beltsville, MD.

In an effort to generate high titer colostrum for immunotherapy of cryptosporidiosis, a study was conducted to test the efficacy of immunizing sheep with recombinant plasmid DNA (pCMV-CP15/60) encoding epitopes of 15 kDa and 60 kDa surface antigens of Cryptosporidium parvum sporozoites. The plasmid DNA was used to immunize preparturient ewes at three dose levels by jet-injection into either hind limb muscle (IM) or mammary tissue (IMAM). Regardless of route of injection, a dose-dependent anti-CP15/60 immunoglobulin response was observed in sera and colostrum from sheep immunized with pCMV-CP15/60 plasmid DNA. High titer antibody responses were observed in one of three animals per group receiving an IM injection of 100 μg or 1000 μg pCMV-CP15/60. IMAM immunization with 100 μg or 1000 μg pCMV-CP15/60 plasmid DNA elicited higher titer colostrum responses and more consistent serum responses compared to IM injections. An eligible serum and colostrum anti-CP15/60 response was observed in control animals. Immunoblotting and immunofluorescence studies using native C. parvum sporozoite/oocyst protein and air-dried sporozoites stained with hyperimmune serum and colostrum corroborated the increased titers against CP15/60 antigen. Preliminary studies showed protective efficacy against cryptosporidiosis in mice given multiple oral doses of anti-CP15/60 colostrum prior to and after parasite challenge.

417 THE INFLUENCE OF ANTI-*CRYPTOSPORIDIUM* ANTIBODIES ON THE OUTCOME OF INFECTION IN HUMANS. Lammie PJ*, Moss DM, Hightower AW, Arrowood MJ, Chappell CL, and DuPont HL. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Center for Infectious Diseases, University of Texas School of Public Health, Houston TX.

Persons infected with *Cryptosporidium parvum* develop characteristic responses to 27- (IgM, IgG), 17- (IgA, IgG) and 15-kDa (IgG) antigen groups as demonstrated by immunoblot. Volunteer studies of *Cryptosporidium* infection provided an opportunity to evaluate the performance of this assay. Volunteers were selected for the study based on low anti-*C. parvum* antibody reactivity by ELISA. Re-screening of initial serum samples by immunoblot revealed that 22 of 29 volunteers had antibody directed against at least two of the 27-, 17-, or 15-kDa antigens. The influence of pre-existing antibody (day 0 post-inoculation) directed against these antigens on the outcome of exposure of volunteers to viable oocysts was analyzed. Band intensities were used as a quantitative measure of antibody level. Day 0 antibody responses did not differ significantly between those who subsequently excreted oocysts following exposure (n=18) and those who did not (n=11). Among persons who developed patent infection, IgG responses to the 17- and 15-kDa antigens were significantly higher at day 0 for persons who remained asymptomatic than for those who developed symptomatic infection (P=0.033 and P=0.018, respectively). Total oocyst excretion was significantly lower in those with pre-existing IgG antibody directed against the 27-kDa antigen (P=0.039). Based on these results, antibody directed against these *C. parvum* antigens is correlated with protection from symptomatic infection, but not necessarily from patent infection.

418 GLUTATHIONE-S-TRANSFERASE ACTIVITY IN *CRYPTOSPORIDIUM PARVUM* OOCYSTS. Okhuysen PC*, Chappell CL, Chakravarthy S, Dang H, and Ali-Osman F. Center for Infectious Diseases, The University of Texas Health Science Center; Experimental Pediatrics, M.D. Anderson Cancer Center, and The University of Texas, Houston, TX.

Cryptosporidium parvum, a common intestinal coccidian parasite of HIV-infected patients with chronic diarrhea, children in day care centers and travelers to certain regions of the world, is also responsible for sporadic outbreaks of diarrhea in normal hosts worldwide. No effective anti-cryptosporidial drug is available at present time. Resistance to antiparasitic agents may be due to detoxifying systems, such as glutathione-s-transferases (GST). These enzymes catalyze the conjugation of exogenous and endogenous toxins and, in other parasites, are important immunogens. The presence of *C. parvum* GST (GST-cp) was investigated using oocysts (Iowa isolate) purified from infected neonatal calves. Enzymatic conjugation was determined using 1-chloro-2,4-dinitrobenzine in a microtiter assay system. Using intact oocysts (10^6) or their homogenates, GST-cp activity was identified in a time and dose dependent fashion. GST-cp was only detected in disrupted oocysts, but not in intact oocysts. Reactivity with α, μ and π GST-subclass specific monoclonal antibodies was studied by western blot technique using 10^5 oocysts. Monoclonal antibodies to π GST reacted with *C. parvum* proteins in the 214,114, 85, 35 and 22 kDa range. We conclude that *C. parvum* oocysts contain one or more GST of the π subclass. The presence of GST-cp may partially explain the ability of this parasite to escape chemotherapeutic agents.

419 AN *IN VITRO* MODEL 0F *CRYPTOSPORIDIUM PARVUM* INFECTION IN HUMAN BILIARY CELLS. Verdon R*, Pereira ME, Tzipori S, Keusch GT, Jefferson DM, and Ward HD. Division of Geographic Medicine and Infectious Diseases, New England Medical Center; Department of Cellular and Molecular Physiology, Tufts University School of Medicine; Division of Infectious Diseases, Tufts University School of Veterinary Medicine.

Intestinal infection by *Cryptosporidium parvum* in immunosuppressed patients is frequently complicated by biliary tract involvement. Biliary cryptosporidiosis has been implicated as a significant cause of AIDS-related cholecystitis and sclerosing cholangitis and may also contribute to resistance or relapse during or after drug therapy. The recent production of immortalized human intrahepatic biliary epithelial cell lines (H69) using retroviral SV40 gene transduction allowed us to investigate the interaction between *C. parvum* and biliary cells *in vitro* using two assay methods. Purified, hypochlorite-treated *C. parvum* oocysts in RPMI were added to confluent H69 monolayers grown in 16 well multichamber slides (immunofluorescence assay) or 96 well tissue culture plates (ELISA-based assay) and incubated at 37°C for 2 hours. Medium containing residual parasites was removed and replaced with fresh culture medium and incubated at 37°C for 24 hours. Cells were washed once and fixed with methanol. In the multichamber slides parasites were detected by indirect immunofluoresence using a polyclonal rabbit antibody to *C. parvum*. Parasitic stages other than sporozoites or oocysts were counted and the results expressed as the number of parasitic stages per high power field. In the 96 well plates, parasites were quantitated using a rabbit polyclonal antibody to *C. parvum* and an avidin/biotin-alkaline phosphatase detection system. The effect of bile on *C. parvum* infection in this model was studied by performing the assay in the presence of increasing amounts of porcine bile.

The results showed that in both the ELISA-based as well as immunofluorescence assays infection with *C. parvum* occurred in a dose dependent fashion. In addition, the presence of bile in concentrations ranging from 50-200 μg/ml resulted in a dose dependent increase in the number of parasitic stages. Concentrations of bile greater than 200 μg/ml resulted in decreased infection and disruption of the monolayer. These studies show that human biliary cells are susceptible to *in vitro* infection by *C. parvum* and may serve as a useful *in vitro* model of biliary cryptosporidiosis.

420 DOGS AS POTENTIAL MECHANICAL VECTORS OF *TOXOPLASMA GONDII*. Frenkel JK*, Lindsay DS, Parker BB. Department of Biology, University Of New Mexico, Albuquerque, NM; Department of Pathobiology, Auburn University, AL; and Arrighetti Animal Hospital, Santa Fe, NM.

Epidemiologic evidence from a study of the transmission of *Toxoplasma* to 1-6 year old children in Panama, Rep. of Panama, indicated that dog contact increased the risk of serocoversion 3.4 to 5.7 fold, whereas cat contact increased it only 2 to 3 fold. A cohort of about 500 children, had been studied prospectively at 3 months intervals over 5 years, for the development of antibody to *Toxoplasma* in the direct agglutination test. After 5 years, 12.6% of children had seroconverted. Mothers had been interviewed concerning the children's meat consumption, contact with soil, cats, dogs, and living conditions. Normally, only cats transmit *Toxoplasma* after a sexual cycle leads to oocyst shedding in their feces. However, dogs do not shed oocysts. It was learned anecdotally, after termination of the field study, that dogs ate and rolled in cat feces in Panama. We conducted a survey of the frequency with which dogs ate or rolled in cat feces in Santa Fe, NM. The owners of 52 dogs observed, that 44.2% ingested cat feces, and 23.1% to rolled in cat feces. In Auburn, AL, 81 students observed 70% of their dogs to ingest cat feces and 23% to roll in cat feces. Fecal contamination of dog fur with cat feces could be important in the mechanical transmission of *Toxoplasma*, because of the desire of small children to pet dogs, their tolerance of fecal smells and the dogs' tolerance to being petted. Ingested oocysts would be less important, because most would excyst, infect the dog and not be shed in the feces. As a precaution, pregnant women and immunocompromised individuals should be advised to wash their hands after contact with dogs, outdoor cats, litterboxes used by them, soil and meat and before eating, until the role of dogs in the transmission of *Toxoplasma* has been further clarified experimentally and epidemiologically.

421 INDUCTION OF IL-12 BY *TOXOPLASMA GONDII* IN INTERFERON-γ KNOCKOUT MICE. Scharton-Kersten TM*, Denkers EY, Gazzinelli RT, Grunvald E, Hieny S, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

Immunity to the protozoan *Toxoplasma gondii* is dependent upon production of IFN-γ during the acute and chronic stages of infection. We previously reported that neutralization of IL-12 in mice infected with the avirulent ME-49 strain of *T. gondii* resulted in decreased IFN-γ production and increased mortality, indicating that early IFN-γ production is IL-12 dependent in this model. Interestingly, IL-12 production by infected macrophages was enhanced *in vitro* in the presence of IFN-γ, thus it was not clear whether IL-12 or IFN-γ initiated many of the early immune responses to *T. gondii*. To address this issue, we evaluated *T. gondii*-induced IL-12 production in interferon-γ knockout (gKO) mice. Infection of thioglycollate-elicited macrophages from BALB/c gKO or wild-type (wt) BALB/c littermates with either *T. gondii* tachyzoites or treatment with soluble *T. gondii* antigen extract resulted in augmented production of IL-12 p40 in cells from both knock-out and wild type mice. Furthermore, intraperitoneal infection of gKO mice resulted in augmented production of IL-12 by spleen and peritoneal cells harvested 3 to 5 days after parasite inoculation. In fact, IL-12 production by peritoneal cells from gKO mice was substantially higher than that by cells from wild-type mice. Nevertheless, gKO mice infected with cysts from the ME-49 strain or with tachyzoites of the ts-4 strain succumbed to infection within 8-12 days. Thus, in the absence of IFN-γ, IL-12 is a necessary but not sufficient component of the immune response to *T. gondii*. To assess potential direct functions of IL-12 in the gKO mice NK cell cytotoxic responses were evaluated following intraperitoneal infection with *T. gondii* tachyzoites. Following infection, NK cell cytotoxicity was elevated in the peritoneum and spleens of infected gKO mice but this response was lower than that detected in either wt or heterozygote control mice. Importantly, *in vivo* neutralization of IL-12 reduced the NK cell cytotoxic response in the gKO mice following infection. Together, these data indicate that *T. gondii* is capable of initiating IL-12 production and function in the absence of IFN-γ.

422 EFFECT OF DINITROANILINES ON *TOXOPLASMA GONDII*. Stokkermans TW*, Roos DS, and Schwartzman JD. Dartmouth Medical School, Hanover NH; and University of Pennsylvania, Philadelphia PA.

Toxoplasma gondii must actively invade host cells to survive, utilizing energy-dependent motility of unknown mechanism, but likely based on cytoskeletal function. We are investigating agents which interfere with cytoskeletal

function as drug targets to interrupt host cell invasion and intracellular growth of *T. gondii*. Agents that are known to disrupt microtubule function include dinitroanilines. We have investigated several members of this class of compounds for their effect on *Toxoplasma*. Dinitroaniline herbicides have been shown to be active against hemoflagellates. We assayed the effect of three of these compounds, trifluralin, ethalfluralin and oryzalin, on invasion and intracellular growth of *T. gondii*. All three agents inhibited intracellular growth of the parasite at concentrations (0.5-1.0 μM) which do not effect the morphology or growth of the host fibroblasts. Host cell growth was affected only at 1000-fold higher drug concentrations. The effect of the these drugs appeared to be on the intracellular growth of the parasite rather than on invasion, as the number of infected host cells did not vary significantly between treated and untreated cultures, but the number of parasites per infected cell was markedly depressed by drug treatment. Mutants resistant to each of these agents were selected after chemical mutagenesis, and each of these mutants was least 10-fold resistant to concentrations of the drugs that appeared to prevent normal intracellular division. Morphological changes of microtubules seen in treated parasites are evidence of the mechanism of drug effect. The dinitroanilines appear to be specific inhibitors of *T. gondii* growth, and therefore are good lead compounds for further development.

423 MOLECULAR MONITORING OF *BABESIA MICROTI* PARASITEMIA: EVIDENCE OF CHRONIC INFECTION IN HUMANS. Persing DH*, Krause PJ, Telford SR, Sikand VJ, Christianson D , Cartter M , Magera J , and Spielman A. Hartford Hospital, Hartford, CT; Harvard School of Public Health, Boston, MA; Mayo Clinic, Rochester, MN; and Connecticut Department of Health, Hartford, CT

Babesiosis is an emergent zoonosis caused by intraerythrocytic protozoa that may cause a severe, malaria-like illness but is more commonly associated with mild disease or asymptomatic infection. Treatment consists of clindamycin and quinine for severe illness but is not recommended for mild to moderate disease because of lack of known sequelae. Untreated patients apparently do well but the frequency with which chronic infection develops in other protozoal infections suggests that chronic parasftemia might be a problem in such patients. The duration of parasitemia and clinical manifestations in patients with severe babesiosis treated with clindamycin and quinine was compared to that of patients with mild babesial illness who were not treated. Subjects were monitored with thin blood smears, hamster inoculation and polymerase chain reaction (PCR) between 2-6 months and 12-16 months after the onset of illness. PCR was superior to blood smear and hamster inoculation for detection of *Babesia microti* infection. *Babesia microti*-specific nucleic acid was found in the blood of 7 of 20 (35%) untreated patients over three months after acute illness but in none of 12 treated patients ($p<0.03$). Three of the seven untreated patients with persistent parasitemia had symptoms for three or more months (and one relapsed) compared with none of the 13 untreated patients without persistent parasftemia ($p<0.03$). More than one-fourth of untreated patients with *Babesia microti* infection may have persistence of parasfte-specffic nucleic acid in their blood for more than three months after acute illness. All patients with *Babesia microti* infection should be treated to prevent long term morbidity and the increased risk of transmission of the babesial parasite through blood transfusion.

424 COMPARATIVE PATHOGENESIS OF A NEW HUMAN *BABESIA* (WA1) AND *BABESIA MICROTI* IN A SYRIAN HAMSTER MODEL. Wozniak EJ, Lowenstine L, Hemmer R, Robinson TW, and Conrad PA*. Department of Pathology, Immunology, and Microbiology, School of Veterinary Medicine, University CA, Davis, CA.

The newly recognized *Babesia* isolate (WA1), obtained from a nonsplenectomized patient in Washington, is morphologically indistinguishable from *Babesia microti* but antigenically and molecularly distinct. In recent years, WA1-type parasites have been identified as the cause of clinical babesiosis in California and Washington, including one fatal case. We utilized a Syrian hamster model to compare the pathogenesis of infection with the WA1 isolate and human *B. microti* isolates. A group of 33 adult female hamsters were intraperitoneally inoculated with either uninfected hamster erythrocytes or hamster erythrocytes infected with WA-1 or *B. microti*. All WA1-infected animals became parasitemic 3-4 days post-infection (PI) and were severely lethargic and dyspneic by 6-9 days PI. Death occurred spontaneously by 10 days PI with parasitemias of 12-90%. Hamsters inoculated with *B. microti* became parasitemic by 7 days PI and by 14 days PI they had developed peak parasitemias (50-70%) which subsequently decreased to low or undetectable levels. Although the *B. microti*-infected hamsters developed a more severe anemia, they generally remained asymptomatic. Postmortem examination of WA1-infected hamsters demonstrated necrotizing leukoclastic phlebitis, most notably in the lung, and multifocal myocardial necrosis. No vascular lesions were demonstrated in any *B. microti*-infected or control hamsters. The results of this study suggest that acute necrotizing phlebitis resulting in DIC, thromboembolism, and infarction may be central to the pathogenesis of WA1 infections. The relevance of these findings to the diagnosis and treatment of human babesiosis, particularly when attributable to WA1-type parasites, will be discussed.

425 INCREASED SEVERITY OF LYME DISEASE ILLNESS DUE TO CONCURRENT BABESIOSIS. Krause PJ*, Telford SR, Spielman A, Sikand VJ, Ryan R, Christianson D, Brassard P, Pollack R, Burke G, and Persing DH. Hartford Hospital, Hartford, CT; Harvard School of Public Health, Boston, MA; University of Connecticut Health Center, Farmington, CT; and Mayo Clinic, Rochester, MN.

Patients with Lyme disease (LD) may also experience babesiosis (B) because both infections perpetuate in North America in the same vector ticks and reservoir mice. To determine whether coinfected patient suffer a more severe illness than occurs with either infection alone, we compared the clinical manifestations of each infection alone with that of coinfected residents of Block Island, RI, and in patients of two Connecticut medical clinics. Block Island residents were subject to an active longitudinal serosurvey with intensified passive case-finding over five years. Clinic patients received enhanced diagnostic services. Resulting blood samples were analyzed serologically and by PCR for evidence of infection by the agents of LD and B. Antibody against both pathogens was noted in 1% of 1,156 surveyed residents and in 14% of 97 residents reacting against spirochete antigen. Of 215 patients on Block Island, and 38 patients in Connecticut identified with LD, 10% and 8%, respectively, had serologic evidence of concomitant B. Coinfected subjects suffered moderate to severe illness (including headache, chills, fever and sweats and persistent fatigue) more than three times as often as did those suffering from LD ($P<0.01$) or B alone ($P<0.05$). Circulating spirochetal DNA were detected more than three times as often in coinfected subjects than in those diagnosed with LD alone ($P=0.06$). In a subset of patients examined for evidence of human ehrlichiosis (E), patients with LD, B and E were more likely to have prolonged LD infection than those with LD alone. The severity and duration of illness accompanying concurrent B and LD is greater than with either infection alone. Because about 10% of New England LD patients are coinfected, the possibility of concomitant babesial infection should be considered when LD has been diagnosed.

426 IMMUNIZATION WITH RECOMBINANT OSPA PROTECTS WHITE-FOOTED MOUSE RESERVOIRS AGAINST THE AGENT OF LYME DISEASE. Rosa Brunet LC*, Katavolos P, Spielman A, and Telford SR. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Immunization with recombinant outer surface protein A (OspA) protects a variety of hosts against the agent of Lyme disease (*Borrelia burgdorferi*). We determined whether the white-footed mouse *(Peromyscus leucopus)* can similarly be protected, because the proposal to actively immunize the main enzootic reservoir by the distribution of vaccine-containing bait would be predicated on the capacity for this mouse to respond to OspA. All mice immunized with rOspA in adjuvant mounted an intense antibody response and were protected from spirochetal infection when challenged by tick-bite. Spirochetes were not retained by ticks that engorged on rOspA-immunized mice, but were present in about 85% of ticks fed on control GT-immunized mice. About 40% of larvae fed on GT-immunized mice acquired infection, while none of the 30 ticks fed on rOspA-immunized mice did so. We conclude that white-footed mice are capable of mounting an antibody response to OspA, and may thereby be rendered incompetent as reservoirs for Lyme disease spirochetes.

427 MACROGEOGRAPHIC ABUNDANCE OF *IXODES SCAPULARIS* (=*I. DAMMINI*) RELATED TO LYME DISEASE INCIDENCE BUT NOT TO DENSITY OF DEER. Wilson ML*, Bertrand MR, Kilpatrick HJ, and Cartter ML. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; Department of Environmental Protection, State of Connecticut, Franklin, CT; and Department of Public Health and Addiction Services, State of Connecticut, Hartford, CT.

Lyme disease risk in the northeastern U.S. is a function of the abundance of the vector *Ixodes scapularis* (=*I. dammini*). Although the distribution of this tick has been expanding, little comparable macrogeographic information on its abundance is available. We systematically sampled 9,012 adult ticks from 1,620 white-tailed deer examined throughout the entire state of Connecticut during November,1994 to define the tick's abundance in different habitats. The spatial pattern of relative tick abundance was compared with that of deer density and diverse environmental variables to evaluate factors that may influence tick survival and distribution. Incidence of Lyme disease cases also was compared. Various spatial scales were examined using a Geographic Information System (GIS) and spatial statistics. Although the small-scale pattern of tick abundance may be related to local deer density, the large-scale distribution of this tick appears to be influenced by environmental factors that affect its microclimate. Results from small-scale observations may be misleading in macrogeographic analyses. The relationship to Lyme disease risk is discussed.

428 *BORRELIA BURGDORERI* OspA EXPRESSION IN *IXODES SCAPULARIS*: SIGNIFICANT DIFFERENCES IN TICK LINES WITHIN AND AMONG DIFFERENT GEOGRAPHIC POPULATIONS. Burkot TR*, and Schriefer M. Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, CO.

A wide range in *Borrelia burgdorferi* OspA levels have been documented in laboratory infected *Ixodes scapularis* using an OspA antigen captureELISA. Differences in OspA levels in ticks could result from differences in the numbers of *B. burgdorferi* ingested or by tick associated factors which either enhance or suppress *B. burgdorferi* and/or OspA expression.To investigate the basis for this variation, wild caught adult *I. scapularis* from New York and Maryland were collected. OspA levels were determined by repeatedly sampling of the same ticks. Ticks were identified which developed and maintained a high level of OspA while other ticks, although infected, failed to develop significant levels of OspA over the 3 week sampling period. Resulting progeny were infected at either the larval or nymphal stages by feeding on JD1 infected mice and their OspA levels determined. Significant differences were found amongthe progeny from a tick population. In addition, there were significant differences in OspA levels between the New York and Maryland tick populations. Of particular interest was the identification of tick lines in which there was very low levels of OspA expression.

429 POSSIBLE ROLE OF A SPIROCHETE FROM LONE STAR TICKS IN AN OUTBREAK OF A LYME DISEASE-LIKE ILLNESS IN MARYLAND. Armstrong PM*, Rich SM, Spielman A, and Telford III SR. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

An outbreak of Lyme disease is said to have emerged among the residents of Gibson Island, MD during the late 1980s. Physicians diagnosed this condition in 17% of the 250 year-round residents of this community. Only one of these diagnoses, however, could be confirmed by Western blot. A survey of the island ticks indicated that Lone Star ticks, *Amblyomma americanum,* were about 20 times as abundant as deer ticks, *Ixodes dammini.* Both kinds of ticks proliferated on the island during the 1980s following an increase in the local abundance of deer during the 1970s, and spirochetal infection was detected in both. About 10% of nymphal deer ticks contained Lyme disease spirochetes, *Borrelia burgdorferi.* To determine how the spirochetes from these diverse ticks may be related, we compared their flagellin and 16s rRNA gene sequences to that of other *Borrelia* species. Lone star ticks were screened by dark-field microscopy and PCR using primers targeting a highly conserved region of the *Borrelia* flagellin gene. Of 128 adult Lone Star ticks, 2 (1.6%) contained spirochetes. Flagellin and 16s rRNA amplification products were sequenced from these ticks. Both sequences shared the most similarity with the same genes from a relapsing fever spirochete, *B. hermsii.* Flagellin and 16s rRNA gene trees were constructed by parsimony methods. Both trees indicate that this spirochete is distinct from *Borrelia burgdorferi* and is more closely related to the borrelias of soft-ticks. It may be that the spirochete present in Lone Star ticks is responsible for the numerous infections that were misdiagnosed as Lyme disease on Gibson Island.

430 NEW RISK FACTORS ASSOCIATED WITH *RICKETTSIA TSUTSUGAMUSHI* INFECTIONS IN NORTHERN THAILAND. Linthicum KJ, Tanskul P, Gordon SW, Suwanabun N, and Prachumsri J. Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

During ecology and epidemiology studies in areas of Chiang Rai Province, Thailand near the houses of patients hospitalized with confirmed serious *Rickettsia tsutsugamushi* infections we identified new associations between chiggers, rodents and *R. tsutsugamushi* within the boundaries of active rice-agriculture ecosystems, an ecological habitat not previously associated with scrub typhus. To determine risk factors associated with humans in these disease foci we conducted a retrospective and prospective cohort study. Residents of 2 villages 10 years of age or older were studied from October 1993-April 1995. Anti-*R. tsutsugamushi* IgG and IgM was found in blood samples of 53.8% and 27.4%, respectively, of the 383 persons who responded to a standard questionnaire. There was no significant difference between the prevalence of IgG antibody in males (58.8%) and females (49.5%); however, the prevalence of IgM antibody in males (35.6%) was significantly higher than females (20.4%) ($P< 0.001$). The prevalence of IgG antibody increased with age for both sexes and the greatest risk factor for past infection with *R. tsutsugamushi* was working in rice fields or plantation areas within rice fields. Active participation in rice field agriculture was also a risk factor strongly associated with recent infections. The overall minimum infection incidence rate was 0.02/person/year. Results indicate that there is considerable risk of infection when people are exposed to infected chiggers in rice fields.

431 PCR DETECTION OF *RICKETTSIA TSUTSUGAMUSHI* IN BLOOD SAMPLES COLLECTED ON FILTER PAPER. A COMPARISON WITH MOUSE ISOLATION AND SEROLOGIC DIAGNOSIS. Gordon SW*, Choksajjawatee N, Rongnoparut P, Linthicum KJ, Watt G, Watcharapichat P, and Eamsila C. Department of Entomology, USAMC, AFRIMS, Bangkok, Thailand; Department of Medicine, USAMC, AFRIMS; Department of Veterinary Medicine, Royal Thai Army Component, AFRIMS; and Department of Epidemiology, Royal Thai Army Component, AFRIMS, Bangkok, Thailand.

Between September 1994 and January 1995 patients presenting with a presumptive diagnosis of uncomplicated scrub typhus in Chiang Rai Province, Thailand were screened for enrollment in an ongoing Rifampin drug

treatment study. Blood samples from pre-enrollment screening were tested for evidence of scrub typhus infection by three methods. Samples for mouse isolation were inoculated intraperitoneally into mice within minutes following collection, while samples for PCR testing were spotted on filter paper, allowed to air dry and held at room temperature until testing. Fresh serum was separated from the sample and immediately tested by IIP. Eighty-eight patients were screened by all three methods. Seventy of 88 patients (79.5%) were positive for scrub typhus by IIP with IgM titers of > 1:400 or IgG titers > 1:1600. Equal numbers of patients 26/88 (29.5%) were positive by either PCR or mouse inoculation. There was an 88% agreement between PCR and mouse inoculation results. Twenty-three patients were positive by both tests, while 3 patients were positive by PCR and negative by mouse inoculation or the converse. All samples that were positive by either PCR or mouse inoculation were also positive by IIP. PCR diagnosis employing a simplified specimen collection, storage, and processing protocol proved to be equally sensitive to mouse inoculation in detecting *Rickettisa tsutsugamushi* in patient blood. These data suggest that the IIP test is well suited for routine use to screen patients with a presumptive diagnosis of scrub typhus. However, while both PCR and mouse isolation detected considerably fewer cases than the IIP test, only the former methods confirm active infection with pathogen.

432 TICK CELL CULTURE SYSTEM FOR PHAGOSOSOMAL RICKETTSIAE. Munderloh UG*, Kurtti TJ, Blouin EF, Kocan KM, Ewing SA, Dumler JS, and Madigan J. University of Minnesota, Department of Entomology, St. Paul, MN; Oklahoma State University, College of Veterinary Medicine, Stillwater, OK; University of Maryland, School of Medicine, Baltimore, MD; and University of California, College of Veterinary Medicine, Davis, CA.

Tick-borne rickettsiae can be divided into two groups: spotted fever rickettsiae escape from the phagosome soon after invasion, and multiply free within the host cytoplasm, while *Anaplasma, Ehrlichia* and *Cowdria* remain and multiply within the phagosome. The latter include pathogens of man and animals some of which are both long known but have not been cultured *in vitro*, or that are among recently emergent disease agents. *Anaplasma marginale*, an important cattle pathogen of world-wide distribution has only recently been cultured continuously. Among the emergent pathogens are the granulocytic ehrlichiae, not yet grown *in vitro*. We have developed a culture system that focuses on the vector stages of these pathogens using tick cell culture. The combination of a permissive cell line isolated from *Ixodes scapularis* and L-15B medium has enabled us to isolate *Anaplasma marginale* in continuous culture for the first time. This system has also proven adaptable to cultivation of *Ehrlichia canis*, and its application to the granulocytic ehrlichiae is now being explored. Infected mammalian blood cells were used to inoculate tick cell cultures. The rickettsiae invaded the cells and multiplied inside phagosomes into large colonies typical of those found in ticks. The rickettsiae were passaged continuously and were infectious for the mammalian host. Using tick cell culture we are now able study the vector phase of hitherto uncultivable tick-borne organisms *in vitro*.

433 PERPETUATION OF THE AGENT OF HUMAN GRANULOCYTIC EHRLICHIOSIS IN A DEER TICK-RODENT CYCLE. Telford III SR*, Dawson JE, Katavolos P, Warner CK, Kolbert CP, and Persing DH. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Department of Laboratory Medicine and Pathology, Mayo Foundation, Rochester, MN.

A human isolate of the agent of human granulocytic ehrlichiosis, a recently described emerging rickettsial disease, has been established by serial blood passage in mouse hosts. Larval deer ticks (*Ixodes dammini*) acquired infection by feeding upon such mice, and efficiently transmitted the ehrlichiae to uninfected mice after molting to nymphs, thereby demonstrating vector competence. The agent was detected by demonstrating Feulgen positive inclusions and by polymerase chain reaction within the salivary glands of the experimentally infected ticks and from field derived adult and nymphal deer ticks. White footed mice (*Peromyscus leucopus*) from a field site infected laboratory reared ticks with the agent of HGE, suggesting that these rodents serve as reservoirs for ehrlichiae as well as for Lyme disease spirochetes and the piroplasm that causes human babesiosis. About 10% of host seeking deer ticks were infected with ehrlichiae, and of these, 20% also contained spirochetes. Cotransmission of diverse pathogens by the aggressively human-biting deer tick may have a unique impact on public health in certain endemic sites.

434 NATURAL KILLER CELL AS AN EFFECTOR OF NON-SPECIFICIMMUNITY TO RICKETTSIAE. Billings AN*, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX.

The natural killer (NK) cell's role in effecting an immune response to an obligate intraendothelial infection is not known. Our study was performed in order to address the importance of NK cells in immunity to a spotted fever group (SFG) rickettsial infection determined by both *in vivo* and *in vitro* models. The NK cell effect on morbidity,

cytotoxicity, and rickettsial content was evaluated, and NK cell activity, histopathology, and immunohistology were analyzed. A new SFG rickettsial murine model was developed in order to specifically examine NK cells. In vitro correlates of NK activity were approached by comparison of the effect of exposure of infected and non-MHC matched endothelial cells to splenocyte populations containing NK cells. We found that NK cell activity is significantly activated and increased in infected mice. Also, treatment of mice with a monoclonal antibody to an NK cell surface protein designed to effect NK cell depletion resulted in earlier onset of symptoms in mice infected with sublethal rickettsial dose. The importance of NK cells in innate immunity to SFG rickettsiae was observed. We have shown that NK cells are activated during a SFG rickettsial infection. We hypothesize that a critical factor in resisting mortality in rickettsial infection may be due to an early strong innate immune response involving NK cells during the pathogen's incubation period.

435 EXPRESSION OF *RICKETTSIA RICKETTSII* rOmpA RECOMBINANT FRAGMENT IN MYCOBACTERIA. Crocquet-Valdes PA*, and Walker DH. Department of Pathology, The University of Texas Medical Branch, Galveston, TX.

Rickettsia rickettsii is an obligately intracellular bacterium in the spotted fever group (SFG). The public health importance of SFG rickettsioses is under-recognized due to the lack of a distinct clinical presentation and timely laboratory diagnostic methods. rOmpA, a surface-exposed immunodominant protein of SFG rickettsiae, is involved in attachment of rickettsiae to the host cell. Vaccine studies have mostly relied on crude recombinant lysates of rOmpA expressed in *E. coli* and yielded partial-to-complete protection in mice and guinea pigs. To determine if a live rOmpA recombinant vaccine would be more effective, mycobacterial integrative vectors were used to express portions of R. rickettsii rOmpA in *Mycobacteria smegmatis*. A pUC19-derived plasmid named pUS909 containing the IS900 insertion sequence for integration in the mycobacterial chromosome was used to subclone a 1.6-kb fragment of rompA downstream from the promoter and in frame with the coding sequence of the 18-kDa antigen of M. leprae. The recombinant construct was electroporated into *M. smegmatis*, and an expected efficiency of transformation of 1.8 102 CFU/μg DNA was obtained. A 75-kDa fusion protein was detected in the transformants containing the rickettsial insert but not in the parental constructs or the untransformed mycobacteria by Western blot analysis with mouse polyclonal sera raised against the rOmpA recombinant protein. Southern blot analysis will determine vector integration into the mycobacterial chromosome and copy number. To achieve the best vaccine potential, the recombinant rickettsial fusion protein of rOmpA and the *M. leprae* 18 kDa antigen will be expressed in *M. bovis* BCG strain.

436 PRELIMINARY RESULTS OF LONGITUDINAL STUDIES OF SIN NOMBRE HANTAVIRUS IN DIVERSE ECOSYSTEMS IN COLORADO, 1994-95. Beaty BJ, Calisher CH*, Sweeney W, Canestorp KM, Davis T, and Mills JN Colorado State University, Fort Collins, CO; U.S. Fish and Wildlife Service, Model, CO; Colorado Department of Health, Denver, CO; and DVRD, NCID, CDC, Atlanta, GA.

Beginning in June 1994, longitudinal studies of Sin Nombre virus (SNV) (Bunyaviridae, Hantavirus) were initiated at three locations in Colorado. Selection of each location was based on proximity to human cases of Hantavirus Pulmonary Syndrome and on ecosystem diversity. Regular mark-recapture trapping of small mammals has revealed the presence of antibody to a hantavirus, presumably SNV or closely related viruses at all sites. At site A, in eastern Colorado, antibody has been detected in *Reithrodontomys megalotis* (western harvest mouse) and in *Peromyscus truei* (piñon mouse) but not in *P. maniculatus* (deer mouse) or in rodents of other species. At site B, in western Colorado, only *P. maniculatus* have been shown to have antibody to SNV. At site C, also in western Colorado, single *P. maniculatus* and *P. truei* have been shown to have antibody to SNV. Seroconversions have been detected in three of 10 recaptured *P. maniculatus* at site B, one between late July and early September 1994 and two between late October 1994 and early May 1995. None of three *P. maniculatus* at site A or one *P. maniculatus* at site C seroconverted. These preliminary results suggest that longitudinal mark-recapture studies at these sites will yield information useful to our understanding of the persistence and transeasonal maintenance and transmission of SNV and other hantaviruses in Colorado.

437 DISTRIBUTION AND PREVALENCE OF ANTIBODY REACTIVE WITH SIN NOMBRE VIRUS AMONG RODENTS IN THE MAJOR HABITAT TYPES IN THE SOUTHWESTERN UNITED STATES. Mills JN*, Ksiazek TG, Rollin PE, Nichol ST, Ellis BA, Yates TL, Gannon WL, Levy CE, Engelthaler DM, Davis T, Tanda D, and Frampton W. Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Department of Molecular Microbiology and Immunology, Johns Hopkins University, Baltimore, MD; Department of Biology, University of New Mexico, Albuquerque, NM; Vector-Borne and Zoonotic Disease Section, Arizona Department of Health Services, Phoenix, AZ; Colorado Department of Public Health and Environment, Denver, CO; and Bureau of Epidemiology, Utah Department of Health, Salt Lake City, UT.

During May through September 1994, we trapped 3175 small mammals of 79 species to determine the distribution and prevalence of infection by Sin Nombre virus (SNV) or related viruses in rodent populations in the 9 major biotic communities of the American southwest. The communities ranged from lower Sonoran desert to alpine tundra in each of 4 states (AZ, NM, CO, UT). Antibody reactive with SNV was found in rodents from all communities (overall prevalence=6.2%), although prevalence was lowest at the altitudinal and climatic extremes [desert (0.4%) and alpine tundra (2.0%)]. Deer mice (*Peromyscus maniculatus*) were present at high densities (>20% of total captures) in all habitats except desert (2.8%). Antibody was found in 10% of 926 deer mice and 20% of 292 *P. boylii* (brush mice). Prevalence of seropositive deer mice varied from 0% in desert, to 4% in chaparral, to 17% in pinyon-juniper woodland. Brush mice were largely restricted to chaparral, pinyon-juniper, and montane forest, but were frequently infected only in chaparral (28%) and montane forest (26%). These were habitats where deer mice were rarely infected (4-5%). Conversely, in pinyon-juniper (where deer mice were most frequently infected), antibody prevalence in brush mice was relatively low (9%). Other species of *Peromyscus* were frequently infected, but captures were rare and restricted to a few habitats [*P. eremicus* --(15%; n=64) in desert and chaparral, *P. leucopus* --(16%; n=32) in grassland and pinyon-juniper, *P. truei* --(4%; n=97) in pinyon-juniper]. Sera from *Reithrodontomys megalotis* commonly reacted with SNV antigen (23%; n=34), and the species was largely restricted to grassland.

438 SURVEILLANCE FOR HANTAVIRUS ANTIBODY IN TEXAS RODENTS. Rawlings JA*, Tabony LJ, Hunt PR, and Regner GD. Infectious Disease Epidemiology and Surveillance Division, Texas Department of Health, Austin, TX; and Bureau of Laboratories, Texas Department of Health, Austin, TX.

In May 1993 an outbreak of illness, now known as hantavirus pulmonary syndrome (HPS), and characterized by flu-like symptoms that rapidly progress to fulminant respiratory failure, was recognized in the western US. Since then, over 100 human cases have been reported; three occurred in Texas. HPS is caused by newly described members of the hantavirus family. The best known is Sin Nombre virus, the cause of the 1993 outbreak. In nature, hantaviruses infect rodents, which shed virus in their urine, feces, and saliva. Because the Texas Department of Health has been interested in determining the prevalence of infection in Texas rodents, 318 banked sera were analyzed by enzyme immunoassay. The specimens, originally tested for antibody to *Borrelia burgdorferi* or *Yersinia pestis*, were collected from February through November 1991. They were from three sites in east, west, and south central Texas. Between February 6 and June 4, 183 specimens from 10 species were collected in Lubbock County; 14 (8%) had significant antibody titers to Sin Nombre virus. Ten of the 14 positive sera were from hispid cotton rats (*Sigmodon hispidus*). Of the 64 specimens from three rodent species collected in Anderson County between March 28 and November 19, 26 (41%) were positive. In this case, all of the positive sera were from hispid cotton rats. None of the 71 specimens collected from Bexar County between June 3 and November 15 had significant antibody titers.

439 SEROLOGICAL SURVEY ON *HANTAVIRUS* HUMAN INFECTION IN JUQUITIBA STATE OF SAO PAULO, BRAZIL. Iversson LB*, Branquinho MS, and Rosa MD. School of Public Health/University of Sao Paulo, Brazil; Department of Endemic Control(SUCEN)/Health Department, Sao Paulo, Brazil.

Hantavirus Pulmonary Syndrome (HPS) caused by new Hantavirus was diagnosed in December 1993 in Juquitiba, one of the 37 districts of the Sao Paulo metropolitan area, Brazil, in three inhabitants of a rural area,who presented respiratory disease with fast evolution and two subsequent deaths. Neutralizing antibodies against Seoul and Puumala hantaviruses have been identified in the State of Sao Paulo since 1991 in human sera which were collected from 1976 to 1990. Our objective was to investigate the previous presence and the prevalence of Hantavirus human infection in Juquitiba. Sixty two sera collected between May-June 1990 for a malaria survey in inhabitants at age 17 to 86, in the district of Senhorinhas II, rural area, were processed by an immunoenzyme technique, for the detection of IgG antibodies against Hantaan antigen (HTN-strain 76118). Thepositive sera were subsequently tested against recombinant nucleoprotein of Sin Nombre virus (SN), the main agent of HPS in the E.U.A. There were antibodies to HTN antigen in 18 sera (29%), (8 in dilution > and = 1/200). Five of these 18 sera also reacted with SN antigen. The prevalence of positive serology was similar in male and female, but it increased gradually according to age. Thirteen of the 18 persons hadinhabited this location for a period of 10 years or more, and the other 5 persons for a period of 3 to 8 years. The result suggests that hantaviruses antigenically similar to Sin Nombre and Hantaan viruses were circulating in humans in that area prior to the diagnosed human disease.

440 INVESTIGATION ON CASE-CONTACTS OF HUMAN DISEASE CAUSED BY *HANTAVIRUS* IN JUQUITIBA, STATE OF SAO PAULO, BRAZIL. Zaparoli MA, Iversson LB*, Rosa MD, Travassos da Rosa E, Pereira LE, Rollin P, and Peters CJ. Health Department of the State of Sao Paulo; School of Public Health/University of Sao Paulo; Evandro Chagas Institute; and Centers for Disease Control and Prevention, Atlanta, GA.

Pulmonary Syndrome by Hantavirus was diagnosed in December 1993 in Juquitiba, Sao Paulo metropolitan area, in three brothers inhabiting the rural area, with subsequent death of two of them. The results of the seroepidemiological investigation carried out in contacts of these cases are presented. Sera of 49 contacts at age 5 months to 65 years, living in the neighbourhood of the cases, were tested by enzyme immunoassay (ELISA) for IgG and IgM antibodies against antigen of Haantan virus (HTN-strain 76118). The positive sera were processed by immunofluorescence against HTN and by ELISA against the antigens of Seoul, Prospect Hill, Sin Nombre (SN) and Puumula viruses. Three contacts were HTN-positive by ELISA IgG or IgM, two of whom with positive ELISA IgG for the antigen and the recombinant nucleoprotein of the Sin Nombre virus. A fourth contact had only IgG antibodies to SN antigen. Two contacts were close friends who used to visit the cases and eat meals which were exposed there to rodent excreta. In this house, internal storage of animal feed may explain the presence of rodents. The other two contacts, father and son, livedabout 6 miles from the cases, also in a rodent-infested house. Apparently, the cases and their contacts became infected in rodent-infested domiciles, in an area where there was no concern about the removal of the excreta of these animals.

441 EMERGENCE OF HANTAVIRUS PULMONARY SYNDROME IN ARGENTINA. Levis SC, Briggiler AM, Cacass M, Peters CJ, Ksiazek TG, Cortes J, Lazaro ME, Resa A, Rollin PE, Pinheiro FP, and Enriz D. Instituto Nacional de Enfermes Virales Humanes (INEVH), pergamino, Argentina; Hospital de Oran, Salta, Argentina; Centers for Disease Control and Prevention, Atlanta, GA; Hospital de Él Bolson, Rio Negro, Argentina; and HCP/HCT-Pan American Health Organization.

Since 1985, isolated acute cases of hantavirus infections were seen at INEVH, presenting with clinical manifestations of hemorrhagic fever with renal syndrome. In 1993, hantavirus pulmonary syndrome (HPS) was first described in the USA as a new disease with a high mortality, induced by a novel Hantavirus (Sin Nombre Virus - SNV). In Argentina we have tried to identify, prospectively and retrospectively, human Hantavirus infections presenting in the form of HPS. As an operational case definition we have used febrile ilness followed by an unexplained respiratory distress syndrome (ARDS) in a previously health person. Serum samples were tested for Hantavirus IgG and IgM antibodies to SNV by ELISA. Up to May 1995, three foci of HPS cases were detected: 1) Oran, Salta province, Northwestern Argentina, a subtropical habitat near the Bermejo rive. Cases of ARDS of unknown etiology have been identified there since early 1980s. From 1991 to 1995, 18/22 identified cases had positive Hantavirus IgM antibodies. Antibody prevalence in a sample of 135 residents in the area was 1.5% (2/135). 2) Central Argentina (North of Buenos Aires province and south of Santa Fe province), a region of humid plains and temperate climate. Between 1990 and 1995, 6/9 cases had positive IgM antibodies to SNV. Interestingly, the last two cases, seen in the spring of 1994, had a common activity (nutria hunters) and lived in the same location: Lechiguanas Islands, a marshy area located in the estuary of the Parana river. In southern Argentina, in a region bodering the Andean range, a family outbreak occurred in March-April 1995 and 2/3 affected members died. During theinvestigation we also identified three other ARDS cases from the previous year. Available serum samples from the last two cases were positive by IgM ELISA. In this area Hantavirus IgG antibody prevalence in 23 HPS case cntacts was 8.7% (2/23). The distribution of these cases, detected due to their presentation in clusters, in three distant locations with very different environmental conditions suggests Argentina has a wide geographic distribution of Hantavirus disease. Studies to identify rodent reservoirs are currently being performed.

442 TOTAL SERUM (S.) CHOLESTEROL (CHOL), HDL CHOLESTEROL AND TRIGLYCERIDES (TG) AS PREDICTORS OF CLINICAL SEVERITY IN HANTAVIRUS (HTV) INFECTION. Clement J*, Colson P, Mc Kenna P, and Heyman P. Belgian Zoonosis Workgroup, Queen Astrid Military Hospital, Brussels, Belgium.; and Centre de Santé des Fagnes, Chimay, Belgium.

Recent reports suggest that disturbed s.lipid levels can give an indirect measure of pro-inflammatory cytokine bioactivity, playing probably a key role in HTV pathogenesis. Decreased s.CHOL and increased s.TG levels have already been reported in French and in Finnish Puumala (PUU) cases. During a 1993 PUU-induced HTV epidemic in the Belgian Ardennes, we found in a total of 42 patients at the peak of the disease a significant decrease of total s.CHOL (mean 141 ± 91 mg%)(N = 58) and of s.HDL chol. (mean 14± 20 mg%)(N = 43), when compared to s. levels after recovery, being 238 ± 106 mg% (N = 57) for s.CHOL, respectively 52 ± 32 mg% (N =56) for s.HDL chol. (X^2 = 35.22, $p < 0.001$, respectively X^2 =26.28, $p < 0.001$). Conversely, and despite an episode of anorexia and vomiting in most patients, s.TG levels during PUU disease rose significantly higher (mean 329 ± 298 mg%)(N = 56) than after recovery (mean 199 ± 407 mg%)(N = 56)(X^2 = 22.13, $p < 0.001$). Lipid perturbations were most outspoken in severe PUU cases, and often went together with major complications. The lowest recorded s.CHOL (53 mg%),respectively the lowest s.HDL (5 mg%) were found each time in the 2 most outspoken cases of PUU-induced non-cardiogenic adult respiratory distress syndrome (ARDS). Significant correlations were found between the degree of thrombocytopenia and s. total CHOL ($R = 0.52$ $p < 0.001$), respectively s.HDL chol. ($R = 0.45$ $p < 0.01$). Infection hypertriglyceridemia may be caused by increased hepatic lipogenesis, together with decreased lipoprotein lipase activity, both induced by pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6. Hypocholesterolemia and low

HDL may be linked in part to cytokine-induced reduction of hepatic lipase. If confirmed in other HTV infections, e.g. ARDS induced by the American Sin Nombre virus, s. lipid perturbations could be used as an easy and quick way of assessing, and even of predicting, clinical severity.

443 SABIÁ VIRUS GENOME STRUCTURE AND PHYLOGENY. Gonzalez JP*, Bowen MD, Nichol ST, and Rico-Hesse R. Institut Franais de Recherche Scientifique pour le Développement en Coopération, Paris, France; Special Pathogens Branch, Centers for Disease Control & Prevention, Atlanta, GA; and Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

Sabiá virus is one of five arenaviruses from South America which are known to be human pathogens, including Junín, Machupo, Flexal, and Guanarito. This virus emerged in 1990, as it was isolated from a fatal case of haemorrhagic fever in Sào Paulo, Brazil. The arenavirus genome consists of two single-stranded RNA segments with one large segment (L) that codes for the viral polymerase and a non structural protein, and a small (S) segment that codes for the nucleocapsid and glycoproteins. We determined the nucleotide sequence of the total S RNA fragment of the Sabià virus prototype strain (SP114202). Direct sequencing of the viral RNA and multiple PCR product sequencing were used to determine the sequence of the Sabiá virus S RNA segment (3366 nucleotides long). This information allowed us to: 1) compare the N and GPC genes to those reported for other arenaviruses to confirm a common structure in this family of viruses, 2) determine genetic relationships between arenaviruses from the New World, using the more conserved N gene sequences in phylogenetic analyses, 3) predict the secondary structure of the intergenic region of the Sabià S RNA which is distinct because it contains 3 stem-loop structures instead of 1 or 2 as described for other arenaviruses, and 4) confirm that the 3'end of all S RNAs of the arenaviruses studied to date contain a conserved 19-nucleotide-long sequence which can be utilized in the design of diagnostic tools. The phylogenetic trees generated by maximum parsimony analysis confirmed that Sabiá virus is distinct from all others in the family and it shares an ancestor with Junín, Machupo, Tacaribe and Guanarito. This suggests that human pathogenic and non-pathogenic arenaviruses have a common origin and have developed pathogenic mechanisms or tropisms independently.

444 EFFECT OF INFECTION WITH GUANARITO VIRUS (ARENAVIRIDAE) ON FECUNDITY IN *ZYGODONTOMYS BREVICAUDA*. Fulhorst CF*, Tesh RB, Ksiazek TG, Salas RA, and Peters CJ. The University of Texas Medical Branch at Galveston, Texas; National Center for Infectious Diseases, Atlanta, Georgia; and Instituto Nacional de Higiene, Caracas, Venezuela.

The cane mouse *Zygodontomys brevicauda* is a natural host for Guanarito virus (GV). In a recent laboratory study on GV infection in *Z. brevicauda*, a majority of the inoculated mice were chronically infected and persistently shed virus in urine and saliva; and few of the infected female mice caged with infected or uninfected mates whelped a litter. The latter finding suggested that GV infection affects fecundity in cane mice. To establish further the effect of GV infection on fecundity in *Z. brevicauda*, 11 adult female mice inoculated with the virus and 11 sham-inoculated counterparts were caged in monogamous pairs with adult male mice. At 92 days post-inoculation, two of the mice inoculated with virus and ten of the sham-inoculated mice had whelped a litter. This finding indicates that GV infection has a strong negative effect on fecundity in experimentally infected cane mice. Results from tests for GV in the progeny and mates of the infected mice will be presented.

445 INVESTIGATION OF A FAMILIAL OUTBREAK OF VENEZUELAN HEMORRHAGIC FEVER. Salas R*, Tesh R, Manzione N, Fulhorst C, Utrera A, Duno G, Aranson J, Miller E, and Ksiazek T. Instituto Nacional de Higiene, Caracas, Venezuela; University of Texas Medical Branch at Galveston, Texas; Universidad de los Llanos, Guanare, Venezuela; and National Center for Infectious Diseases, Atlanta, GA.

After a 15 month period of quiescence, 11 cases of Venezuelan hemorrhagic fever (VHF) occurred between November 1994 and February 1995 in rural areas of Portuguesa and Barinas states. Two of the cases (one fatal) were husband and wife. The husband (the index case) worked as a day laborer clearing land prior to the onset of his illness; the wife (a homemaker) became ill 15 days after her husband was hospitalized; and the four children in the family remained healthy and did not develop antibodies to Guanarito virus (GV), the etiologic agent of VHF. Histopathological examination of autopsy material from the wife revealed focal hepatic necrosis with eosinophilic deposits in hepatocytes similar to that described for other arenaviral hemorrhagic fevers. During the epidemiological investigation of the outbreak, several isolations of GV were made from wild rodents trapped within 200 m of the family's house. Although all members of the family potentially were exposed to infected rodents in the vicinity of the house, the short period of time between the husband's illness and theonset of the wife's illness suggests that the wife acquired the virus from her husband. Results from the epidemiological investigation and autopsy of the fatal case will be presented.

446 BOLIVIAN HEMORRHAGIC FEVER: INTRAFAMILIAL OUTBREAK IN THE ABSENCE OF EPIDEMIC DISEASE. Kilgore PE, Ksiazek TG, Mills JN, Rollin PE, Peters CJ, Pinheiro FP, Enria DA, McKee KT, Glass RI, Villagra MR, Pozo SI, and Arce RB. Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Instituto Nacional de Enfermedades Virales Humanas (INEVH), Pergamino, Argentina; Division of Communicable Disease Division, Pan American Health Organization, Washington, DC; Communicable Disease Unit, Womack Army Medical Center, Fort Bragg, NC; National Health Secretary for Bolivia, La Paz, Bolivia; and University of San Simon de Major Medical School, Cochabamba, Bolivia; Magdalena Hospital, Magdalena, Beni Department, Bolivia.

In July and August 1994, an outbreak of BHF occurred in Magdalena, Bolivia among seven members of an extended family. We investigated the family outbreak and determined the prevalence of Machupo antibody among community members of Magdalena and surveyed rodent populations to identify potential reservoirs for the virus. BHF was confirmed by virus isolation or detection of circulating viral antigen in 5 family members from whom material was available and the index case was diagnosed by ELISA detection of IgM and IgG antibody. Rodent trapping in areas of potential exposure for the index case yielded nine *Calomys callosus*, the rodent host of Machupo virus and no *C. callosus* were trapped in Magdalena. BHF occurred in this extended family following the infection of the index case in agricultural areas where few rodent hosts were trapped. A clear mode of virus spread within the family could not be identified but aerosol transmission and direct contact appeared to be most likely. Primary and secondary transmission of BHF may be prevented if medical staff were trained to use barrier nursing precautions routinely and identify BHF early. Health education focused on agricultural workers in endemic areas may prevent transmission of Machupo virus in rural areas. Control of *C. callosus* in the endemic area appears effective in preventing community outbreaks of BHF which have been absent since the 1970s.

447 CASE CONTACT TRACING DURING AN EPIDEMIOLOGIC INVESTIGATION OF AN ARENAVIRUS INFECTION. Armstrong LR*, Khan AS, Russi MB, Rollin PE, and Peters CJ. Special Pathogens Branch, Division of Viral and Rickettsial Diseases, National Centers for Infectious Disease, Centers for Disease Control and Prevention (CDC), Atlanta, GA; Epidemology Program Office, Division of Training, CDC, Atlanta, GA; and Occupational Medicine Program, Internal Medicine, Yale University, New Haven, CT.

In August 1994, a laboratory worker in the United States was infected with Sabia virus, a newly discovered arenavirus. Sabia virus, previously known to cause hemorrhagic fever disease in two individuals, was first isolated from a fatal case in Brazil. Arenavirus diseases are usually transmitted by close personal contact but occasionally are associated with intrafamilial or nosocomial epidemics. This case is the third known occurrence of infection with Sabia virus and the second infection caused by a laboratory exposure. Measures were quickly initiated to prevent transmission of the disease to the patient's contacts and health care workers. All individuals who had come into contact with the patient or his laboratory specimens were administered a questionnaire and were instructed to monitor their temperature and symptoms twice daily for 3 weeks. In the event of fever of 101°F or greater, case-contacts were instructed to contact on-call physicians who were familiar with the index case and who had set up a formal triage system for the evaluation of possible secondary cases. Five categories based on graduated levels of risk were established for case-contacts. No case-contact had an exposure which would place them in the highest risk group (level 1). Ten case-contacts were categorized at level 2, 66 at level 3, 53 at level 4, and 10 at level 5. No significant illness occurred and blood samples collected from casecontacts at the time of enrollment and at 6 weeks followup were negative for Sabia virus antibodies by enzyme-linked immunosorbent assay.

448 ABRUPT ONSET OF MALARIA AMONG THE YANOMAMI AMERINDIANS. Laserson KF*, Petralanda I, Almera R, Alvarez R, Matos A, Bolivar M, Gonzalez M, Jank M, Lopez A, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Centro Amaz. para la Invest. y Control de Enferm. Trop., "Simon Bolivar", C.A.I.C.E.T., Pto. Ayacucho, VZ; SNEM, Santo Domingo, Rep. Dominicana; New Tribes Mission, Pto. Ayacucho, VZ.

The Yanomami Amerindians of the Brazilian and Venezuelan Amazon live in isolated communal housing units *(shabonos)*. Previous epidemiological data suggested that this population of 10,000 people was susceptible to epidemics of malaria. We sought to determine whether the traditional lifestyle of Yanomami communities and their isolation from each other renders these people vulnerable to epidemic malaria. An 18 month prospective cohort study of malaria in three distinct Yanomami villages confirmed that infections of *Plasmodium vivax* were present among this population at low levels (0.5-7%), often asymptomatically, throughout the study period. By contrast, *P. falciparum* appeared only in outbreaks: one of these struck an isolated village that was in the process of being abandoned. The epidemic curve of *P. falciparum* was steep and involved 46% of the population (33/71). All age groups were equally affected and 94% of those infected were symptomatic (31/33). Many of those affected walked 5 days to another village seeking treatment. In this village a second outbreak of *P. falciparum* struck 45% of the

residents three weeks later (25/55). Again, all age groups were affected and 92% of those infected were symptomatic (23/25). We conclude that *P. vivax* malaria is endemic in these *shabono* communities, but that *P. falciparum* malaria is unstable. The resulting outbreaks develop rapidly and cause disease in large portions of each community. The communal style of living and the isolation of the *shabonos* contribute to abrupt outbreaks of malaria among the Yanomami.

449 CONGENITAL MALARIA: A PAN-AFRICAN SURVEY. Fischer PR*. Department of Pediatrics, University of Utah, Salt Lake City, UT.

For decades, congenital malaria has been considered to be "rare." Recent reports, however, show that plasmodial infection may occur in up to 29% of newborns in Africa. This study was designed to identify the current incidence of congenital malaria at several sites spanning sub-Saharan Africa and to explore factors that could be influencing the incidence of congenital malaria. Blood from 100 consecutive mother-newborn pairs was tested for malaria at each of five sites in tropical Africa. Rates of maternal and neonatal infection were compared with epidemiologic information from each site. Key results are shown in the table. It is concluded that: 1) congenital malaria is not uncommon, but there is a wide variation in its incidence in sub-Saharan Africa, and, 2) there is an apparent inverse relationship between the risk of congenital malaria and the use of prenatal prophylaxis, but neither maternal infection rate nor season is closely associated with the risk of neonatal infection.

Site	Season	Prophylaxis Used	Mothers +	Newborn +	Ratio
Nigeria	dry	88%	5	3	0.60
Zaire	wet	80%	23	17	0.74
Tanzania	dry	0%	14	0	0.00
Zimbabwe	wet	rare	4	0	0.00
Madagascar	dry	64%	24	23	0.96

450 EFFECT OF NEW AND PERSISTENT MALARIA INFECTIONS ON THE HEMATOLOGIC STATUS OF CHILDREN AND WOMEN IN WESTERN KENYA. Bloland PB*, Boriga DA, Ruebush TK, Oloo AJ, McCormick JB, Lal AA, Nahlen BL, and Campbell CC. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

To better understand the natural history of *Plasmodium falciparum* associated anemia, data collected from a large cohort of Kenyan mothers, infants, and older children followed monthly between July 1992 and June 1994 were analyzed. Parasitologic status at each visit was categorized into parasite-free, recently cleared, recently infected, and persistently infected based on blood smear results from that visit and the previous monthly visit. Clinical and hematologic outcomes and potential risk factors were compared within and between groups. The number of new *P. falciparum* infections/person-year was 2.7 among infants; incidence did not differ significantly for children > 5 years or non-pregnant women (between 1.6 and 1.7 infections/person-year). While no difference in mean hemoglobin (Hb) concentration was noted in children > 5 years between parasitologic status categories, children < 5 showed large differences, with the lowest mean Hb concentration in infants with persistent infections (8.5 g/dl). This study suggests that persistent infections may account for much of the anemia in children < 5 years and has important implications for the continued use of ineffective antimalarials in areas with multi-drug resistant malaria and for anti-disease vaccines which limit, but do not clear, parasitemia.

451 PARASITOLOGIC AND HEMATOLOGIC VARIABLES ASSOCIATED WITH MORTALITY AMONG INFANTS IN WESTERN KENYA. Koumans EH*, Hightower AW, Bloland PB, Lal AA, Oloo AJ, and Nahlen BL. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

As part of a larger prospective study of malaria in children in western Kenya, an area with hyperendemic *Plasmodium falciparum* transmission, we conducted a nested case-control study to identify parasitologic and hematologic variables associated with death. Infants who died since the start of enrollment (cases) were matched with randomly selected live infants by month of birth and sex (controls); controls were followed until the death of the matched case. All had monthly blood smears and hemoglobin (hb) determinations from birth. The overall infant mortality rate in the area is 145/1000. The analysis included 117 cases and 566 controls. Risk of dying was associated with the number of months of parasitemic episodes, OR=1.13 (95%CI 1.04, 1.23). Each additional month with infection contributed significantly to mortality. First or last parasitemia density (before death) did not differ among cases or controls. A single hemoglobin of <5.0, 8.0, or <11 g/dl at any time was not a risk factor for death,

nor were the first or last hemoglobin determinations (before death) significantly different. However, the risk of dying was associated with the number of months with mild (hb <11.0g/dl) or moderate (hb <11.0g/dl) anemia, OR=1.2 (95%CI 1.08, 1.33), and OR=1.3 (95%CI 1.12, 1.53). Results suggest that early intervention among infants to prevent malaria and associated anemia may reduce mortality in this area.

452 *PLASMODIUM FALCIPARUM* GAMETOCYTEMIA IN KENYAN CHILDREN. Jones TR*, McElroy PD, Oster CN, Beier JC, Oloo AJ, Onyango FK, Chumo DK, Sherwood JA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; U.S. Army Medical Research Unit-Kenya; School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA; and Kenya Medical Research Institute, Kisumu, Kenya.

A recent study showed that asexual *Plasmodium falciparum* parasitemia density in Kenyan children is associated with entomologic inoculation rate (EIR) measured prior to measurement of asexual parasitemia. This study examines whether sporozoite burden, as represented by EIR, associates with the prevalence or density of gametocytemia in Kenyan children. Approximately 50 children (0.5 to 6 y.o.) were enrolled and a new cohort entered the study each month for 19 months. Upon entry, each subject had blood films taken on days 0, 7, and 14. All subjects were treated with Fansidar on day 0. EIR was calculated for the 28 day period ending 14 days prior to enrollment; the relationship between blood film data from day 7 and exposure variables was explored. As reported by others, we found younger children (<2 y.o.) were more likely to be gametocytemic than older children (4-6 y.o.) ($p=0.004$) and, if gametocytemic, were more likely ($p<0.001$) to have a dense gametocytemia (>72 gametocytes/μl). Interestingly, there was also an inverse relationship between the number of infective bites received and prevalence but not density of gametocytemia, even after age adjustment. Concordance between gametocytemia prevalences on days 0 (64%), 14 (48%), and 7 (66%) was poor; 84% of children were positive on at least one day. This indicates that many subjects moved in and out of detectable gametocytemia over 14 days. Under these holoendemic transmission conditions, EIR can inversely correlate with prevalence of gametocytemia, and point measurements of the gametocytemia by conventional microscopy underestimate the size of the pool of infective donor hosts.

453 FIELD EVALUATION OF A POLYMERASE CHAIN REACTION-BASED NONISOTOPIC LIQUID HYBRIDIZATION FOR MALARIA DIAGNOSIS. Oliveira DA*, Shi YP, Oloo A, Anyona D, Nahlen B, Hawley W, Holloway BP, and Lal AA. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Vector Biology and Control Research Center, Kenya Medical Research Institute, Kisumu, Kenya; and Biotechnology Core Facility, Scientific Resources Program, CDC, Atlanta, GA.

We recently reported the development of a PCR-based nonisotopic liquid hybridization for detection of malaria parasite. Here we report results of a blind field study to assess the sensitivity and specificity of this assay. A Giemsa-stained thick smear reading of 100 (1,000x) microscopic field was used as the gold standard reference test. A total of 100 bloodsamples from a malaria endemic region in Kenya were tested. Sensitivity, specificity, positive and negative predictive values of the liquidhybridization assay were 98.6%, 100%, 100% and 96%, respectively. One sample negative by microscopy and positive by the liquid hybridization method tested positive upon re-examination by microscopy. The detection limit of the test was 0.0003% parasitemia (1 parasite/300 white blood cells). Eight samples were diagnosed by the hybridization method as being mixed infections. The re-examination of thick smears showed that 50% of these samples had been misdiagnosed as single species by microscopy. Four samples diagnosed as mixed infections by microscopy and single infection by the hybridization test showed no evidences of a second *Plasmodium* species upon re-examination of the slides. We conclude that the PCR-based nonisotopic liquid hybridization assay performed better than conventional light microscopy in detecting low grade parasite infection and offers a significant advantage when detecting mixed infections.

454 GIS/DGPS APPLIED TO THE ASEMBO BAY COHORT PROJECT. Hightower AW*, Hawley WA, Nahlen BL, Koumans EH, Lal AA, and Oloo A. Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; Division of Parasitic Diseases, NCID, CDC, Nairobi, Kenya; Division of Parasitic Diseases, NCID, CDC, Kisumu, Kenya; Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; and Kenya Medical Research Institute, Kisumu, Kenya.

Differential Global Positioning System (DGPS) technology was used to map six adjacent villages participating in the Asembo Bay Cohort Project in Western Kenya for use in a Geographic Information System (GIS). This technology, which requires simultaneous use of two GPS units reduces errors of measurement to less than five meters versus 100 meters for readings from a single GPS unit. The longitudinal nature of this malaria cohort study and the fact that all data are collected with household identifiers present unique opportunities for multi-disciplinary spatial analyses. The longitude, latitude, and altitude of study households, mosquito breeding sites, local health care clinics, roads, rivers, the shoreline of Lake Victoria, and other features was computed and entered into a database file.

Study databases were then linked to the map feature databases via GIS software. Buffer zones were created around the lake shore and mosquito breeding sites to summarize data for households inside and outside the buffers. We will review how DGPS technology works, steps taken to verify the accuracy of the points, personnel and time commitments needed, and how the DGPS readings are used to create a GIS map file. Steps needed to link GIS maps to study databases will be presented. Preliminary data from two studies (entomology and childhood mortality) will be used to show how GIS is being used to investigate spatial hypotheses.

455 MALARIA AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AMONG SUGAR ESTATE WORKERS, M ALAWI, 1994. Nwanyanwu OC*, Kumwneda N, Jemu S, Ziba C, Kazembe PN, and Redd SC. Malawi Ministry of Health, Lilongwe, Malawi; and Centers for Disease Control and Prevention, Atlanta, GA.

In sub-Saharan African countries where malaria is endemic, fever presumed to be malaria constitutes a major public health problem; the proportion of fevers not related to malaria is unknown. Between February and April 1994, 643 consenting adult male employees of the Sugar Corporation of Malawi were enrolled in a study to detemine the frequency and causes of fever. Data collected included: age, oral temperature, history of fever or other illness in the previous 2 weeks, and results of routine physical examination. Blood was collected and tested for malaria parasites and for HIV antibodies. Among the 643 adult male sugar estate workers, 248 (41%) reported a history of fever in the 2 weeks before enrolhnent. Fever reporters (FR) were slightly younger (32.1 v 33.8 years old), were more likely to be febrile at enrollment (8.5% v 2.2%), and were more likely to be HIV infected (27.7% v 19.9%) compared to non-fever reporters (NF). Among parasitemic persons, the density of parasitemia was similar among FR and NF. Parasitemia was present in 10.3% (7/68) of HIV+/FR and 12.8% (23/180) HIV-/FR. None of the HIV+/FR (0/68) reported ingesting an antimalarial drug in the 2 weeks before enrollment compared with 11.1% (20/180) of HIV-/FR. Among the HIV-/FR patients who were aparasitemic (n= 137), the cause of fever was found for 114 (83.2%); 78 had respiratory tract infection (including 8 with pneumonia), 14 gastroenteritis, 8 tuberculosis, and 12 other. The attributable risk for malaria as a cause of fever was 29.4%. In this study population, about 70% of fevers were erroneously attributed to and treated as malaria. The policy of treating only laboratory confirmed malaria in adults should be adopted wherever microscopy is available; this policy may be especially important in populations with a high proportion of HIV infection. Increased used of laboratory confirmation for malaria should aid malaria control efforts by delaying the time it takes for malaria parasites to develop drug resistance.

456 EROLOGIC INVESTIGATION OF HUMAN *PLASMODIUM VIVAX*-LIKE MALARIA IN SEVERAL LOCALITIES IN THE STATE OF SAO PAULO. Curado I, Duarte AM, Lal AA, Nussenzweig RS, Oliveira DA, Oliveira S, and Kloetzel JK*. Instituto Butantan de Sao Paulo, Sao Paulo, Brazil; Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Department of Medical and Molecular Parasitology, New York, University School of Medicine, New York, NY; Instituto Evandro Chagas, Belem, PA, Brazil; and Department of Parasitology, Institute of Medical Sciences, Sao Paulo University, Sao Paulo, Brazil.

Serological surveys were conducted in several localities in the State of Sao Paulo, Brazil in areas where malaria was eradicated but a few atypical cases, identified as *Plasmodium vivax* (Pv), are sporadically reported. Monkeys are found in woods close to the houses in this area. In 475 sera, collected between 1992-4 within a 5-10 km range of malaria cases, we found a high prevalence (29.5%) of positive IFA reactions with asexual Pv forms. An ELISA using circumsporozoite (CS) repeat peptides of various *Plasmodia* revealed a 4.3% positivity for the classical Pv, 5.3% for *P. vivax*-like (Pvl)/*P. simiovale* (Ps), 7.1% for *P. vivax* VK247, and 3.6% for *P. brasilianum/P. malariae* (Pm). The positivity with repeat CS peptides of different parasites varied greatly in neighboring areas, and most sera reacted with a single peptide. Out of 292 sera collected from 5 localities, 25 were Pvl/Ps positive. Of these 25 sera, 11 reacted with asexual forms of either Pv or Pm, frequently at high titers, confirming the occurrence of cross-reactivity between different stages. The detection of anti Pvl/Ps repeat antibodies demonstrates the ongoing transmission ofthis parasite in these areas. The lack of anti-blood stage antibodies to Pvl/Ps in many of these sera, may reflect aborted infections due to a parasite of simian origin. All 25 control sera from blood donors were negative.

457 PREVALENCE OF *PLASMODIUM VIVAX*-LIKE HUMAN MALARIA PARASITE IN A MALARIA ENDEMIC REGION OF BRAZIL. Oliveira DA, Qari S*, Machado RL, Oliveira S, Povoa MM, Collins W, and Lal AA. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; and Instituto Evandro Chagas, Belem, PA, Brazil.

We have recently identified a *Plasmodium vivax*-like human malaria parasite,which bears morphologic resemblance to *P. vivax*, but differs in the composition of its CS protein repeat with type 1 and type 2 vivax repeats. At present, it is not clear whether the *P. vivax*-like type is a member of the *P. vivax* species complex or a new human malaria parasite. An epidemiologic study of the prevalence of *P. vivax* type 1, 2 and *P. vivax*-like was

conducted in different regions of the state of Para, Brazil. Of the total samples (n=125) tested by a polymerase chain reaction nonisotopic liquid hybridization, 118 (94%) were found to be positive for the *P. vivax* parasite. Mixed infection with two or all three types accounted for 60 (50.8%) of the samples. Type 1 parasites were detected in114 samples, an overall prevalence of 96%, followed by a prevalence of 42% (50 samples) and 39% (47 samples) for the vivax-like and type 2 parasites, respectively. Single infection with type 1 was seen in 58 (49.2%) samples, but no pure type 2 nor vivax-like infection was found in any of the samples. The prevalence of *P. vivax*-like was further confirmed by hybridization with ^{32}P-labeled type 1, 2 and *vivax*-like oligonucleotideprobes. Attempts are being made to isolate a pure line of *P. vivax*-like parasite. Six field collected samples showing the predominance of *P. vivax*-like parasites have been inoculated in *Aotus* monkeys.

458 EFFICACY OF λ-CYHALOTHRIN TREATED BED NETS IN PREVENTING MALARIA IN A COHORT OF RADICALLY CURED VOLUNTEERS. Church CJ, Richie TL, Ohrt C, Tjitra E, Subianto B, Sandjaya B, Gomez E*, Baird JK, Fryauff DJ, and Richards AL. U.S. Naval Medical Research Unit #2 Jakarta, Indonesia; Walter Reed Army Institute of Research, Washington DC; National Institute of Health Research and Development, Jakarta, Indonesia; and Provincial Health Service, Irian Jaya, Indonesia.

The efficacy of λ-cyhalothrin treated bed nets for malaria prevention was compared against untreated bed nets during an eight-month study in the village of Arso Pir IV, Irian Jaya. Two hundred thirty-two volunteers received quinine + doxycycline + primaquine following a physical examination and G6PD screening. Following radical cure, treated or untreated bed nets were randomly distributed to households with volunteer subjects. Standardized questionnaires were used to document bed net usage and health on a weekly and biweekly basis, respectively. Biweekly bioassays were performed to document residual activity of the insecticide applied to the bed nets. Parasitologic follow-up was weekly microscopic examination of Giemsa-stained bloodfilms. Compliance to nightly bed net usage was greater than 90%. Treated bed nets remained effective, killing > 90% of laboratory reared Anopheles farauti, during the course of the study. The 35 week cumulative incidence of malaria was 68.8% and 72.3% (P>0.9) for untreated and treated bed nets, respectively. Forty-three percent of the malaria infections were due to *Plasmodium falciparum*. The other malaria infections were due to *P. vivax* except for one *P. malariae* infection. In this study, one of the first to measure the efficacy of insecticide treated bed nets as determined by malaria incidence, the insecticide treated bed nets offered no protective advantage over untreated bed nets. This may because the principal vector(s) is predominately exophilic, exophagic, and begins feeding before most people retire to bed for the night.

459 EPIDEMIOLOGY OF MALARIA VECTORS IN THE LACANDON FOREST OF CHIAPAS, MEXICO. Arredondo-Jiménez JI*, Danis-Lozano R, González-Cerón L, Rodríguez MH, Hernández-Avila M, and Washino RK. CIP-INSP, Chiapas, Mexico; CISP-INSP, Cuernavaca, Morelos, Mexico; and Department of Entomology, University of California, Davis, CA.

We conducted a two-year longitudinal epidemiological study (1992-1993) in the Lacandón Forest of Chiapas, México, an area experiencing intense malaria transmission in comparison with other regions in southern México. Our aims were to investigate the anopheline vectors involved in the transmission of *Plasmodium falciparum* and *P. vivax*, the seroprevalence of antibodies against *P. vivax* in the human population, as well as the risk factors of contracting malaria. Seven anopheline species were found feeding on man, but only 2 *Anopheles vestitipennis* and 4 *An. albimanus* were infected with both *P. falciparum* and *P. vivax* polymorph strain VK247 (overall infection prevalence=6/12958=0.00046). Seroprevalence of antibodies ranged from 8.1 to 22.1%; however, no correlation was found with patent transmission which ranged from 0 to 7.9 cases per 1,000 inhabitants. Potential risk-factors, adjusted by place of residence and sex, showed significant risks associated with illiteracy, place of birth, insecticide/bednet usage, non-utilization of health services and areas were vegetation cover surrounding households exceeded 70%. Intensive large-scale malaria control activities in the area, initiated since 1991, are now suspended due to indian uprising. However, malaria outbreaks are not expected because migration from endemic areas of neighboring Guatemala has been sharply reduced.

460 ABANDONING DDT: A BURGEONING GLOBAL MALARIA CONTROL CRISIS. Roberts D*, Sherman S, and Vanzie E. Uniformed Services University of the Health Sciences, Bethesda, MD; Ministry of Health, Belize City, Belize C.A.

Numbers of malaria cases in the Americas have grown progressively since the mid-1970s. Of the multiple causes for increased malaria, the least appreciated factor has been the inexorable decline in numbers of houses sprayed with insecticide. The rate of decline in house spraying is accelerating due to increasing operational costs and increasing concerns about the potential adverse health effects of DDT. The inverse relationships between numbers of houses sprayed and malaria cases are examined with hemispherical, regional and country data. A similar analysis of

malaria data from Belize will be combined with costing data for DDT, alternative insecticides, spray operations, case treatments, health impacts of malaria infections, plus published estimates of adverse health effects of DDT. This analysis of data from the Belize malaria control program will be used as a case study for cost-benefit analyses of different options for malaria prevention. Analyses are based on the assumption that no additional funds are available for augmenting national malaria control programs. Under this assumption, the rapid elimination of DDT for malaria control imposes health burdens on endemic countries that must switch to more expensive insecticides. Regardless, this global phenomenon is underway and observations will be presented on possible approaches to stratifying the application of control measures to reduce numbers of malaria cases and numbers of houses sprayed with insecticide.

461 EPIDEMIOLOGY OF TRAVELER'S DIARRHEA IN JAMAICA. Steffen R*, Stucki A, DuPont HL, Mathewson JJ, Ashley DV, Ashley D, and Campbell-Forrester S. Division of Epidemiology and Prevention of Communicable Diseases, University of Zurich, Switzerland; St. Lukes Episcopal Hospital, Texas Medical Center, Houston, TX; and Ministry of Health, Epidemiology Unit, Kingston, Jamaica.

In Jamaica, an outbreak of typhoid and reports on traveler's diarrhea (TD) attracted the attention of the US Travel Advisory which cited the island as a high risk tourist destination. Thus an expert group was invited to evaluate TD. Within a two arm study self-administered questionnaires in six languages were distibuted to returning visitors at the main airport in Jamaica and stool samples were collected for microbiological assessment from TD patients in various hotels. TD was defined as three or more unformed stools per 24h, or any number of unformed stools, when accompanied by abdominal cramps, nausea, vomiting, or fever. From 3,416 questionnaires distributed to air passengers, 3,067 were completed (answer rate 86.1%). The incidence rate of TD during the first week of stay was 18.6% with a range of 0-36.8% at hotels visited by ≥40 tourists. Those aged 20-29y showed a significantly higher incidence rate as compared to other age groups, Canadian and British visitors were at higher risk than those residing in other countries. Other risk factors for TD included alcohol abuse, consuming dairy products, but not eating outside the hotel. Among the 523 patients, 17.6% were incapacitated for 1-12h, 6.3% for 13-24h, 2.9% for 24-48h, while 2.7% consulted a doctor in Jamaica and 0.2% were hospitalized. The microbiological results are pending. In conclusion, TD continues to be a considerable health problem in Jamaica (similar incidence rate as 1979). Strategies to be considered include hygienic measures in the hotels, more vigorous advice on food and beverages tourists should avoid, and/or ETEC vaccine — when it will become available.

462 REVIEW OF THE BACTERICIDAL AND VIRICIDAL EFFICACY OF IODINATED RESINS. Hart PE*, and Hembree D. Recovery Engineering, Inc. Minneapolis, MN.

There are four major infectious agents which cause water borne illnesses; bacteria, viruses, protozoa, and nematodes. Of these agents, bacteria and viruses represent the more significant health risk due to their higher mortality rates especially among infants and children. Unlike protozoa and nematodes, bacteria and viruses cannot be eliminated by traditional water filtration methods but require effective disinfection. Delivery of effective drinking water disinfection is problematic for residents and travellers to countries where the drinking water is of unknown quality. This research was undertaken to demonstrate the effectiveness of devices containing iodinated resins at eliminating pathogenic bacteria and viruses in both residential and field applications. Several devices were tested according to the E.P.A. purifier test protocol which uses a variety of water conditions known to impact kill rate (pH, temperature, turbidity and temperature). These challenge tests included a broad range of pathogenic organisms; Salmonella typhi (ATCC 6539), *Shigella flexneri* (ATCC12022) *Vibrio cholerae* (ATCC11623), Poliovirus type I, and Rotavirus (SA-11). The results demonstrate that iodinated resin devices can be designed which provide broad spectrum biocidal capability and relatively little residual iodine. These devices represent a low cost method of providing a safe source of drinking water for both travellers and residents of developing countries.

463 A DOUBLE BLIND CONTROLLED TRIAL COMPARING STANDARD AND LOW OSMOLARITY ORAL REHYDRATION SOLUTION FOR MAINTENANCE THERAPY OF ADULT CHOLERA PATIENTS. Punjabi NH*, Pulungsih SP, Rifajati A, Kumala S, O'Hanley P, Simanjuntak CH, Juwono, and Lesmana M. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Prof. Dr. Sulianto Saroso Infectious Diseases Hospital, Jakarta; and National Institute of Health Research and Development, Jakarta, Indonesia.

Results of clinical trials indicated superiority of diluted oral rehydration solution for maintenance therapy of infants and children with acute non cholera diarrhea. To evaluate the efficacy of Low Osmolarity Oral Rehydration solution (LORS) and its association with any side effect for maintenance therapy of cholera, a double blind controlled trial with comparison to Standard Oral Rehydration Solution (SORS) was conducted at Prof Dr. Sulianti Saroso Infectious Disease Hospital of Jakarta from January 94 to January 95. Only adult cholera patients with severe dehydration were enrolled into the study. All patients received initial rehydration with intravenous Ringers

Lactate solution for 6 hours, followed by randomization to receive either ORS. Total of 180 patients with severe diarrhea were enrolled into the study, however only 160 patients fulfilled the study criteria for analysis. Seventy eight patients received LORS and 82 patients received the SORS. Both groups of patients were comparable in age, sex, body weight, episodes and duration of vomiting and diarrhea prior to admission, amount of mean intravenous fluid required and stool output during initial rehydration period. The study results showed that both, LORS and SORS had almost similar mean absolute volume of stool output (4451 ± 3251 vs 4450 ± 2712 ml) or as ml/kg BW (150.5 ± 87.6 vs 152.8 ± 76.5), and mean duration of diarrhea (44.4 ± 13.3 vs 42.7 ± 13.5 hours). Although statistically still not significant, mean volume (206 ml ± 416 vs 417 ± 769 ml) and mean duration of emesis (8.1 ± 12.6 vs 9.6 ± 12.6 hours) as well as treatment failure (16/78 vs 22/82) were less in patients who received LORS. This group of patients (LORS) also had higher mean ORS consumption (6964 ± 3018 vs 6464 ± 3972 ml) and mean urine output (1761 ± 935 vs 1484 ± 894 ml). Serum electrolyte measurement at 24 hours after admission were comparable (i.e. mean serum Na 139.3 ± 7.1 vs 140.4 ±10.1 mEq/L). These results indicate that LORS is as efficacious as SORS for maintenance therapy of adult cholera patients.

464 LEPTOSPIROSIS: AN ENDEMIC DISEASE IN BALTIMORE, MARYLAND. Vinetz JM*, Glass GG, Bragg S, Mueller P, and Kaslow DC. Johns Hopkins School of Medicine, Baltimore, MD; Johns Hopkins School of Public Health and Hygiene, Baltimore, MD; Centers for Disease Control and Prevention, Atlanta, GA; and National Institute of Allergy and Infectious Diseases, Bethesda, MD.

Leptospirosis, a worldwide zoonosis, is not recognized to occur sporadically in American cities in the absence of typical epidemiological exposures such as keeping pets, sanitation work or swimming in contaminated fresh water. We report a newly recognized epidemiology of leptospirosis and the use of PCR to establish early diagnosis. A 23-year-old woman presented with fever, jaundice, renal failure, and a hemorrhagic diathesis, 10d after cutting her foot on glass in a Baltimore alley. Microscopic agglutination titers (MAT) were highest for two serovars of *L. interrogans* serogroup Icterohemorrhagiae: icterohemorrhagiae (1/3200) and mankarso (1/12,800). A 36-year-old man was admitted with fever, jaundice and hemoptysis, 11d after cutting his hand on glass in a Baltimore alley. His MAT rose from 1/1600 to 1/6400 for the same two serovars as patient #1. PCR demonstrated a 285-bp band characteristic of *L. interrogans* from CSF of patient#1 and acute serum of patient #2. Rats from the alleys of patients #1 (18/19) and #2 (1/3) carried *L. interrogans* , as determined by PCR. MAT was ≥ 1/200 for serovar icterohemorrhagiae in 8/19 rats. Although rats are known to carry *L. interrogans* , sporadic urban leptospirosis without recognizable occupational or avocational risk factors has not been reported previously. These two patients experienced percutaneous exposure to potentially contaminated glass in Baltimore alleys, where rats carry leptospires. PCR demonstrated the presence of the organism in the patients' CSF and serum. These results suggest that leptospirosis is endemic in Baltimore and that PCR can rapidly establish the diagnosis.

465 LEPTOSPIROSIS IN HAWAII, 1971-1990: CLINICAL AND EPIDEMIOLOGIC ANALYSIS OF 187 LABORATORY CONFIRMED CASES. Katz AR*, Sasaki DM, Odello LM, and Ansdell VE. Department of Public Health Sciences, University of Hawaii School of Public Health, Honolulu, HI; Communicable Disease Division, State of Hawaii Department of Health, Honolulu, HI; and Kaiser Permanente Medical Center, Honolulu, HI.

The State of Hawaii has consistently reported the highest annual rates of leptospirosis nationally. From 1971-1990, a total of 404 cases were reported, of which 384 were acquired through exposures within the state. Laboratory confirmation, defined by either: (1) positive culture; (2) four-fold rise in serum antibody titer by microscopic agglutination test on paired acute and convalescent serum samples; or (3) positive direct fluorescent antibody test on autopsy tissue, was obtained for 187 of the 384 cases (49%). Confirmed cases were predominately male (92%), with an age range of 1-77 years (median age=32 years). Exposures leading to disease were most often related to either recreational, avocational, or habitational activities, as opposed to activities of an occupational nature. Clinical manifestations included fever (96%), myalgias (86%), nausea or vomiting (65%), and jaundice (35%). Hematuria was present in 50% of cases. Duration of illness ranged from 3-77 days (median=14 days). The case fatality rate was 2% (4/187). In contrast to previously reported case series, leptospirosis was correctly identified as the initial clinical impression in over 50% of cases in this series. The analysis of large case series which have laboratory confirmation, as opposed to those which are predominately "presumptive" or "suspect", helps to better delineate the clinical and epidemiologic features of this widespread zoonotic illness.

466 PLAGUE *YERSINIA PESTIS* IN INDIA, 1994. Bhattacharjee SJ, Biswas G, Chu MC, Datta KK, Dennis DT, Gage KL, Khera AK, Kumar K, Orloski KA*, and Sehgal S. National Institute of Communicable Diseases, New Delhi, India; and Division of Vector-Borne Infectious Diseases, NCID, Centers for Disease Control & Prevention, Ft. Collins, CO.

In August, 1994, rat deaths, flea nuisance and a disease characterized by mild lymphadenitis and fever was reported in Beed District, Maharashtra State, followed in late September, 1994, by a reported outbreak of acute respiratory illness, characterized by fever, cough, bloody sputum, pneumonia, and a high fatality rate in Surat, Gujurat State. Ecologic studies conducted in Beed in October 1994 and in March 1995 identified several potential sylvatic and commensal rodent reservoirs of *Yersinia pestis* and vector flea species, mostly *Xenopsylla cheopis*. Seroepidemiologic studies in Beed in October 1994 provided presumptive evidence of recent infection in suspect cases and in dogs; follow-up studies in March 1995 were negative. In Surat, hospital and surveillance records were reviewed and retrospective seroepidemiologic studies of suspect cases and controls, and a serosurvey of dogs were conducted; serum from 4/30 (13%) suspect cases, 1/34 (3%) controls, and 2/10 (20%) dogs were positive for antibodies to *Y. pestis*. Cultures provided by the National Institute of Communicable Diseases, New Delhi, reportedly from 3 suspect pneumonic plague cases, were confirmed as *Y. pestis*.

467 EVALUATION OF THE TOLERANCE OF ANTIVENIN IN TROPICAL AFRICA. Rage V, Chippaux JP*, Amadi-Eddine S, Fagot P, and Lang J. Centre Pasteur du Cameroun, Yaounde, Cameroun; Centre Pasteur du Cameroun, Garoua, Cameroun; Hopital Provincial, Garoua, Cameroun; and Pasteur Merieux Serums & Vaccins, Lyon, France.

A multicenter trial was conducted in North Cameroon to evaluate the tolerance of the antivenin (AV) Ipser Africa® (Pasteur Merieux Serums & Vaccins) through venous route. Eight health centres were chosen in Northern Cameroon savannah where *Echis ocellatus* is responsible for most of snakebites. All people bitten by a snake and presenting either oedema or bleedings and who accepted antivenin therapy were included. Two AV doses were administered in drip infusion after dilution in 250 ml dextrose solution and renewed according to the results of clinic and biological examinations. These examinations were made one hour after each of the first two administrations of AV then three hours after further administrations of AV. The immediate tolerance has been evaluated all along the hospitalisation that lasted at least five days according to the protocole. Each patient has been summoned four weeks after the first administration of AV to evaluate the delayed tolerance. Data from only 7 centres were explored. 295 bitten people were admitted out of which 223 patients (76%) were included (81 females/142 males). Age range was 1 - 67. *Echis ocellatus* was involved in 90% out of the 127 snakebites for which snake was identified. The average number of administered AV vials was 4.6 per patient (range 2-24). 49% of patients presented local or systemic bleedings and 65% presented a coagulation time higher than 30 minutes. 5 patients presented severe side effects out of which 3 died. Analyses of data concerning AV tolerance are in progress.

468 HANSEN'S DISEASE- THE TEXAS EXPERIENCE. Joyce MP*, and Longfield RN. Texas Center for Infectious Disease, Texas Department of Health, San Antonio, TX.

Since 1953, the Texas Center for Infectious Disease (TCID) has provided services in Hansen's Disease (HD), tuberculosis and pulmonary diseases. Of 482 Hansen's Disease cases currently followed by the Texas Department of Health, ninety-two patients are followed directly or indirectly by TCID for medical care and routine screening. A review of medical records was conducted in fifty-seven patients followed in the HD outpatient clinic to determine demographic and risk factor information. Patients ranged from seven to seventy-seven years old. Males outnumbered females 2.6:1. The patients included four persons of oriental descent, six whites, one black, and forty-six hispanics. Multibacillary (MB) disease was found on diagnosis in forty-two persons. Paucibacillary infection was present in thirteen. An observation was made that MB disease was found in thirty-five (83%) of the hispanics, and in seventeen (77%) of the twenty-two hispanic patients born in Mexico. Analysis of risk factors revealed eight persons had family members with HD. Seven persons had history of US military travel to HD-endemic areas. Possible exposure to armadillos was found in thirty-one persons (54%). Direct contact with an armadillo was found in twenty-two persons, and another nine had indirect contact, usually through agricultural exposure. A history of armadillo consumption was given by thirteen persons (23%).

469 EFFECTS OF THE VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION IN THE BIOGENIC AMINES CONCENTRATIONS OF MOUSE BRAIN. Estévez J*, Singh R, Arrieta A, and Teruel-López M. Instituto de Investigaciones Clínicas, Universidad del Zulia, Maracaibo, Venezuela.

Venezuelan equine encephalomyelitis (VEE) is a viral infection which produces limited but irreversible damage in the brain. This preliminary report seek to elucidate the upsetting in monoamine metabolism during the infection lapse. Male albino mice (20-25 g) were inoculated i.p. with 100 LD_{50} of the Guajira strain of VEE. The animals were killed at 24, 48, 72 and 96 hours post infection (p.i.). The brain was quickly removed and processed. Levels of norepinephrine (NE), epinephrine (E), dopamine (DA),3,4-dihydroxyphenylacetic (DOPAC), 5-hydroxyindolacetic acid (5-HIAA), homovanillic acid (HVA) and serotonin (5-HT) were determined by HPLC by method described previously. Brain samples were taken for determination of virus titers determination by a plaque method. We

found a significant increase of 5-HIAA 24 hours p.i. and of HVA since 48 hours p.i., in the infected mice brain. Virus titers were detected 24 hours p.i. The increase of HVA and 5-HIAA, the principal metabolites of DA and 5-HT, seem to be due to a enhanced turnover of these amines. Although it had been reported that a significant decrease in the tyrosine-hydroxylase activity in brain of rats infected with VEE virus, this determination was carried out in the final stage of disease, where neuronal mechanisms may be altered by cellular death. Our findings suggest an early vulnerability of the dopaminergic and serotonergic pathways to the infection produced by VEE virus.

470 THE GENERATION OF MOSQUITOES RESISTANT TO FLAVIVIRUS INFECTION VIA EXPRESSION OF FLAVIVIRUS SEQUENCES USING SINDBIS VIRUS VECTORS. Higgs S*, Olson KE, Gaines PJ, Powers AM, Beaty BJ, and Blair CB. Arthropod-borne and Infectious Diseases Laboratory, Microbiology Department, Colorado State University, Ft. Collins, CO.

It is estimated that several million human cases of dengue fever occur annually. *Aedes aegypti* is the principal vector of Dengue (DEN) viruses. Mosquito-virus interactions are poorly understood, and the genetic basis of mosquito susceptibility to arboviruses is unknown. Double subgenomic Sindbis (dsSIN) virus expression vectors (pTE/3'2J) can express genes in mosquitoes, and can generate pathogen derived resistance (PDR) to La Crosse virus in mosquito cells. Complimentary DNA derived from the premembrane (prM) coding region of the DEN type 2 (Jamaica) virus genome has been inserted in either sense (D2prMs) or antisense (D2prMa) orientations downstream of the second internal initiation site of dsSIN virus vector pTE/3'2J. Northern blots of infected C6/36 cells confirmed the presence of D2prMs and D2prMa RNA. Protein analysis confirmed translational expression from TE/3'2J/D2prMs. Experiments to demonstrate PDR in *Aedes aegypti* showed that dsSIN viruses can knock out DEN-2 replication in salivary glands. Whole body DEN-2 titers in mosquitoes concurrently infected with TE/3'2J/D2prMa were up to 100 fold less than in those infected with DEN-2 only, or concurrently infected with a control TE/3'2J virus. Knock out of flavivirus replication in salivary glands may effectively prevent transmission. Using constructs expressing yellow fever virus (YF) sequences, we have also demonstrated PDR against YF infection. We are currently investigating sequences (eg NS5) which may generate broadly protective heterologous PDR. dsSIN viruses have been used to identify sequences that might be utilized to genetically manipulate mosquito vector competence for arboviruses.

471 NATURAL IMMUNITY OF ROTAVIRUS INFECTION. Mahmud MA*, Hossain MM, Mathewson JJ, Habib M, and DuPont HL. Beth Israel Medical Center, Division of Infectious Disease, New York; United Arab Emirates University; University of Texas School of Public Health; The Center for Applied Research, Ministry of Health, Cairo, Egypt; Baylor College of Medicine.

Data on natural immunity of rotavirus infection, such as symptomatic or asymptomatic infection acquired at early life period and breast-feeding could be very important to our understanding of future development of effective vaccine against rotavirus. We conducted a prospective study to document the role of such immunity against rotavirus gastroenteritis among infants followed from birth to the first year of life in a rural community of Egypt. Natural rotavirus infection (first rotavirus diarrhea) did not appear to protect (RR : 1.04, CI: 0.54-2.03) the infants from subsequent (second symptomatic episode) infection. Similarly, antibodies expressed in maternal breast milk did not confer such protection. Antibodies to rotavirus was more commonly (39.9% vs 22.1%, $p<0.05$) found in breast milk of mothers whose infants had rotavirus diarrhea except infants under 3 months (20% vs 29%, $p>0.05$). However, longitudinal data on feeding mode revealed an approximately 63% protection (RR : 0.37; CI : 0.17-0.75) by exclusive breast milk. No other socio-demographic factor appeared to be related to rotavirus gastroenteritis except age of infants which showed younger infants (under 3 months) were more (RR : 2.34; CI : 1.03-5.33) likely to develop rotavirus gastroenteritis than older (9 months or above) infants.

472 TOWARDS THE UTILIZATION OF CHAPERONIN 60 AS AN ANTIVIRAL TARGET: A STRUCTURAL STUDY. Braig K*, and Braig HR. Department of Genetics, Yale University School of Medicine, New Haven, CT; and Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

Chaperonins are ribosome size multi-subunit complexes that play an essential role in mediating the ATP-dependent folding of nascent polypeptide chains. Although they play an important role in the cellular heat shock response, genetic studies indicate that they are essential for *de novo* protein folding at all temperatures. Chaperonin-60, also known as heat shock protein 60 (hsp60) or groEL, assists together with its co-factor chaperonin-10 in the folding of newly synthesized proteins. Despite the fact that it has been known for more than 15 years that some bacteriophages code for their own chaperonin-60, little or no research has been undertaken to study animal or human viruses regarding their need for viral or host coded chaperonin during their replication. In order to explore the possibilities of chaperonin-60 as a potential target for antiviral intervention, the differences between viral and

host chaperonin have to be identified and the mechanism of assisted protein folding has to be further elucidated. The determination of the crystal structure of groEL, a 14 member homo-oligomer with a molecular mass of ~ 720,000 Da, to 2.8 Å has provided valuable information. The refined structure in combination with extensive mutational analysis allowed the functional assignment to different regions and the identification of amino acids which might represent future targets for an antiviral therapy.

473 SILENT TRANSMISSION OF DENGUE VIRUS IN SOUTHERN TAIWAN. Chen WJ*, Chen SL, Chien LJ, and King CC. Department of Parasitology, Chang Gung College of Medicine and Technology, Kwei-San, Tao-Yuan, Taiwan; and School of Public Health, National Taiwan University, Taipei, Taiwan.

This study was conducted to investigate the IgM antibody prevalence in southern Taiwan by using MAC-ELISA. Of 1252 serum specimens collected from a junior high school and 4 preliminary schools of Liuchiu island in 1990, 35 were detected to be IgM-positive. This demonstrated that dengue virus has been circulating on the island in spite no outbreak occurred in the past few years. Since the positives tended to aggregate around the port area, fishing boats which have stopped by ports in endemic countries were presumed to introduce the virus into the island periodically. The investigation in Tunkung, 1991, showed that 16 out of 925 serum serum specimens collected from preliminary school children were IgM-positive. In addition, 2 of 192 randomly sampled serum specimens from local population were positive. The results also showed that the school close to the port possessed a higher positive rate. It indicated that the dengue virus may be introduced via fishing boats as well. Furthermore, 2 out of 108 suspected clinical cases from Kaohsiung area were detected to be positive while as 4 out of 642 specimens collected from the general population were positive. This revealed that the dengue virus was definitely circulating around the southern part of Taiwan. Presumably, the traditional rural living style and crowded population have provided an appropriate environment for vector breeding, leading to continuation of virus transmission in this area. For more effective monitoring of the disease, active serological or virological surveillance, as well as a reduction of vector larval breeding sites will need to be taken.

474 EXPERIMENTAL BUNYAVIRUS INFECTIONS OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*). Blackmore CG*, and Grimstad PR. Laboratory for Arbovirus Research and Surveillance, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

Cache Valley virus (CVV) and Potosi virus (POTV) are mosquito-borne members of the Bunyamwera serogroup (Bunyaviridae). Their endemic transmission cycles are not fully understood, however antibody surveys in the Midwest have shown that white-tailed deer (*Odocoileus virginianus)* are common vertebrate hosts. This study was conducted to determine whether deer can serve as reservoir hosts transmitting CVV or POTV to mosquitoes. A second objective was to examine the extent to which deer antibodies to CVV and POTV crossreact *in vitro* and *in vivo*. Captive white-tailed deer were infected subcutaneously with either CVV or POTV during the winters of 1992-93 and 1993-94. The animals were bled daily for 7 days. These samples were used for virus and antibody detection assays. Viremic bloods were identified using Virus Plaque Assays and CVV or POTV specific antibody titers were measured in the Serum Dilution Neutralization Test. White-tailed deer developed a 2-3 day viremia when infected with either virus. Specific antibodies were detectable after 3-5 days. Low titers of crossreactive antibodies also were seen. Deer that had been immunized by exposure to one virus produced lower viremia than naive animals when they were infected with the second virus.Titers of antibodies specific to each virus increased in superinfected animals. Because deer become viremic, these results indicate that infected individuals are capable of transmitting CVV and POTV to susceptible mosquitoes. In vitro, antibody crossreactivity to these viruses is low unless animals have experienced dual infections.

475 THE EFFECT OF GLOBAL WARMING ON THE GEOGRAPHIC AND SEASONAL DISTRIBUTION OF *PHLEBOTOMUS PAPATASI* IN SOUTHWEST ASIA. Cross ER*, and Hyams KC. Infectious Diseases Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD.

The distribution in Southwest Asia of *Phlebotomus papatasi* is thought to be dependent on temperature and relative humidity. A computer model based on weather data was developed to predict the seasonal and geographic distribution of *P. papatasi* in Southwest Asia. For the model, information on the presence or absence of the vector, as well as human cases of cutaneous and visceral leishmaniasis and sandfly fever, was compiled from 136 articles. Data were available from the Federal Climate Complex for 115 weather stations in Southwest Asia, including measurement of mean high, mean minimum and mean temperature, extreme high and extreme minimum temperature, relative humidity and dew point. To simulate global warming, temperature values for each weather station were increased by 1°C, by 3°C and by 5°C, and the dependent variable coded as unknown. The original model was run and from this model, probability of occurrence values were predicted for those locations where the temperatures had been increased. Those stations with positive probability of occurrence values for May, June, July,

and August were considered as locations where two or more life cycles of *P. papatasi* could occur and therefore locations that could be endemic. Seventy-one (61.7%) of the 115 stations would be considered endemic with no change in temperatures, 14 (12.2%) additional stations would become endemic with an increase of 1°C, 17 (14.8%) with a 3°C increase and 12 (10.4%) with a 5°C increase. With at least a 3°C rise in temperature, seasonality would be extended throughout a 12-month period in certain locations.

476 DEVELOPMENT OF A PURIFIED, INACTIVATED, DENGUE-2 VIRUS VACCINE PROTOTYPE IN VERO CELLS: IMMUNOGENICITY IN MICE AND RHESUS MONKEYS. Putnak JR*, Barvir D, Burrous MJ, D'Andrea VM, Dubois D, Hoke CH, Sadoff J, and Eckels KH. Division of Communicable Diseases and Immunology, The Walter Reed Army Institute of Research, Washington DC; Division of Clinical Laboratory Devices, The United States Food and Drug Administration, Rockville, MD.

The feasibility of a purified, inactivated dengue (DEN) vaccine made in Vero cells was explored. Virus strains representing each of the four serotypes were selected based on their ability to replicate to high titers in Vero cells. A DEN-2 virus candidate was chosen for production of a monotypic, purified, inactivated vaccine (PIV). Virus was harvested from roller bottle culture supernates, concentrated, and purified on sucrose gradients. Analysis of purified virus preparations by SDS-PAGE and Western blotting demonstrated predominately viral structural antigens, E and prM/M with traces of nonstructural antigen NS1. The purified virus was inactivated with 0.05% formalin at 22°C. Inactivation was confirmed by negative attempts to amplify infectious virus in C6/36 mosquito cells. After inactivation, the virus retained its antigenicity and was immunogenic in mice and rhesus monkeys where it elicited high titers of DEN-2 virus neutralizing antibody. Mice were completely protected against challenge with live, virulent virus after receiving two, 0.15 µg doses. These results warrant further testing of this PIV and development of PIVs for other DEN virus serotypes.

477 DETECTION OF ANTI-DENGUE VIRUS ANTIBODY USING RECOMBINANT FUSION PROTEINS CONTAINING THE B DOMAIN OF DENGUE ENVELOPE. Simmons M*, Porter KR, Escamilla J, Shope RE, Eckels KH, Watts DM, Burans JP, and Hayes CG. Naval Medical Research Institute, Bethesda, MD; NEPMU-6, Pearl Harbor, HI; Naval Medical Research Institute Detachment, Lima, Peru; Walter Reed Army Institute of Research, Washington, DC; and Yale Arbovirus Research Unit, New Haven, CT.

Dengue (DEN) fever is caused by any one of 4 serotypes of DEN virus which are members of the family *Flaviviridae*. The DEN viruses are widely distributed in the Tropics, and their range overlaps with a number of other flaviviruses such as yellow fever (YF) and Japaneses encephalitis(JE). In such areas, it can be difficult to diagnose DEN infections using standard serological tests such as the HI or ELISA because of cross reactivity among the members of the *Flaviviridae*. This is particulary true for individuals who have experienced more than one flavivirus infection. To increase the specificity of DEN diagnosis based on antibody detection, we have evaluated recombinant fusion proteins that span the B domain of the envelope protein of DEN virus as antigens. A pooled antigen consisting of recombinant proteins representing all four serotypes of DEN virus was compared to a DEN virus infected cell culture pooled antigen in a standard indirect ELISA format to detect IgG. Both antigen preparations gave eqivalent OD values with a collection of sera from Peru representing mostly primary DEN-1 infections. Sera from individuals that had been sequentially vaccinated with YF and JE also reacted with the cell culture antigens, but did not react with the recombinant antigen. Based on these initial results the recombinant antigen may provide a more specific diagnosis of past or current DEN infection in some situations.

478 EVALUATION OF DENGUE VIRUS ANTIGENS FOR USE IN A RAPID DIP-STICK IMMUNOASSAY. Wu SL*, Hanson B, Paxton H, Simmons M, and Hayes CG. Infectious Diseases Department, Naval Medical Research Institute, Bethesda, MD; Integrated Diagnostics, Inc., Baltimore, MD.

Dengue (DEN) fever, caused by any of the 4 serotypes of DEN virus, is widely distributed in the world's tropical regions. To facilitate its diagnosis in unsophisticated settings, we are seeking to develop a rapid, inexpensive serological test which can be performed in the field without special instrumentation or technical expertise. Preliminary steps in assay development require selection of suitable antigen and comparison to standard methods for determination of sensitivity and specificity. The format used is an enzyme immunoassay performed with nitrocelluose dip-sticks (DS) containing preapplied dots of serially diluted antigen. The DS are processed through a series of reaction cuvettes containing the test human serum, enzyme-conjugated anti-human IgG and IgM antibody, and enzyme substrate. The semi-quantitative results, expressed as reactive antigen titers, were compared with results from a standard microtiter plate enzyme-linked immunosorbent assay (ELISA). Both crude and purified native DEN virus and recombinant DEN envelope protein (REC-E) could be used to identify human DEN immune sera. Native DEN antigen titers determined by DS correlated well with results from standard ELISA with a panel of convalescent sera from individuals with confirmed DEN infections. A limited number of DS tests with

REC-E indicated that this antigen is DEN virus group specific and does not react with sera from individuals exposed to other flaviviruses. These preminary tests suggest that DS dotted with native virus antigen may be useful in screening for DEN antibodies. More extensive testing is planned to confirm this and to ascertain the usefulness of DS in detecting recent infections in flavivirus-endemic areas.

479 PURIFICATION OF BACULOVIRUS PRODUCED DENGUE-2 ENVELOPE GLYCOPROTEIN. Kelly EP*, King AD, and Bailey SL. Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

Dengue-2 virus envelope glycoprotein (rEgp) was expressed by baculovirus and purified as a particle from Trichoplusia ni (Tini) cells. The rEgp self-aggregated into particles that ranged in size, however, the majority of rEgp assembled into large, multimeric particles. Neither sarkosyl nor sonication disrupted the particles, however treatment altered chromatographic profiles of other cellular proteins. The rEgp particles were purified by pelleting microsomal fractions of recombinant baculovirus-infected cells through a sucrose cushion. Purity of the rEgp fraction was assessed by SDS-polyacrylamide gel electrophoresis and western blotting. In a dose response immunization trial, purified rEgp induced high levels of neutralizing antibody in immunized mice compared to levels achieved with nonpurified rEgp. This response was boosted byusing adjuvant. Adult mice immunized with the purified rEgp were protected from morbidity and mortality following challenge with live dengus virus. Adult, immunized, mice were protected from intracerebral challenge resulting in a 77-99% reduction in plaque forming units of virus in brain tissue. Studies of effects of different adjuvants on the immunogenicity and immunoglobulin isotype profile achieved with purified rEgp are planned. Furthermore, it is important to assess the immunogenicity of purified rEgp in primates.

480 EFFECTS OF FOLLICULAR QUIESCENCE ON REPLICATION OF LA CROSSE VIRUS IN *AEDES TRISERIATUS* OVARIES. Chandler LJ*, Wasieloski LP, and Beaty BJ. Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

La Crosse (LAC) virus (Family Bunyaviridae, genus Bunyavirus) is efficiently transmitted orally and transovarially by the mosquito *Aedes triseriatus*. Transovarial transmission (TOT) is the mechanism by which the LAC virus cycle is maintained during the winter: the virus survives in diapausing mosquito eggs. LAC virus infects the ovaries of 95-100% of the mosquitoes following oral infection. When mosquitoes do not take regular blood feedings to stimulate egg development, the ovaries are in an inactive state (follicular quiescence). To investigate the effects of follicular quiescence on viral replication in the ovaries of infected mosquitoes, a transcript-specific RT-PCR technique was used to detect LAC virus genome or replicative forms of RNA. Viral activity was monitored over a course of 30 days following oral infection of the mosquito by flood feeding. At day 8, 100% of the mosquitoes had virus present and replicating in the ovaries.At day 14, a loss of mRNA was seen in 2/3 ovaries. At day 21, no mRNA or vcRNA was detectable in 3/3 ovaries. At day 30, 9/10 mosquitoes had no replicative forms of RNA detectable in the ovaries. Interestingly, 3/10 mosquitoes had no virus detectable in the ovaries. These results indicate that viral replication is restricted in mosquito ovaries that are not metabolically active (developing eggs). It is possible that the virus requires a certain level of host cell activity in order to maintain a persistent infection in the ovaries of infected mosquitoes.

481 MISSOURI POST FLOOD MOSQUITO SURVEILLANCE - 1994. Frazier CL*, and Robins JH. Southeast Missouri State University, Cape Girardeau, MO; and Southeast Missouri State University, Cape Girardeau, MO.

Mosquitoes were collected during summer 1994 in areas of Missouri effected by flooding in 1993, and assayed for SLE, WEE, EEE, and LAC antigens by ELISA. State wide 2,128 *Aedes albopictus*, 7,075 *Aedes triseriatus*, 82,555 *Culex pipiens* complex, 1,016 *Culex tarsalis* and 1,269 *Coq. perturbans* were tested. No positives were found upon confirmatory testing. The state was divided into five regions, each with two or three sections. An initial rotation of sites was established, but non productive sites were eliminated from the collecting cycle and new sites added. Most of the trapping used light traps baited with CO^2, but gravid traps were used, and mosquitoes were aspirated from resting places. Between 6 and 534 collections were made per section from 3 to 102 sites. Collection indexes(CI) (mosquitoes collected/number of collections) were calculated for each site and statistical analysis revealed significant differences between the productivity of sites. The mean CI by section varied from 1 to 67 while the high CI ranged from 2 to 382. By region, for *Culex pipiens* complex, between 33 and 94% of sites had CIs below ten while 0 to 20% had CIs above 100. Descriptions were made of the sites in one region to determine if a set of visible attributes predictive of high productivity could be determined. Preliminary evaluation suggests sites combining foul water, mown area and vertical structure are more productive.

482 PANTROPIC RETROVIRAL INFECTION OF MOSQUITO CELL LINES. Matsubara T*, Beeman RW, Besansky NJ, Mukabayire O, Higgs S, Burns JC, and James AA. Department of Pediatrics, UCSD School of Medicine, La Jolla, CA; USDA, US Grain Marketing Research Laboratory, Manhattan, KS; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; AIDL, Colorado State University, Fort Collins, CO; and Department of Molecular Biology and Biochemistry, UC Irvine, Irvine, CA.

Pantropic retroviral vectors infect a broad range of host cells and infection of insect cell lines has been previously reported. To investigate whether these vectors can support stable gene expression in infected cells, we created two vectors in which either β-galactosidase (Lac Z) or hygromycin phosphotransferase (hyg) was expressed from the hsp70 *Drosophila* heat shock promoter. Infection of *Aedes albopictus* cell lines with the vector expressing hyg resulted in stable clones growing in selection with 50 μg/ml Hygromycin B. Infection efficiency was estimated at 104-fold lower than mammalian cells. Infection of the *Anopheles gambiae* cell line, MOS-55, with the Lac Z expressing virus resulted in enzyme activity detected by chemiluminescent assay. Retroviral integration was documented by amplification and sequencing of junctional fragments using a hemi-nested PCR with 2 nested 5'-end primers complimentary to the LTR of the provirus and a highly degenerate 3'-end primer. A cloned junctional fragment hybridized in situ to chromosome 2R, subdivision 7B of polytene chromosomes from adult female *A. gambiae* mosquitoes, thus confirming the identity of the cloned flanking sequence as *A. gambiae* DNA. These data confirm retroviral vector-mediated stable integration and foreign gene expression in two species of vector mosquitoes and suggest the possibility of insect transgenesis with pantropic retroviral vectors.

483 NEW VEE VACCINE CANDIDATE INDUCES PE2-SPECIFIC ANTIBODIES AND RETAINS CONFORMATION OF A DOMINANT NEUTRALIZING EPITOPE. Buckley MJ*, Ludwig GV, Davis NL, Johnston RE, and Hart MK. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC.

TC83, the live-attenuated Venezuelan equine encephalitis (VEE) virus vaccine currently used under IND status, is reactogenic in 20% of recipients and nonimmunogenic in another 20%. As part of an ongoing program to develop an improved live-attenuated vaccine for VEE, site-directed mutagenesis was used to develop a vaccine candidate (V3526) in which the PE2 cleavage site was deleted. In the cleavage deletion virus V3526, the glycoprotein spikes are comprised of PE2-E1 heterodimers instead of E2-E1 heterodimers. Including the E3 sequence in the spike could have profound effects on the immune response by providing new epitopes or by sterically or conformationally altering important epitopes on E1 or E2 glycoproteins. To address this issue, a panel of monoclonal antibodies (MAbs) was developed from the fusion of V3526-immune Balb/c spleen cells and mouse myeloma cells. MAbs were tested for the ability to bind or neutralize V3526 and Trinidad donkey viral strains. Likewise, a panel of existing MAbs directed at defined epitopes on Trinidad donkey virus was tested for the ability to bind or neutralize the mutated V3526 virus. Results indicated that immunization with V3526 produced some antibodies directed at the PE2 molecule which did not bind to gradient-purified TrD virus. Some of these antibodies were able to neutralize the V3526 virus. A dominant neutralizing epitope on TrD (E2c) was conformationally conserved on V3526 as a MAb directed against this epitope efficiently neutralized both TrD and V3526 viruses. Despite the inclusion of the E3 protein in the spike structure, V3526 induced in mice protective immunity that was equivalent to or better than that induced by TC83.

484 EVALUATION OF A NEUTRALIZATION-ELISA ASSAY (NT-ELISA) TO MEASURE NEUTRALIZING ANTIBODIES TO HANTAAN VIRUS. Summers PL*, and McClain DJ. Virology Division, U.S. Army Medical Research Institute of Research, Ft Detrick, MD.

Hemorrhagic fever with renal syndrome (HFRS) is caused by a variety of viruses belonging to the genus Hantavirus. Hantaan virus is the prototype virus and causes severe human disease in much of Asia and Europe. Plaque-reduction neutralization tests (PRNT) used to measure neutralizing antibodies to some Hantaviruses have been hampered by the inability of virus to form visible plaques. We have adapted a NT-ELISA assay for the detection of neutralizing antibodies to Hantaan virus using a 96-well microtiter plate, and compared it with the PRNT currently employed in our laboratory. A panel of 131 sera were analyzed from individuals vaccinated with an experimental vaccinia-vectored Hantaan vaccine, or persons who seroconverted after a natural or laboratory acquired infection. Twenty-eight sera were positive by PRNT, 27 of these 28 were positive by NT-ELISA. Endpoint (50% reduction) titers determined by the NT-ELISA test compared very well (r= 0.79) with those of the PRNT. The NT-ELISA sensitivity was 96% and specificity was greater than 99%. Besides being more economical, the NT-ELISA is a simple, sensitive and specific method to measure neutralizing antibodies to Hantaviruses.

485 CLINICAL AND EPIDEMIOLOGICAL FEATURES OF DENGUE FEVER INFECTIONS AMONG US TROOPS IN HAITI. Smoak BL*, DeFraites RF, Trofa AF, Kanesa-thasan N, King AD, Burrous JM, MacArthy PO, Putnak R, Quan J, Scheutte J, Longacre J, and Hoke, Jr. CH. Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC; Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC; and 28th Combat Support Hospital, Port-au-Prince, Haiti.

In September 1994, over 20,000 US troops deployed to Haiti in support of Operation Uphold Democracy. During the first six weeks of the operation, 106 service members with febrile illnesses were admitted to the US Army hospital in Port-au-Prince and evaluated using a standard clinical and laboratory protocol. Twenty-two (20.8%) had dengue fever (DF) virus cultured from serum drawn during the febrile phase of their illnesses. DF types 1,2, and 4 were isolated. Eight other patients from whom no virus was isolated developed IgM antibody to the DF. DF accounted for a significant proportion (25%) of febrile illness among US troops hospitalized in Haiti. Patients with DF had a median hospital stay of 3 days and there was no evidence of hemorrhagic complications among patients treated in Haiti. The percentage of febrile patients reporting headaches, arthralgias and myalgias were not different between the DF and non-DF groups, but rashes and conjunctival irritation were observed more frequently in the DF group. Infections were acquired throughout the country with no obvious focal outbreaks. This is the first confirmation of DF in Haiti since the mid 80's and suggest that it is highly endemic throughout the country.

486 A COLORIMETRIC MICROPLATE ASSAY FOR DETECTING PCR-AMPLIFIED DENGUE VIRUS RNA. Ibrahim MS*, Baird JB, Lofts RS, Roberts LW, and Henchal EA. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Procedures to detect and type dengue viruses using the reverse transcriptase-polymerase chain reaction (RT-PCR) have been described. The four dengue serotypes are typically distinguished by molecular size analysis of PCR products in agarose gels or by membrane-based oligonucleotide probe hybridization. We describe a sensitive, type-specific procedure combining the advantages of quick solution hybridization and colorimetric detection in a microtiter plate format to analyze RT-PCR products. A genomic segment of about 400 base pairs located within the NS1 coding region was targeted by a single set of cross-reactive primers that amplified all four dengue serotypes. Antigenic targets were incorporated into amplicons during the PCR by partial substitution of digoxygenin-11-dUTP for dTTP. The digoxigenin-labeled amplicons were hybridized in solution to serotype-specific biotinylated probes and captured onto streptavidin-coated microtiter plates. The captured hybrids were detected by the enzymatic activity of HRP-labeled anti-digoxygenin antibody on a chromogenic substrate. The four dengue serotypes were easily detected and differentiated using this method, and no cross-reactive interference was noted from RNA of other flaviviruses. A detection sensitivity of between 1 and 20 plaque forming units or approximately 50 picograms of amplified PCR product was achieved. The assay is simple to perform, more sensitive than electrophoretic procedures, readily configured for rapid processing of large number of samples, and easily adaptable to field applications.

487 RECOGNITION OF DENGUE-2 VIRUS E PROTEIN BY MURINE CD8+ CYTOTOXIC T LYMPHOCYTES. Rothman AL*, Kurane I, lai CJ, Bray M, and Ennis FA. University of Massachusetts Medical Center, Worcester, MA; and Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD.

Dengue hemorrhagic fever (DHF) has been increasing in incidence and geographic range in recent years, reinforcing interest in the development of vaccines against dengue. We have been studying the murine T cell responses to dengue virus to identify potential protective epitopes We reported that CD8+ CTL from dengue virus-immunized BALB/c mice recognize target cells expressing the dengue structural proteins. To study the recognition of these proteins further, we cloned CD8+ CTL from dengue-2 virus-immunized BALB/c mice. Three of ten clones established recognized target cells expressing the dengue-2 structural proteins. We tested the protein specificity of these clones using recombinant vaccinia viruses expressing individual dengue structural proteins. The epitope recognized by these CTL clones mapped to the dengue-2 E protein. The clones did not recognize target cells expressing the dengue-4 E protein. Recognition by these CTL clones was restricted by the H-2L^d antigen. These results show that the dengue-2 E protein is recognized by serotype-specific CD8+ CTL. However, our previous studies showed that the dengue-4 E protein is not recognized by CTL in bulk culture. Candidate vaccines based on the dengue E protein are protective against dengue encephalitis in mice. CD8+ CTL might be one mechanism for this protective effect, but this effect may differ between the four dengue serotypes.

488 A RECOMBINANT BCG VACCINE EXPRESSING THE *LEISHMANIA CHAGASI* ANTIGEN Lcr1. Streit JA*, Donelson JE, and Wilson ME. Department of Internal Medicine, University of Iowa, and VAMC, Iowa City, IA; Department of Biochemistry, University of Iowa, and Howard Hughes Medical Institute, Iowa City, IA; and Departments of Internal Medicine and Microbiology, University Iowa and VAMC, Iowa City, IA.

Recovery from visceral leishmaniasis leads to protection against reinfection. In humans and mice, such protection is associated with T cells producing interferon-γ (IFN-γ). This suggests immunization may be feasible if parasite-reactive lymphocytes producing IFN-γ are stimulated. Expression of *Leishmania* antigens in an organism such as the *Mycobacterium bovis* strain BCG which elicits IFN-γ production might bias toward expansion of IFN-γ producing cells responsive to these antigens. Recombinant *L. chagasi* antigen *Lcr1* stimulated T cell proliferation and IFN-γ production and provided partial protection against *L. chagasi* infection. The *Lcr1* gene was subcloned into the mycobacterial vector pMV261 3' to the promoter and in frame with the first ten amino acids of the mycobacterial hsp60 gene. This construct was introduced by electroporation into BCG. Recombinant BCG produced Lcr1 protein seen on immunoblots. Genetically susceptible BALB/c mice were immunized with recombinant BCG expressing Lcr1. Eight weeks later BCG isolated from murine spleens retained their kanamycin resistance. We conclude that *L. chagasi* antigens can be expressed in BCG, and that BCG retain antigen-encoding plasmids in a murine host. Thus BCG is a reasonable vaccine vector for immunizing against *L. chagasi* infection.

489 EVIDENCE OF GENETIC EXCHANGE DURING SILVATIC TRANSMISSION OF *TRYPANOSOMA CRUZI*. Carrasco HJ*, Frame IA, Valente SA, and Miles MA. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, UK; and Chagas' Disease Control Division, Institute Evandro Chagas, Belem, Para, Brazil.

Extensive phenotypic and genotypic diversity between natural populations of *Trypanosoma cruzi* has been reported using biochemical and molecular methods. A theory of clonal reproduction has been sustained, based on pronounced genotype linkage disequilibrium observed in natural circulating populations. We have studied, using enzyme electrophoresis (isoenzyme) and random amplified polymorphic DNA (RAPD), 36 different *T. cruzi* strains which were isolated from mammals and triatomine bugs in a silvatic cycle in the Amazon basin of Brazil, and which were characterised as belonging to the principal zymodeme 1 (Z1). With the enzyme phosphoglucomutase (E.C.2.7.5.1., PGM), two different homozygous and the corresponding heterozygous characters were found within this population, presenting a phenotypic distribution as predicted by the theoretical Hardy-Weinberg equilibrium. Similar homozygous and heterozygous profiles were shown using RAPD, which also indicated the absence of mixed populations in the putative hybrid isolates. These results indicate that genetic exchange could contribute to the generation of genetic diversity during the silvatic cycle of *T. cruzi*, and this may have important medical, epidemiological, and taxonomic implications.

490 IMMUNIZATION OF MICE WITH MOLECULAR ANTIGENS OF *LEISHMANIA (LEISHMANIA) DONOVANI* AND EFFECTS ON ANTIBODY AND LYMPHOKINE PRODUCTION. Shin SS*, Hanson WL, Russell DG, and McMaster WR. Department of Parasitology, College of Veterinary Medicine, The College of Georgia, Athens, GA; Department of Molecular Microbiology, Washington University Medical School, St. Louis, MO; and Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia.

Mice (DBA-2N) were immunized with liposome-encapsulated, biochemically purified molecular leishmanial antigens, GP63 and LPG, plus BCG as an adjuvant either separately or in combination and studies for resistance to challenge with virulent amastigostes of *Leishmania (Leishmania) donovani*. Mice immunized with the combination of liposome-Gp63/LPG plus BCG had slightly, but significantly ($p < 0.05$), lower mean numbers of amastigotes in the liver than control mice (27.4% reduction) receiving empty liposomes plus BCG. In another experiment C3H mice were immunized with recombinant GP63 plus BCG and studied for the effect of immunization on protective immunity against challenge infection, antibody response, and production of interferon-γ (IFN-γ) and interleukin-5 (IL-5). Immunization with recombinant GP63 plus BCG resulted in no significant protection of these mice against challenge infection when compared to the BCG control group. However, spleen cells from mice immunized with recombinant GP63 plus BCG and then exposed to leishmanial antigens *in vitro* produced 10-fold greater quantities of IFN-γ and 3-fold greater quantities of IL-5 than mice receing BCG only or saline. Although immunization with GP63 plus BCG failed to protect mice against challenge, the results indicated that the immunization procedure stimulated an immune response with possible activation of both TH1 and TH2 cells of the CD4+ T-cell subset.

491 EVOLUTIONARY GENETICS OF PARASITIC PROTOZOA AND OTHER PATHOGENIC MICROORGANISMS. IMPLICATIONS FOR STRAIN IDENTIFICATION AND MOLECULAR TAXONOMY. Tibayrenc M*. ORSTOM, Montpellier Cedex 01, France.

It is proposed here that evolutionary genetics, apart its interest for our basic knowledge on evolution of microorganisms, can allow substantial pay-offs for applied research too. The 3 main potential applications of evolutionary genetics in microbiology are: (i) Strain typing for epidemiological tracking: Evaluating the impact of genetic recombination on the stability of microbial genotypes in the long run; (ii) Molecular taxonomy: (a) confronting the genetic data with the presently-accepted taxonomy. (b) Looking for hidden discrete genetic subdivisions within the presently-described species; (iii) Downstream studies: exploring the impact of the genetic diversity of microorganisms on their relevant medical properties such as virulence or resistance to drugs. Whatever the microorganism under study (either eukaryotic: parasitic protozoa and fungi, or prokaryotic: bacteria), evolutionary genetics is required to address the above questions. We hence propose to build a global approach aiming at the comparison of various microorganisms with the same techniques and the same statistics. this will help codifying this field of research and render the use of these methods routine in microbiology. Preliminary comparisons drawn between *Trypanosoma* and *Leishmania* show that divergent taxonomic conclusions have been deducted for these two groups of parasite from sets of data that are nevertheless comparable.

492 MURINE MODEL OF *LEISHMANIA INFANTUM* DISSEMINATED VISCERAL LEISHMANIASIS. Garin YJ*, Sulahian A, and Derouin F. Hôpital St-Louis, Paris, France.

Leishmania infantum, is responsible for most European cases of visceral leishmaniasis (VL) in AIDS patients but the pathogeny of the multiple organ involvement in these patients is still poorly understood due to the lack of convenient experimental model. In this study we developed a murine model of VL due to *L. infantum* and describe the tissue kinetics of visceral leishmaniasis in Swiss-OF1 mice. At day 0 (d0), infections were obtained by IV injection of 10^7 promastigotes of the MON2259 strain isolated from an AIDS patient. Mice were sacrificed at intervals after infection and parasite burdens were determined in blood and tissues using a highly sensitive culture micromethod. Infection resulted in an early and predominant involvement of the liver. Parasites were then detected in the spleen at d7 and in the lungs at d14. Subsequently infection was demonstrated in the bone marrow, the pancreas and the kidney at d60, the blood and the brain at d100, and the small intestine at d135. In all organs, parasite burdens progressively increased until the end of the experiment (d135), reaching a level of 10^7 parasites/g in the spleen, 10^5 parasites/g in the lungs, pancreas and brain, 10^4 parasites/g in the kidney and small intestine, 10^4 parasites/femoral bone in the bone marrow and 10^2 parasites/ml in the blood. Comparing the results obtained in Swiss-OF1, Balb/c and C57Bl mice inoculated with different strains of *L. infantum*, we also showed that this model allowed to clearly differentiate the virulence of the infection according to the parasite or the mouse strains. This is the first experimental model in which the multiple visceralization of *L. infantum* can be evidenced; it proved reliable to study the pathophysiology of VL and represents a new experimental tool for pharmacological and immunological studies.

493 THE INOCULATION SITE CONDITIONS RESPONSE ON THE HAMSTER MODEL TO *LEISHMANIA VIANNIA* SPP. Travi BL, Osorio Y, Valderrama L, and Guarin N. Centro Internacional de Entrenamiento e Investigaciones Medicas-CIDEIM, Cali, Colombia; and Universidad del Valle, Cali, Colombia.

Golden hamsters are exquisitely susceptible to all *Leishmania* species of the subgenus *Viannia* . Other authors have shown that nice and hamsters infected with *L. major* at different body sites will display distinct clinical and immunological responses; caudal body areas being the most susceptible. In our hamster-*Leishmania Viannia* spp. system we observed that, rather than a cranial-caudal increase in susceptibility to *Leishmania* infection, a site specific response occurs. Hamsters (n=7/group) inoculated with $1x10^6$ promastigotes of *L. panamensis* (MHOM/COL/84/1099) or *L. guyanensis* (WHI/BR/78/M5313) consistently showed a faster and more severe lesion evolution in individuals infected in the snout. Lymphocyte proliferation of cells obtained from the draining lymph nodes of hamsters infected with *L. guyanensis* , and exposed to the specific antigen, indicated that the strongest cell-mediated response was exerted by hamsters infected in the foot (p=0.002). Histopathology showed that 30 days p.i. small doses (1x 10^3) of *L. panamensis* produced ulceration only in the snout. In addition, the mononuclear and polymorphonuclear infiltrate was more extensive and necrotizing in the snout than in the foot. When *L. panamensis* infections were treated with antimonials, lesion reduction was faster in hamsters infected in the foot than individuals infected in the snout. These results considered together, indicated that the clinical and immunological response of the hamster-*Leishmania Viannia* system could be modulated to obtain different "sub-models" appropriate for various experimental approaches regarding pathogenesis, immunization or chemotherapy.

494 NO TITLE. Traub-Cseko.

Leishmania are parasitic protozoa that cause a spectrum fo diseases that go from cutaneous to visceral leishmaniasis, and that affect millions of people, mostly in developing countries. Leishmaniasis is a serious Public Health problem in Brazil. One striking aspect of these parasites is their capacity to escape destruction in the hostile

environment of the macrophage, where they reside and multiply. The knowledge of the molecules responsible for these escape mechanisms can lead to the development of drugs that interfere with these processes and/or vaccines. Our objective was to study amastigote-specific cysteine proteinases, that can have a role in these processes. In previous work we have identified and characterized two cysteine proteinase genes of *L. pifanoi*. We are presently carrying on studies on the mechanisms of targetting of these proteinases to the lysosome. One possible mechanism might involve the C-terminal extension characteristic of trypanosomatid cysteine proteinases. We have identified a gene that is divergent in this region from the other copies of the repeat. We are investigating the functional differences of these two proteinase types. As an alternative mechanism, we are also investigating the possible function of N-glycosilation in targetting, through mutagenesis. We have also constructed recombinant clones of cysteine proteinase genes containing molecular tags and started transfection experiments that will allow to follow the targetting of these molecules to the lysosome, in homologous and heterologous systems.

495 A RAPID DIAGNOSTIC ASSAY FOR VISCERALIZING *LEISHMANIA TROPICA*. Alwen A*, Francies WM, Campbell JR, Hall E, and Mansour M. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt.

The protozoan *Leishmania* is transmitted by the sandfly (*Phlebotomus*). It causes leishmaniasis, a disease widely spread in developing countries, against which no effective vaccine has yet been developed. The clinical presentation of the various forms of leishmaniasis ranges in gravity from self-healing cutaneous lesions to fatal visceral infections. Until recently, the species *L. tropica* was believed to generate a relatively innocuous form of cutaneous leishmaniasis only. However, in recent years, *L. tropica* was found in the bone marrow and/or in splenic biopsies from returning Gulf War veterans, an indication of the visceralization of the organism. It has become crucial to find a means of differentiating cutaneous from visceralizing forms of *L. tropica*. We are developing a reliable diagnostic assay by making subtractive genomic and cDNA libraries. These libraries are then screened by differential hybridization and/or Southern and northern blotting for clones specific to the visceralizing *L. tropica* isolates. The clones of interest are then sequenced and analyzed. To date, thirteen clones have been chosen after Southern blot analysis and are currently being sequenced. The primers designed on the basis of these sequences are finally tested as to their specificity for the visceralizing *L. tropica* isolates in Polymerase Chain Reaction (PCR) assays. If positive, these primers can then be used in a rapid, sensitive and reliable laboratory assay to determine whether a *L. tropica* isolate is a visceralizing form or not.

496 IMMUNE RESPONSE TO *TRYPANOSOMA CRUZI* GRP78 DELIVERED INTRAMUSCULARLY AS NAKED cDNA IN A EUCARYOTIC EXPRESSION VECTOR. Chen Z, and Rowland EC*. Department of Biological Sciences, College of Osteopathic Medicine, Ohio University, Athens, OH.

Previous studies have shown that the glucose regulated protein (Grp78) derived from *Trypanosoma cruzi* is immunogenic in infected mice. Sera from mice surviving acute infection react with recombinant Grp78 proteins in both ELISA and immunoblot. In this report, cDNA encoding Grp78 is examined for its ability to induce immune responses in mice using naked DNA gene transfer technology. The cDNA of Grp78 was subcloned into a eucaryotic gene expression vector, pCR3, controlled by the CMV promoter. Mouse fibroblasts were transfected with this plasmid and were found to express this protein *in vitro*. A single injection of 100 microgram of this construct was introduced intramuscularly into the quadriceps muscle of BALB/c mice. These mice were found to produce strong IgG1, IgG2a, and IgG2b antibody responses against recombinant Grp78 as measured by ELISA. Using the dermal ear test, these mice were also able to display a strong delayed hypersensitivity response to Grp78. The mice which showed the highest cellular reactivity also had a decrease in parasitemia during infection. These results, when compared to data from mice immunized with recombinant Grp78 protein in combination with Freunds adjuvant or incorporated into ISCOMs, suggests the naked DNA introduction of *T. cruzi* antigens is a promising approach for screening vaccine candidate antigens. Studies involving multiple injections of the Grp78 cDNA in the same expression vector are presently underway.

497 CHARACTERIZATION OF INTRALESIONAL CYTOKINE PRODUCTION IN LOCALIZED CUTANEOUS LEISHMANIASIS. Isaza DM*, Restrepo MI, Restrepo M, and Melby PC. Instituto de Colombiano de Medicina Tropical, Medellin, Colombia; The University of Texas Health Science Center, San Antonio, TX.

The goal of the present study was to characterize the intralesional expression of IFN-γ, IL- I 0, and IL-12 in human localized cutaneous leishmaniasis (LCL). These cytokines play a key role in resistance and susceptibility in experimental leishmaniasis. To date, biopsies from the lesions of 8 patients with active disease due to *Leishmania panamensis* have been studied by single and double immunostaining for cytokines and cell surface markers. Monoclonal antibodies against CD4, CD8, CD14 (macrophage), and CDS 6 (NK cell), and polygonal antibodies against the cytokines were used. Intralesional CD8+ and CD4+ T cells were prominent, and CD14+ macrophages and CD56+ NK cells less common. Cells producing each of the cytokines were present in the dermal infiltrates at approximately

equal density, and positively staining cells for each cytokine could be identified within the same granuloma. There was no cytokine staining in the epidermis. IFN-γ producing cells were primarily CD4+ T cells, but IL-10 staining was common in both CD4+ and CD 1 4+ cells. IL-12 staining co-localized with several of the cell markers, raising questions about the specificity of the polygonal antibody we used. These studies indicate that in active lesions of LCL there is a mixture of protective (IFN-γ and IL-12) and counter-protective (IL- 10) cytokines. The concomitant production of IL-10 with IFN-γ and IL- 12, suggests that it is not sufficient to block their production, as shown in *in vitro* studies. The intralesional immune response appears to be a balance between cytokines which have an anti-leishmanial effect, but may also promote tissue damage, and those which may enhance *Leishmania* survival, but restrain tissue inflammation and damage.

498 PCR AMPLIFICATION OF SERCA LIKE ATPase MOLECULES IN *LEISHMANIA*. Xue L*, and Meade JC. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

The maintenance of tightly controlled intracellular ion concentrations is a physiologically crucial process in all eukaryotic cells, including *Leishmania*. Membrane bound ion motive ATPase proteins are the primary regulators of ion movement in cells. *Leishmania donovani* possess a tandemly linked pair of proton pumping ATPase genes and evidence also indicates the presence of calcium stimulated, vanadate sensitive ATPase activity. We have used primers designed to bind the highly conserved ATP binding (FSBA site) and phosphorylation domains of ion motive ATPase genes to PCR amplify calcium motive ATPase sequences from the genomic DNA of *Leishmania donovani* and *L. mexicana pifanoi*. PCR products of 1026 and 1029 bp respectively were obtained. The PCR products are nearly identical, 97% by protein sequence and 95% in nucleotide sequence. Comparison of the *Leishmania* peptides with the analogous region from other ATPases reveals homology of 43%, 37%, and 34% with sarcoplasma-endoplasmic reticulum calcium ATPases (SERCA) from three protozoan parasites; *Plasmodium falciparum, Trypanosoma brucei*, and *Trichomonas vaginalis*. Homology scores in alignment with other ion motive ATPases gave significantly lower values. We conclude that the PCR products we have amplified encode portions of the *Leishmania* SERCA pump.

499 RESERVOSOMES OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES AND THE *IN VITRO* EFFECTS OF EXTRACELLULAR ATP ON THEIR SEQUESTRATION OF HORSERADISH PEROXIDASE. Ribeiro-Rodrigues R*, Carter CE, and Bogitsh BJ. Department of Biology, Vanderbilt University, Nashville, TN.

The marker protein, horseradish peroxidase (HRP), is transported into the cytosol of *Trypanosoma cruzi* epimastigotes by a combination of receptor- mediated and fluid phase pinocytosis. Subsequently, HRP is sequestered in reservosomes, organelles peculiar to this stage of the parasite's life cycle. It is likely that these organelles provide much of the nutritional energy for metacyclogenesis by the parasite. Incubation for 18-24 hrs in LIT medium containing 50 mM ATP produced distinct alterations in the ultrastructure of the epimastigotes, most obvious of which was the formation of large, membrane-bounded vacuoles in the cytosol. Upon removal from an ATP-HRP enriched medium, HRP reaction product was observed in these vacuoles. Twenty mins after removal from the ATP-HRP medium, the HRP_{+VE} vacuole began separating into discrete structures so that after 60 mins post-removal, obvious reservosomes were observed. It is hypothesized that extracellular ATP increased the permeability of the cytostomal and/or flagellar pocket membranes, allowing HRP to accumulate much more rapidly than usual which resulted in the formation of these large vacuoles. Unlike the intracellular pathway in mammalian phagocytic cells whereby endocytosed dyes are secreted into the extracellular medium once the cells are removed from the ATP-enriched medium, results of the present investigation lends credibility to the view that reservosomes are retained by the epimastigote until metacyclogenesis occurs.

500 EXPRESSION AND LOCALIZATION OF THE 24 KDA FLAGELLAR CALCIUM BINDING PROTEIN OF *TRYPANOSOMA CRUZI*. Godsel LM*, Olson CL, Maldonado RA, Goldenberg S, and Engman DM. Departments of Pathology and Microbiology-Immunology, Northwestern University Medical School, Chicago, IL; and Department of Biochemistry and Molecular Biology, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil.

The flagellar calcium-binding protein (FCaBP) of the protozoan parasite*Trypanosoma cruzi* is a member of the newly named "calflagin" family of FCaBPs,EF-hand calcium binding proteins found in the flagella of a variety of trypanosome species. Immunoblot and immunofluorescence assays were performed to assess the expression and localization of FCaBP in different life cycle stages of *T. cruzi*. These studies showed that FCaBP is found exclusively in the flagellum of the epimastigote, but in the cytoplasm as well in the trypomastigote and amastigote. This has been confirmed by immunogold electron microscopic studies which further demonstrate that FCaBP is associated with the axoneme and paraflagellar rod; cytoplasmic staining seen in all forms of the parasite does not seem to correspond to any particular cellular structure. To determine the amino acid sequences necessary for the specific

targeting of FCaBP to the flagellum, we transfected *T. cruzi* epimastigotes with various epitope-tagged deletion mutants of FCaBP and assessed their localization by immunofluorescence assays using a tag-specific monoclonal antibody. These studies showed thatthe amino terminus of the protein is critical for proper flagellar targeting and/or association with flagellar components. Further work is underway to determine the exact amino acid residues that mediate flagellar localization as well as the biochemical basis for this localization.

501 IDENTIFICATION OF AN ENHANCER-LIKE ELEMENT IN THE *TRYPANOSOMA CRUZI* RIBOSOMAL RNA GENE PROMOTER. Harris-Kerr CL*, and Buck GA. Department of Microbiology and Immunology, Medical College of Virginia Campus, V.C.U., Richmond, VA.

We have previously demonstrated the ability of the cloned *Trypanosoma cruzi* strain CL rRNA gene promoter to drive the expression of the CAT reporter gene after transient transfection of the appropriate plasmid constructs into epimastigotes. Deletion analysis of the promoter sequence strongly suggests that the most critical element necessary for efficient *in vivo* transcription is located around -360 relative to the transcription start point. Loss of a ten nucleotide sequence between -356 and -346 results in a drop of CAT activity 100-fold relative to the full-length construct beginning at -379. The ten nucleotide sequence between -356 and -346 includes a TTTTTG motif which represents one half site of a direct repeat element located between -367 and -346. This motif is also present at other downstream locations in the promoter sequence and is also found in the large repeated units R1 and R2 found upstream in the IGS (intergenic spacer). Gel-shift assays with *T. cruzi* nuclear extracts suggest the presence of proteins which recognize the enhancer-like element at -360. Understanding how this element and its binding proteins interact will help us better define transcriptional regulation in *T. cruzi*.

502 DIFFERENTIAL EXPRESSION OF ATPase 1B PROTON PUMP IN *LEISHMANIA DONOVANI*. Hicock PI*, Kong L, Stiles JK, and Meade JC. Department of Clinical Laboratory Sciences, School of Health Related Professions, University of Mississippi Medical Center Jackson MS; and Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

Leishmania donovani, a member of the Kinetoplastida, is a parasitic hemoflagelate. The organism cycles between two morphologically and biochemically distinct forms; an extracellular promastigote residing in the insect vector and an intracellular amastigote residing in the acidic phagolysosome of host reticulendothelial cells. *L. donovani* contains a proton pumping ATPase gene duplicated in tandem. Northern blot analysisof mRNA from the two forms of *L. donovani* demonstrates that these two ATPase genes are differentially regulated; transcripts of the downstream (1b) gene are not detected in log phase promastigotes but are abundant in amastigote forms. However, reverse transcription of cellular RNA followed by polymerase chain reaction (RT-PCR) shows that the 1b ATPase is transcribed in the promastigote and both ATPase genes (1a & 1b) are transcribed as a polycistronic precursor. This precursor is processed by trans-splicing into distinct 1a and 1b mRNAs. This evidence indicates that message abundance of the 1b ATPase is not controlled by differential promoter activity but is regulated by posttranscriptional events which may include trans-splicing efficiency and mRNA degradation.

503 TRANS-SPLICING AND POLYADENYLATION AT NASCENT RNA TRANSCRIPTS FROM CATION TRANSLOCATING ATPASE 1A LOCUS IN *LEISHMANIA DONOVANI*. Stiles J*, Kong L, Hicock P, Xue L, and Meade JC. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS.

Pathogenic trypanosomes and leishmanias evade or adapt to host defenses during their cyclical transition from the vector to vertebrate stages. Evidence suggests that *Leishmania donovani* may rely on proton pumps such as cation transport ATPases to drive the uptake of nutrients and to regulate ion flow to allow for adaptation and differentiation. The mechanism of transcriptional regulation of these genes in *Leishmania* sp. has not been clearly defined. We analyzed one of the ATPases (ATPase 1A) in *L. donovani* at different stages of development (amastigote, promastigote; log and stationary phases) and culture conditions for analogous transcription regulatory elements which feature prominently in eukaryotes. Using 3'and 5'RACE (Rapid Amplification of cDNA Ends) experiments followed by cloning and sequencing of the 5' and 3'ends of mRNA we determined the *trans* spliced miniexon and polyadenylation sites of the ATPase 1A message respectively. The results indicate that the 39 nt 5' miniexon is spliced 676 nt upstream of the start codon and appear to shift to an alternate site 15 nt downstream when the site is mutated. The poly(A) site of the 1A message was variable in log and stationary phase promastigotes and occured within a 32 nt stretch 1325 bases downstream of the stop codon. A polypyrimidine tract was located 239 bases upstream of the poly(A) site.

504 THE USE OF *scid*-SOURCE OF *LEISHMANIA MAJOR* TO STUDY THE HOST CELL-PARASITE INTERFACE. Guy RA*, and Belosevic M. Departments of Biological Sciences and Immunology, University of Alberta, Edmonton, Canada.

The amastigote stage of *Leishmania major* has not been extensively studied due to the difficulties in obtaining sufficient amounts of parasite material. We propose that the parasites obtained from *scid* mice can be used to study the biology of host cell-parasite interactions. The course of the infection in susceptible BALB/c and BALB/c congenic *scid* mice was examined: a delay in both the development of footpad lesions and the multiplication of the parasite in the first 3 weeks of infection occurred in *scid* mice. By week 5, the lesion size was similar in the two strains of mice, however, there were 10 fold greater numbers of parasites in the footpads of *scid* compared to BALB/c mice. The surface of amastigotes obtained from BALB/c mice was covered with host immunoglobulin, whereas that of parasites from *scid* mice was immunoglobulin-free. Macrophages internalized significantly more BALB/c-source amastigotes *in vitro* compared to *scid*-source parasites. Opsonization of the *scid* amastigotes with parasite-specific IgG increased the uptake of *scid*-source organisms, which was similar to the phagocytosis of BALB/c-source amastigotes. Surface labeling of the *scid*-source amastigotes using iodination revealed a few surface molecules, the major bands were 13.8, 11.5 and 4-9 kDa. Biotinylation revealed additional bands, including a major band of 60 kDa. A low molecular weight, water soluble molecule isolated from *scid*-source amastigotes significantly reduced the uptake of parasites by macrophages. Thus, the *scid* source amastigotes will be usefull for studies of the host cell-parasite interface in leishmaniasis.

505 EVIDENCE THAT A UNIQUE GENE IS CONTAINED IN THE REGION BETWEEN STATIONARY MSP (GP63) GENES OF *LEISHMANIA CHAGASI*. McCoy JJ*, Donelson JE, Wilson ME. Department of Internal Medicine, University of Iowa, Iowa City, IA; Department of Biochemistry, University of Iowa, and Howard Highes Med Institute, Iowa City, IA; Departments of Internal Medicine and Microbiology, University of Iowa, and VA Medical Center, Iowa City, IA.

The surface protease gp63 of *Leishmania chagasi* is encoded by at least 18 tandem *msp* genes belonging to three classes (*msp*L, *msp*S, *msp*C) that differ in their 31 untranslated sequences (3'UTRs). RNAs from *msp*L genes are expressed primarily during log phase of promastigote (PM) growth *in vitro* when PM virulence is low; RNAs from *msp*s genes are expressed primarily during stationary phase when PMs are highly virulent; and RNAs from *msp*c genes are expressed throughout growth. The amount of *L. chagasi* gp63 protein increases 11-fold as PMs grow from log to stationary phase, in part due to accumulation of the product of one p*msp*s gene (*msp*S2). Because the 51 UTRs and coding regions of ims genes are highly homologous, we studied the 31 UTR and downstream intergenic region (IR) of *msp*S2 to identify sequences responsible for high level gp63 expression. This 3' UTR + IR, when cloned downstream of a β-galactosidase reporter gene, caused increased β-gal activity in stationary versus log phase PMs. Sequence analysis of the 2.8 kb *msp*S2 IR showed several open reading frames, the longest of which is 1263 bp, suggesting the presence of (an)other gene(s). A [^{32}P]-labeled 2.3 kb PCR product amplified from the IR hybridized to a 2.5 kb RNA species in log and stationary PM RNA. These data suggest the presence of genes downstream of *msp*S2, within a region likely involved in gp63 regulation.

506 INFECTIVITY OF PROCYCLIC AND METACYCLIC PROMASTIGOTES OF AN HUMAN VISCERAL STRAIN OF *LEISHMANIA INFANTUM*. Louassini M*, Adroher FJ, and Benítez R. Department of Parasitology, Faculty of Pharmacy, University of Granada, Granada, Spain.

Agglutination by peanut agglutinin (PNA) is mostly used to separate procyclic (PNA+) and metacyclic (PNA-) promastigotes of *Leishmania* spp. Previously this technique, has only been used to study the infective capacity of promastigotes during growth or to determine the infectivity of inetacyclic cells. The present study aimed to follow, in part, the variation in infective capacity during growth and, on the other hand, to determine the infectivity of agglutinated (PNA+) and non-agglutinated (PNA-) parasites. Proniastigotes of an human visceral strain of *L. infantum* were cultured at 23°C in RPMI-1640 medium supplemented with 20% IFCS and modified by us. The ability of parasites to invade and multiply within J744 marine macrophages was assessed in RPMI-1640 supplemented with 10% IFCS. %I express percentage of parasites/macrophage at time "x" postinteraction (p.i.) divided by the number of parasites/macrophage at 0 h p.i. (after 2 h of interaction), and is used to discuss the following results. Amastigotes from the promastigotes collected on 2nd day of culture (2nd d) showed a dramatically decreasing tendency of %I, with 33% and 25% at 24 h and 72 h p.i. respectively. Whereas, by the 4th day of growth (4th d), %I increased to a value of 172% at 24 h p.i. showing a maximum of 172% at 72 h p.i. However, by the 6th day of growth (61 d), %I had overtaken this maximum at 24 h p.i. with a value of I'/ 2%, decreasing later to 109% at 72 h p.i. The PNA- cells showed an increasing tendency similar to that of the 4th d, but the %I values at 72 h p.i. were greater, with 237%, 397% and 312% at the 2nd, 4th and 6th day of growth respectively. At the 2nd, 4th and 6th d both the D-galactose treated and the untreated groups of PNA+ cells, treated in order to eliminate the PNA macromolecules, showed a decreasing tendency similar to that seen on the 2 nd d overtaking minimum values at

71 h p.i. These findings oblige us to review the infective concept in *Leishmania* at least in *L. infantum*, when the PNA technique is used, to include the supposedly procyclic PNA+ cells, which showed, at 72 h p.i., 89% parasitized niacrophages and the %I equal to or superior to that showed by promastigotes of logarithmic phase of growth. We propose the variation of parasitic mass to know the progression or recession of infection. This term expresses the ratio between parasites/macrophage by % parasitized macrophages at 72 h to the same at 0 h p.i.

507 CANINE LEISHMANIASIS: ESTABLISHMENT OF A DOG *LEISHMANIA* MODEL AND STUDY OF THE VIRULENCE OF PARASITES. Rhalem A*, Sahibi H, Guessous-Idrissi N, Natami A, Lasri S, and Berrag B. Departement de Parasitologie, Institut Agronomique et Veterinaire Hassan II, Rabat, Morocco; and Unite d'Etudes et de Recherche sur les Leishmanioses, Faculte de Medicine et Pharmacie de Casablanca, Morocco.

Little work on vaccination against visceral leishmaniasis has been carried out. A vaccine for canids will reduce human disease, should be easier to accomplish than a vaccine for human disease and provide insights into a human vaccine. Development of dog-*Leishmania* model should be an adequate way to achieve this goal. In this study, 3 *Leishmania* strains were isolated from naturally infected dogs: 2 from Khemisset region cultured and typed as *L. infantum*, the third one from Taounate is still under culture and typing. Nine naive dogs were used and infected by a dose rate of 10^5 amastigotes per Kg body weight by intravenous route. Parameters used to assess the infection were clinical signs, parasitemia, antibodies and histological changes. The infections induced severe visceral and cutaneous symptoms similar to signs recorded from sick dogs in the field, except absence of adenopathy which may be explained by the route of infection. Of the total used, 7 dogs died within 2 to 5 months post-infection (P.I.). The two remaining dogs are still alive and continue to exhibit moderate signs. The parasites were isolated from spleen and skin by biopsy. Circulating antibodies appeared 7 days P.I. Antibody titres (IFAT) being low at the beginning of infection increased in parallel to exhibition and aggravation of clinical signs. The investigations revealed the high virulence of autochthonous *Leishmania* strains. The results obtained are especially striking, as published reports suggest that laboratory infected dogs frequently self-cure and may not be good models for acute clinical disease.

508 CLONING AND OVEREXPRESSION OF CASEIN KINASE IIα SUBUNIT FROM *LEISHMANIA DONOVANI CHAGASI*. Bhatia A*, Ismail SO, Paramchuk W, Gedamu L. Department of Biological Science, University of Calgary, Canada.

Casein kinase II (CKII) is a second messenger-independent serine/threonine protein kinase which is ubiquitously present in all eukaryotic cells. The holoenzyme exists as a heterotetramer comprising of two α and two β subunits. The α subunits provide the catalytic activity of the kinase subunit. Recent studies have shown that CKII plays a very important role in the phosphorylation of various transcription factors, oncogenes, anti-oncogenes and signal transduction proteins. CKII might also play a significant role in intra-cellular signal transduction during cell proliferation and differentiation. In order to study the functional role of CKII α subunit during *Leishmania* differentiation and pathogenesis, we have recently isolated and sequenced a full length cDNA clone from *L. donovani chagasi*. The CKIIα gene has been localised to a single chromosome using PFGE. Southern blot analysis reveals that CKIIα subunit is encoded by a single copy gene. The CKIIα cDNA has been successfully expressed as a GST fusion protein for antibody production. Northern blot analysis has revealed that CKIIα transcript levels change during the various stages of *Leishmania* differentiation as well as in reponse to various oxidative stress agents. We have overexpressed the CKIIα cDNA in L donovani chagasi using pX expression vector. Studies are currently underway to investigate the response of *Leishmania* overexpressing CKIIa to oxidative stress agents. The effect of CKIIα gene disruption in Leishmania is also being investigated.

509 MOLECULAR CLONING AND EXPRESSION OF A *TRYPANOSOMA BRUCEI* RIBONUCLEASE H (RNase H2) GENE. Campbell AG*, and Bodnick JS. Division of Biology and Medicine, Brown University, Providence, RI.

Ribonucleases H (RNases H) are enzymes which degrade the RNA strand of RNA:DNA duplexes and as such may satisfy roles in both DNA replication and transcript processing. We are interested in the events which control and regulate parasite DNA replication and transcriptional activities. Recently we cloned an RNase H gene from the kinetoplastidae, *Trypanosoma brucei*, by complementing bacterial mutants deficient in this activity and have expressed its encoded enzyme. This RNase H gene represents only the second eukaryotic RNase H gene for which complete nucleotide sequence information is available and reagent quantities of the enzyme has been produced. Our current studies are aimed at exploring the range of catalytic functioning of the enzyme and its potential role in replication and transcript processing including its ability to interact with snRNAs. In situ localization studies of this

enzyme in the parasite will also provide important clues into its resident sites of action and specific biological roles. By understanding the mechanism of RNases H action, their specific roles in gene expression and parasite growth and replication will become apparent.

510 INTERFERON-γ AND INTERLEUKIN-5 PRODUCTION IN MICE IMMUNIZED WITH FORMALIN-KILLED PROMASTIGOTES OF *LEISHMANIA (LEISHMANIA) DONOVANI* AND CHALLENGED. Shin SS*, and Hanson WL. Department of Parasitology, College of Veterinary Medicine, The College of Georgia, Athens, GA.

Studies were conducted to investigate the interferon-γ and interleukin-5 (IL-5) response in mice following immunization with formalin-killed promastigotes in aluminum hydroxide (FKP/AH) or following immunization and challenge with homologous virulent *Leishmania (Leishmania) donovani.* Spleen cells obtained at the end of the immunization schedule form mice receiving FKP/AH produced lower levels of IFN-γ (25.0 ± 6.7 compared to 104 ± 3.5 U/ml) and higher levels of IL-5 (675.7 ± 34.4 compared to 488.7 ± 55.7 pg/ml) in the presence of Con A (5 μg/ml) *in vitro* than spleen cells from control mice receiving AH alone. Following challenge infection, however, spleen cells from mice immunized with FKP/AH produced considerably greater quantities of IFN-γ (179.6 ± 7.9 compared to 105.2 ± 11.3 U/ml) and lower levels of IL-5 (248.3 ± 16.0 compared to 747.7 ± 14.3 pg/ml) than cells receiving AH alone. Increased *in vitro* IFN-γ and lowered levels were accompanied by an 83.2% reduction in the numbers of the liver amastigotes in mice immunized with FKP/AH. These studies suggest that protective immunization with FKP/AH enhanced the activity of TH1-like response accompanied by possible inhibition of the TH2-like response by spleen cells only after challenge infection but not prior to challenge infection.

511 ALTERATIONS OF G-PROTEINS IN ACUTE MURINE CHAGAS' DISEASE. Huang H*, Tanowitz HB, Chen B, Wittner M, and Morris SA. Albert Einstein College of Medicine, Bronx, N.Y.

In order to characterize the changes in myocardial signal transduction in Chagas' disease we examined myocardial G-proteins (Gp) in CD-1 mice infected with the Brazil strain of *T. cruzi* at 7, 21, 30 and 60 days (d) post-infection. Western blot (WB) analysis revealed that Gi_2, Gi_3 and Gβ subunits were not altered at 7 d. However, at 21 and 30 d they were significantly increased. In those mice that survived to 60 d there were no changes in these Gp subunits. In contrast, throughout the course of infection, Go was unchanged; Gi_1 was not detected and Gs was decreased at 21 and 30 d and unchanged at 60 d. Northern blot (NB) analysis revealed that Gi_2 and Gi_3 mRNAs were unchanged at 7 d but significantly increased at 21 and 30 d. There were no changes in the mRNAs for these subunits in those mice surviving to 60 d. Go and Gs mRNAs were unchanged during the course of infection. The ratio of Gs:Gi by WB and NB decreased during infection. Verapamil treatment did not reverse the infection-associated changes in myocardial Gp subunits. Our studies suggest that acute Chagas' disease is associated with significant alterations in myocardial signal transduction pathways and that the cardioprotective effect of verapamil may be independent of these pathways.

512 MOLECULAR CLONING AND CHARACTERIZATION OF AN IMMUNODOMINANT *ONCHOCERCA VOLVULUS* LARVAL ANTIGEN RECOGNIZED BY PUTATIVELY IMMUNE INDIVIDUALS. Joseph GT*, Mair KF, Kass PH, Huima T, and Lustigman S. Virology and Parasitology, The Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY; and Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA.

Western blot analysis of *Onchocerca volvulus* L4 extracts with sera from putatively immune and infected individuals has indicated that the putatively immune sera recognized a unique set of specific antigens. In an effort to clone these L4 antigens, a pool of putatively immune sera was used to immunoscreen an *O. volvulus* L4 cDNA expression library. One of the isolated clones, OV73K, was ubiquitous. The antigen corresponding to OV73K cDNA clone is expressed in the L4, L3 and adult stages of the parasite. The humoral immune response to the recombinant OV73K antigen was analyzed in putatively immune (PI) and infected (INF) individuals from Liberia and Ecuador. While 90-100% of the Liberian PI and INF recognized the antigen, only 50-60% of the PI and INF from Ecuador reacted. The geographic difference was also observed when sera of infected individuals from Ivory Coast and Guatemala were tested and the antigen recognition was 85 and 40%, respectively. Analysis of the specific IgG subclass response to OV73K antigen among the Liberian PI and INF subjects showed that although the PI had similar levels of IgG1 and IgG3 antibodies as the INF, the PI individuals had significantly lower levels of OV73K-specific IgG2 and IgG4 responses. Antibodies raised against OV73K antigen localized the protein in the cuticle, hypodermis and in the secretory vesicles of L4. Despite its strong recognition by the infected individuals, the immune recognition of OV73K antigen by the putatively immune individuals makes it an intriguing candidate for protection against *O. volvulus* infection.

513 CHARACTERIZATION OF FILARIAL RETINOL-BINDING PROTEINS. Gundlapalli AV*, and Rajan TV. Department of Pathology, University of Connecticut Health Center, CT.

Filarial parasites are a major public health problem in a number of third world countries, where there are an estimated 120 million cases of lymphatic filariasis. In an attempt to generate a recombinant DNA based subunit vaccine for this disease, our laboratory has focused on aspects of the parasite's metabolism that are unique to it and distinct from that of the host. One function that appears to be critical in parasite physiology is the accumulation of retinoids. Considerable evidence in the literature suggests that filarial parasites sequester retinoids by synthesizing high affinity retinoid binding proteins (RBP). The current work is based on the hypothesis that an immune response against parasite RBP should prevent the sequestration of retinoids by filarial parasites and thereby prevent their growth in the mammalian host. Using a combination of ion-exchange and size separation chromatography, we have purified RBP to homogeneity from the dog heartworm, *Dirofilaria immitis*, and obtained the N-terminal amino acid sequence of the purified protein. The resultant 37 amino acid sequence does not match any of the RBP's catalogued in GenBank, which are all of vertebrate origin. The amino acid sequence does, however, show almost complete identity with a sequence previously reported, to be that of a highly immunogenic antigen derived from the human filarial parasite, *Onchocerca volvulus*. We are currently cloning full length cDNAs of this antigen from various filarial parasites. This will enable us to express the recombinant proteins, assay their retinol binding properties, and perform animal protection studies to determine their efficacy as vaccine candidates.

514 CONSTRUCTION AND ANALYSIS OF A cDNA LIBRARY OF *ONCHOCERCA VOLVULUS* INFECTIVE STAGE (L3) LARVAE. Lu W*, and Williams SA. Molecular and Cellular Biology, University of Massachusetts at Amherst, MA; and Department of Biological Sciences, Smith College, Northampton, MA.

About 20 million individuals worldwide are infected with *Onchocerca volvulus*, the filarial parasite that causes African River Blindness. Due to a limited amount of available parasite material and the lack of a transformation system for filarial parasites, it is impossible to identify filarial genes by direct functional analysis. To aid in the discovery of genes involved in immune evasion and pathogenesis, and to identify genes that code for proteins that could serve as targets for new drugs or that will be useful for vaccines, a cDNA library of *Onchocerca volvulus* infective larva (L3) was constructed. The cDNA library has an original titer of 180,000 pfu and an average insert size of 900 base pairs. To characterize the library and to identify some of the important genes of the organism, EST (Expressed Sequence Tag) analysis was employed. EST analysis involves the sequencing of random cDNA clones and the comparison of the resulting data with sequences from other organisms via computer database searches. Several hundred ESTs have been analyzed to date. Many interesting sequences showing excellent matches with genes of known function have been identified using EST analysis. Examples of these genes include macrophage migration inhibitory factor, thiol-specific antioxidant protein, and onchocystatin precursor Ag. Sequences with similarity to vespid venom allergen, *Drosophila* bicoid protein, and others have also been identified. About 50% of the ESTs do not match with any functionally identified genes in any database and represent parasite genes of unknown function. A variety of interesting clones have also been identified by immunoscreening. Further information will be obtained by ongoing EST analysis and the study of newly discovered genes.

515 CHARACTERIZATION OF ECDYSONE RECEPTOR TARGET GENES IN A FILARIAL PARASITE, *DIROFILARIA IMMITIS*. Crossgrove K*, and Maina CV. Molecular Parasitology Group, New England Biolabs, Inc., Beverly, MA.

Filarial parasites are responsible for several serious human diseases. A useful model system for the study of these parasites is *Dirofilaria immitis*, the causative agent of dog heartworm disease. Study of the development of this parasite may suggest mechanisms for combating filarial parasites. *D. immitis* undergoes four molts from the microfilarial to the adult stage. Many insects undergo a similar series of molts during their development. The steroid hormone ecdysone has a well-characterized developmental role in insects, where it is involved in the control of molting and metamorphosis. In *D. immitis*, molting from the third to the fourth larval stage can be induced *in vitro* by ecdysone, suggesting that ecdysone may play a similar role in the development of *D. immitis*. Recent work in *D. immitis* has identified homologues to the *EcR* and *usp* genes, which compose the functional ecdysone receptor in *Drosophila*. The ecdysone signal in *Drosophila* is modulated by a number of transcription factors that are induced as a primary response to ecdysone. Since *EcR* and *usp* homologues are found in *D. immitis*, it is possible that homologues of ecdysone receptor target genes also play a developmental role in *D. immitis*. We are searching for homologues of these target genes in *D. immitis* by PCR with degenerate primers, Southern hybridization, and library screening. We are also undertaking an unbiased search for target genes of the *D. immitis* ecdysone receptor using the yeast two-hybrid system. Using degenerate primers to the *Drosophila E75* gene, an ecdysone-inducible primary response gene, we have identified a putative *EcR* homologue in *D. immitis*.

516 ANTIBODY RESPONSES TO RECOMBINANT PARASITE ANTIGENS IN PATIENTS WITH ONCHOCERCAL DERMATITIS. Chandrashekar R*, Murdoch ME, Weil GJ, Hay RJ, Maizels RM, Jones BR, and Abiose A. Kaduna/London/St. Louis Collaboration for Research on Onchocerciasis.

The pathogenesis of onchocercal dermatitis is poorly understood, but autoimmunity may be involved in some forms of the disease. We have recently shown that immune complexes in sera from onchocerciasis patients contain a parasite intermediate filament (OvIF) with significant homology to human keratin. Some onchocerciasis patient sera contain antibodies to OvIF and autoantibodies to human keratin. The present study measured IgG and IgG4 antibodies to *O. volvulus* adult worm extract (OvA) and to recombinant *O. volvulus* antigens (OvIF, OC3.6, and OC9.3) in sera from people in mesoendemic savannah areas of northern Nigeria with various types of onchocercal dermatitis (n=98) and from people with normal skin and high MF loads (n=30). Interestingly, 5 of 5 sera from patients with lichenified onchodermatitis (sowda) contained antibodies to OvIF. There was no obvious association between antibodies to OvIF and other types of dermatitis. Paradoxically, antibodies to OvIF were more common in patients without dermatitis than in patients with dermatitis (64% vs. 46% P=0.001). Conversely, antibodies to OC3.6 and OC9.3 (both prevalence rates and levels) tended to be higher in patients with dermatitis than in people with normal skin. Antibodies to OvA were the same in these groups. Additional studies are needed to determine whether autoantibodies play a role in the pathogenesis of the sowda form of onchocercal dermatitis.

517 HELMINTH INFECTION WITH EOSINOPHILIA IS ASSOCIATED WITH INCREASED SERUM PHOSPHOLIPASE A_2 LEVELS. Mawhorter SD*, Bernton EW, Hershey J, and Nutman TB. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Allergy/Immunology Service, Walter Reed Army Medical Center, Washington, DC.

The host response to helminth infection is characterized by eosinophilia and tissue inflammation. Secretory Phospholipase A_2 (PLA2) is a 14 kDa protein which is a mediator of inflammation and tissue injury in many infectious and non-infectious conditions such as sepsis, asthma, anaphylaxis, and inflammatory bowel disease. PLA2 has numerous cellular sources and is also an abundant protein antigen of some filarial parasites. Further, eosinophil degranulation may be induced by endogenous PLA2. This information led us to investigate the role PLA2 may play in mediating inflammation in helminth infections. Serum PLA2 levels were measured in 12 patients with helminth infections (filarial n=10, other helminth n=2) and 9 normal donors using a PLA2-specific ELISA. Helminth infected individuals, with eosinophilia ranging from 100 to 16,409 cells/ml (geometric mean [GM]=869 cells/ml), had significantly increased serum levels of PLA2 (GM=21.5 vs. 11.8, p=0.037) compared to normal individuals. Peripheral blood eosinophilia was shown to correlate with serum PLA2 elevations (p=0.027). Although we have previously described significant differences in eosinophil surface activation molecule (CD66, CD69, CD25, and CD23) expression between these same helminth infected patients and normal donors, there was no correlation between the expression of these molecules and serum PLA2 levels. The data suggest secretory PLA2 may give insights into tissue inflammation and eosinophil activation in helminth parasite infections, although the precise mechanism awaits further clarification.

518 EFFECT OF FGAMMA RADIATION ON *BRUGIA* L3 DEVELOPMENT *IN VIVO* AND THE KINETICS OF GRANULOMATOUS INFLAMMATION INDUCED BY THESE PARASITES. Nasarre C*, Coleman SU, Rao UR, and Klei TR. Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton, Rouge, LA.

In previous studies we have shown that the downregulation of parasite specific cellular immune responses in *Brugia*-infected jirds is not dependant on microfilariae for either induction or maintenance. It is not clear, however, that the adult stage must be reached to induce this downregulated state. To examine this question L3 were exposed to 0, 15, 25, 35, 45, or 90 krads of gamma radiation to alter L3 development and inoculated IP into jirds. Necropsies were performed at 7, 14, 28, and 120 days post-inoculation (DPI). The degree of parasite development, intraperitoneal inflammation, and pulmonary granulomatous inflammation (PGI) to parasite antigen coated beads embolized in the lungs were monitored at these DPI. Parasites irradiated with 15-25 krads did not decrease in number until 28 DPI. Numbers of those irradiated at 35-90 krads were reduced by 7 DPI, although some larvae were recovered from all groups at all times. Larvae which survived were L4. The PGI of all groups except those receiving L3 irradiated with 45-90 krads were decreased at 28 DPI suggesting that development beyond the early L4 is necessary to induce the hyporesponsive state. Data to be collected at 120 DPI will clarify this point.

519 DOWN REGULATION OF T-CELL PROLIFERATIVE RESPONSES BY *BRUGIA MALAYI* ADULT ANTIGEN IN RHESUS MONKEYS. Giambartolomei GH*, Dennis VA, Lasater BL, Grab DJ, and Philipp MT. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

Human and experimental animals infected with the filarial parasite *Brugia malayi* exhibit T-cell hyporesponsiveness. Parasite antigen(s) that may be involved in downregulating T-cell responses are not fully characterized. Earlier studies in our laboratory showed that 10 μg/ml of *Brugia malayi* excretory/secretory (E/S) antigen suppressed *in vitro* the Con A-induced blastogenic response of peripheral blood mononuclear cells (PBMCs) from naive rhesus monkeys (37-42% inhibition). In contrast only 5-15% suppression was obtained when 10 μg/ml adult worm somatic antigens (ASA) were used rather than E/S. However, an increment in the dose of ASA was able to increase the percent suppression of Con A-induced blastogenic response of PBMCs from normal rhesus monkeys. Cells (viability > 95%) treated with different concentrations of ASA (10, 20, 40 and 80 μg/ml) for five days were washed and subsequently co-cultured with freshly obtained syngeneic cells in the presence of Con A. These concentrations rendered an inhibition of 4, 6, 29 and 41% respectively. ASA was fractionated by size exclusion HPLC and a fraction was obtained that was able to induce 30% suppression at a concentration of only 5 μg/ml. These results suggest that *B. malayi* worms contain a suppressive component that may be shed in their E/S products. Current studies are being conducted to further purify and biochemically characterize this component and also to determine the mechanism(s) of the ASA-E/S suppression on the Con A-induced blastogenic response.

520 IMMUNE RESPONSE STUDIES IN BANCROFTIAN FILARIASIS. Regunathan J, and Kaliraj P*. Center for Biotechnology, Anna University, Madras, India.

To charactarize filarial antigens responsible for the diverse immune response noted in the disease filariasis, T cell responsiveness was examined among individuals living in an area in which *Wuchereria bancrofti* is endemic.The parasite antigens used were of *Brugia malayi* adult worm extract (BMA) and recombinant *W. bancrofti* parasite antigen. In order to prepare the recombinant filarial antigens a 1.8 Kb filarial DNA fragment obtained after screening the lambda gt11 *W. bancrofti* genomic library (using pooled filarial sera) was subcloned in two different vectors. The peripheral blood mononuclear cells (PBMC) from microfilaraemic (MF), chronic pathology (CP) and endemic normal (EN) had mounted a similar lymphocyte response to the non-parasite antigen like purified protein derivative (PPD) and mitogen like phytohaemagglutinin (PHA). Whereas the lymphocyte response by the PBMC of MF was significantly very low ($p<0.001$)to BMA compared to CPand EN. The antigen specific cellular unresponsiveness seen in MF was not reversed by the addition of recombinant IL1α, IL1β, and IFNγ. It was found that the PBMC from MF had secreted more IL1to BMA ($p<0.001$) than CP and EN. The two recombinant fusion proteins expressed i.e of 98 and 58 Kda were found to induce lymphocyte response in all the three groups, especially in MF with higher proliferative index ($p<0.001$) than BMA. Since, this recombinant antigens possess T cell stimulatory properties, further analysis of this antigen should help to elucidate the host parasite interaction at cellular and molecular levels.

521 RECOMBINANT *ONCHOCERCA VOLVULUS* ANTIGENS AND THE DIAGNOSIS OF PREPATENCY IN CHILDREN FROM AN ENDEMIC AREA. Gbakima AA*, and Scott AL. Tropical Diseases Research Unit, Njala University College, University of Sierra Leone, Sierra Leone; and Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

Ochocerciasis is hyperendemic in Sierra Leone where it poses a major public health problem. The onset of *Onchocerca volvulus* infection in humans is usually unknown and data from children to define the ontogeny of antibody response is scare. This study was undertaken to determine changes in the humoral antibody response of children and monitor the ontogeny of their IgG and IgG4 profiles every six months over a three year period. Sera from 16 microfilaria positive (mf+) and 369 microfilaria negative (mf-) children (ages 1-15 years) were tested by ELISA to determine the IgG and IgG4 responses against a crude aqueous extract of *O. volvulus* adult and recombinant *O. volvulus* antigens OC 3.6 and OC 9.3. These recombinant antigens are known to be important in detecting prepatent (OC 3.6) and patent (OC 9.3) *O. volvulus* infection in chimpanzees. Fifteen (93.8%) of the 16 mf+ children reacted against the crude adult extract, while 80% of the mf- children reacted. Nine (56.3%) of mf+ children and 95(25.7%) of mf- children had detectable levels of IgG against OC 3.6; the reactivity with OC 9.3 was 31.3% for mf+ children and 21.4% for mf- children. Only 25% of the mf+ children had IgG4 reactivities against OC 3.6, but these responses were significantly elevated. Sixteen percent of mf- children also showed significantly high IgG4 reactivities against OC 3.6. The IgG4 response against OC 9.3 was 6.3% and 8.7% for mf+ and mf- children respectively. These data suggest that enzyme immunoassays based on the recombinant antigens OC 3.6 and OC 9.3 may be useful for the diagnosis of early and prepatent *O. volvulus* infection, especially in early childhood. These antigens may prove more useful in longitudinal studies.

522 MULTIPLEX PCR AND ELISA-BASED DETECTION STRATEGY FOR THE DIAGNOSIS OF *LOA LOA*. Kubofcik J*, Williams SA, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD; and Department of Biological Sciences, Smith College, Northampton, MA.

Currently, the diagnosis of *Loa loa* infection has relied on membrane filtration of whole blood and staining for microfilariae. To improve the sensitivity and specificity of the diagnosis of infection, we have developed a multiplex PCR-based strategy using three sets of Loa-specific sequences that target a ribosomal 5S intergenic spacer region, the subunit of the polyprotein *Loa loa* antigen, and a microsatellite repeat sequence of *Loa loa*. Using ELISA-based detection of these three sequences -- a process that uses one biotinylated and one unbiotinylated primer coupled with a fluorescinated oligonucleotide probe for each target sequence -- we have detected *Loa loa* genomic DNA in amounts as low as 400 fg. This PCR-based diagnostic technique shows no cross reactivity to any of the other filarial parasites pathogenic for humans, including the bloodborne *Wuchereria bancrofti*, *Brugia malayi*, and *Mansonella perstans*. This approach has been used to cofirm *Loa loa* infection in patients whose microfilarial counts have ranged from 0.6 to > 200 per 100 µl. Further testing of this technique is being undertaken to assess formally its efficacy in the diagnosis of loiasis.

523 DIAGNOSIS AND NINE YEAR IMMUNOMONITORING OF FILARIAL PATIENTS DURING DEC THERAPY IN FILARIA ENDEMIC AREA IN INDIA. Padigel UM, Devi KK, Chenthamarakshan V, Reddy MV, and Harinath BC*. Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram, India.

Bancroftian filariasis is a major public health problem in India with about 25 million harbouring microfilariae and 19 million people sufferingwith disease manifestations. ELISA with penicillinase as marker,has been developed using mf ES antigen for detecting antibody and antigen. A nine year followup study was done on the effect of DEC therapy on microfilaraemia, antibody and antigen levels and recurrence of infection in 27 mf patients. One course of DEC treatment (75 mg/kg body wt.) followed by yearly dose (6 mg/kg body wt.) showed disappearance of microfilariaemia in most of the cases excepting for the three cases. Similarly there was significant decrease, followed by gradual increase in antigen and antibody levels during the course of 9 years. None of the cases developed any clinical symptoms suggesting the need for long term treatment and follow up of mf patients in an endemic area to interrupt transmission and prevent disease. ELISA was found to be very useful in indicating filarial aetiology and followup of amicrofilaraemic conditions such as acute, chronic and occult filarial infections. Immunomonitoring of clinical cases with lymphoedema, hydrocoele, lymphadenopathy, TPE, monoarthritis, tenosynovitis, etc with long term DEC therapy (6 mg/kg for 21 days in a month) for as long as 12 months showed considerable relief in clinical signs and symptoms.

524 EFFECT OF REPEATED LARGE SCALE IVERMECTIN TREATMENT ON THE TRANSMISSION OF *LOA LOA* IN SOUTHERN CAMEROON. Bouchite B, Demanou M, Prud'hom JM, Boussinesq M, and Chippaux JP*. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon.

The impact of repeated large scale ivermectin treatment on the transmission of *Loa loa* was evaluated in a village of Southern Cameroon by comparing the infection rates in vector *Chrysops* one year and two years after the first treatment round. The initial prevalence of microfilaraemia in the community was 31%. Pretreatment studies have shown that *Loa loa* was transmitted mainly by *Chrysops dimidiata*, less frequently by *C. silacea*, and that high transmission occurred during rainy season due to density of the vectors. Ivermectin (200 mg. kg-1) was distributed in the community at 3-month intervals from April 1993 to April 1995 and the mean drug coverage was 60%. The entomological method used for all the surveys was the same. Flies were collected during 4 days twice a month, then identified and dissected to count *Loa* larvae in each dissected fly, especially third stage larvae (L3) found in the head of vectors. The proportion of parous flies and the mean infection rate were calculated after each catching round. After two years of ivermectin treatments, the proportion of flies harbouring larvae decreased significantly in comparison with the pretreatment results as did the number of L3 in the head of vectors. Consequently, we may assume that the decrease of the mean microfilarial load following ivermectin treatments led to a decrease of the transmission of loiasis, and that the infection risk in the community was lowered. Analyses of data of the last entomological round (one month before and one month after the April 1995 treatment) are in progress and would have to allow us to tell whether this trend is confirmed and if one can consider a control of loiasis with ivermectin is realistic.

525 SAFETY OF IVERMECTIN FOR TREATING ONCHOCERCIASIS IN POPULATIONS WHERE LOIASIS IS CO-ENDEMIC (CENTRAL AFRICA - GABON). Kombila M*, Duong TI, and Richard-Lenoble D. Department of Parasitology and Tropical Medicine, University School of Medicine, Libreville, Gabon and Tours, France.

Large scale ivermectin treatment for onchocerciasis in Gabon is confronted to the co-filariasis infections which occurs in this region. We know the interesting efficacy of ivemectin on microfilaria *Loa loa* but do not understand the potential secondary effects, on the association of *Onchocerca volvulus* and *Loa loa*. After more than 5000 onchocercian patients were treated in mass campaign (under control of the National Committee on Onchocerciasis) and after the results of individual schedule treatments at the hospital have been completed, we have identified severe neurological adverse events in only 4 cases with asthenia with a degree of confusion or obnubilation partially explain among 2 of them by an alcoholic impregnation. However, pruritus, allergic reactions, oedema, lymphatic infiltration, were often reported after treatment, are in relation with the microfilarial level in skin and blood. Additional studies among the highest microfilaria carriers living in the rural part of the country where the association of main filariasis is frequent and field campaigns with ivermectin are appropriate.

526 SEASONAL SHIFTS IN CIRCULATING *WUCHERERIA BANCROFTI* ANTIFILARIAL IgG ANTIBODY ISOTYPES IN AN ENDEMIC AREA: KWALE DISTRICT, KENYA. Wamae CN*, and Lammie PJ. Center for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya; Parasitic Diseases Division, Centers for Disease Control and Prevention, Atlanta, GA.

The relationship of transmission intensity to anti-filarial antibody responsiveness is not well defined. We studied antibody levels in two communities in Coastal Kenya before and after a transmission season. Blood samples were collected from 294 persons at the initial time point. Microfilaria and antigen prevalence and intensity were significantly higher in Kilore than in Mvumoni. Antifilarial antibody levels prior to the rainy season were dependent upon both age and infection status and all four IgG isotypes were significantly higher in Kilore than in Mvumoni among antigen-negative persons. In antigen-positive persons, levels of IgG4 were also significantly higher in Kilore. Paired serum samples wereavailable from a subset of 123 patients. In comparing pre- and post-transmission samples in Mvumoni, the low prevalence village, there were no significant changes in IgG1, IgG2 or IgG3 levels but IgG4 antibody responses increased significantly. In contrast, antifilarial IgG3 responses decreased significantly following the rainy season in Kilore. Antifilarial antibody responses may be dependent upon the timing of sample collection since changes in antibody levels occur over a transmission period.

527 DETECTION OF A 26-28 KD COPROANTIGEN OF *FASCIOLA HEPATICA* USING A CAPTURE ELISA. Abdel-Rahman SM*, O'Reilly KL, and Malone JB. Department of Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, LA.

Standard fecal sedimentation procedures for quantitating fecal egg counts are time consuming and prone to technical error. Four monoclonal antibodies (MAb) were prepared to a *Fasciola hepatica* 26-28 KD excretory-secretory (ES) protein and were used to characterize the molecule. All four Mab bind the 8 KD protein core of a stable 26-28 KD O-glycosylated glycoprotein present in the feces of infected animals. Using Western blot we have demonstrated that the 26-28 KD ES protein can be detected in bovine feces and that this protein is present in the feces at levels proportional to the fluke burden. Further, in indirect immunofluorescence the antigen was localized to gut cells and tegument of the adult fluke. Potential protease activity of this glycoprotein was also assayed using 1% gelatin SDS-PAGE; no protease activity was detected. Using monoclonal and polyclonal antibodies to the 26-28 KD protein we have developed a capture ELISA to detect and quantitiate the coproantigen in feces. Because this assay is not dependent on the presence of eggs in the feces, this assay will provide a rapid screening test for *Fasciola hepatica* infection of cattle and has potential for diagnosis of human fascioliasis.

528 RECOMBINANT ANTIGENS FOR DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* IN TURKANA, KENYA. Blanton RE*, Aman RA, Wachira TM, and Zeyhle E. Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH; Department of Molecular Genetics, National Museums of Cleveland, Nairobi, Kenya; and Nomadic Health Unit, African Medical and Research Foundation, Nairobi, Kenya.

The Turkana of Kenya are a nomadic people some of whom have the highest prevalence of hydatid in the world. They also have reacted poorly in some immunodiagnostic assays for infection. Most immunodiagnostic assays based on crude cyst fluid contain many cross-reacting antigens making ELISA sometimes non-specific. The products of *in vitro* translation, however, appeared to be highly specific, therefore it was predicted that recombinant antigens produced in bacteria would likewise be specific. Further, there was a pattern to the appearance and disappearance of antigen recognition after chemotherapy that might suggest that the clinical status of individuals could be determined serologically. A longitudinal study of a population of Turkana was initiated to obtain sera for screening an *E. granulosus* cDNA library and for analysis of serologic responses to defined antigens. In a 400 km^2 study area in central Turkana Province, Kenya, 950 individuals were examined by ultrasound. The prevalence of

hydatid cysts was 2-5% depending on the group examined. The mean estimated age of the infected was 38.5 years. The distribution of cysts was equal among individuals >20, but there were few cases under 15 years of age and none under 11. Eighteen clones were identified using pooled sera from Turkana with ultrasound evidence of cysts. By plaque lifts, all of the sera recognized at least one of 4 clones. The clone 7-1 was recognized by 86% of sera, clone 6-3 by 73%, clone 7-5 by 73% and clone 8-1 by 69%. It is hoped that these antigens will allow us to predict at an early stage which seropositive individuals will go on to develop cysts and might therefore benefit from chemotherapy.

529 ESTABLISHMENT AND APPLICATION OF DOT COLLOIDAL IMMUNOASSAY (DIA) FOR ANTIBODY DETECTION IN PATIENTS OF SCHISTOSOMIASIS JAPONICA. Zhu YC*, Yu CX, Yin XR, Liu YJ, and Xu YL. Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu, P.R. China.

In order to develop a simple, cheaper and effective immunodiagnosis method for schistosomiasis in the field use, we established the Dot Colloidal Dye Immunoassay (DIA) and have obtained satisfactory results. One kind of colloidal blue dye (D-1) used as a stain reagent for immunoassay was first selected from the dyes made in China in the study. The optimum condition for labelling the dye onto sheep anti-human Ig G antibody had been explored. The antibody labelled with the dye could react and stain with relative antigens. Minimal concentration of human IgG protein, 25-10 ng/ml, could be detected with the labelled dye by Dot Double Antibody Sandwich Assay. For antibody detection with Dot Colloidal Dye Immunoassay (DIA), 61 of 62 sera samples of schistosomiasis japonica were positive, positive rate was 98.4%. Just one sample in 30 health people sera was positive, the false positive rate was 3.3%. All of 40 Fasciolopsiasis sera samples were negative. After that all 288 serum samples of schistosomiasis, including 69 of acute cases, 110 of chronic cases and 19 of advanced cases were detected with the method, the positive rates were 95.65%, 96.36% and 47.37%, respectively. Among 40 of clonorchiasis cases two cases were positive. The results were similar to that of Dot-ELISA. The activity of antibody labelled with colloidal dye could be maintained for one week in the room temperature and be reserved in lyophilized condition. The assay was cheaper, simple to use and did not require special equipment. It suggested that the Dot Colloidal Dye Immunoassay (DIA) might have potential value for use in schistosomiasis endemic areas.

530 ANTIBODY REACTIVITY OF SMB, A RECOMBINANT *SCHISTOSOMA MANSONI* PROTEIN. Hancock K, Noh JC, and Tsang V. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Immunodiagnosis of *Schistosoma* infections is dependent upon the detection of parasite-specific antibodies. A variety of *Schistosoma* antigens, ranging from crude egg and adult worm preparations to microsomal membrane fractions and recombinant proteins, have been used to detect anti- *Schistosoma* antibodies, with varying degrees of sensitivity and specificity. In order to obtain a ready supply of highly specific *Schistosoma* antigens, we targeted the genus- and species-specific proteins for gene cloning. We selected the *S. mansoni* protein, SMB, from a cDNA expression library, expressed this protein in *E. coli* as a 6X histidine fusion protein, and purified it on a Ni-NTA affinity column. When assayed with a battery of well-defined sera, the purified protein was shown to be 100% specific for *S. mansoni* and *S. haematobium* infections with a sensitivity of 80% for mansoni and 95% for haematobium. The 100% specificity indicates that the 12 amino acid (including 6 histidines), vector encoded, N-terminal sequence of the recombinant protein does not form an antibody epitope. This system may be useful for the expression of other diagnostically important proteins. Recombinant SMB, alone or in conjunction with other cloned proteins, may be a valuable diagnostic tool for detecting *S. mansoni* and *S. haematobium* infections.

531 RATES OF *SCHISTOSOMA MANSONI* INFECTION AND REINFECTION IN THE NILE DELTA. El-Morshedy H, Barakat R*, El Masry AG, ElSayed MK, Farghaly A, Husein MH, and Miller FD. High Institute of Public Health, Alexandria University, Alexandria, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Cairo University, Cairo, Egypt; and School of Public Health, University of Hawaii, HI.

There are no recent data on transmission rates of *Schistosoma mansoni* infection in the Nile Delta. In this study, two annual follow-up measures of incidence, reinfection, and reversion rates were estimated in a large prospective study of *S. mansoni* infection in the northern Nile Delta of Kafr El Sheikh. Rates were estimated in a cohort established from a probability sample of the entire rural area of Kafr El Sheikh. Infection was determined by the examination of two Kato stool slides. The weighted first and second overall annual incidence rates were 20.4% SE ±1.4 and 15.9% SE±1.4, respectively. Geometric mean egg counts in incident cases were 35.6 epg SE ±1.2, and 30.97 SE±1.6 in the first and second follow ups, respectively. Village specific incidence rates was strongly associated with baseline prevalence (r^2= 0.34, $p<0.01$). First and second annual reinfection rates were much higher: 64.8% SE±3.4 and 53.2% SE±3.6, respectively. Village specific reinfection rates were associated with village specific incidence rates (r^2 = 0.32, $p<0.01$). Reversion [loss of infection] rates were highest in children 0 to 4 years old, and increased from the

first to second annual follow-up: 37.2%, SE±3.4 and 47.0%, SE±3.7, respectively. Patterns of these rates by village, hamlet, age and sex were also studied. Selective treatment of infected individuals with praziquantel (40 mg/kg body weight) in each round of examination had little impact on measures of transmission. This is the first report of multiple measures of infection rates of *S. mansoni* in Egypt, and provides important benchmarks for evaluation of control programs. Comparison with limited data on rates of *S. mansoni* infection from previous studies in the Nile Delta, suggests a stable pattern of *S. mansoni* transmission over time.

532 DETAILED AGE SEX SPECIFIC PREVALENCE OF *SCHISTOSOMA MANSONI* IN THE NILE DELTA FROM A LARGE SAMPLE. El-Sayed MK, Barakat R, El Masry AG, Husein MH, Farghaly A, and Miller FD*. High Institute of Public Health, Alexandria University, Alexandria, Egypt; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Cairo University, Cairo, Egypt; and School of Public Health, University of Hawaii, HI.

This is an early descriptive report of the "Epidemiology 123" project in Egypt which makes use of large probability sampling methods. These results focus on *S. mansoni* infection in the northern Nile Delta governorate of Kafr El Sheikh. A probability sample of 18,777 persons, representing the rural population of the entire governorate, was drawn. The sample was designed not to exclude villages based on location or presences of health care facilities and to include representation of the smaller ezbas or hamlets. The objective was to obtain detailed estimates on age and sex specific patterns of *S. mansoni* infection, and to provide a baseline for prospective studies. Stool specimens were examined by the Kato method. The estimated prevalence of *S. mansoni* infection in the rural population was 39.3% (SE±3.3) in 44 villages and ezbas after weighing for the effects of the sample design. The estimated geometric mean egg count per gram stool (GMEC) was 72.9 (SE±7.3). Prevalence and GMEC varied considerably by village and ezba, with ezbas having a small but significantly higher prevalence. Villages and ezba specific prevalence was strongly associated with GMEC (r^2= 0.61, $p<0.001$). The prevalence of *S. mansoni* infection increased by age to 59% at age 15, without significant decline in the adult ages. There was no gender differences until age six, after which males were consistently higher until middle age, when the differences converged. These estimates provide the basis for evaluating control measures for reducing prevalence, intensity of infection, and transmission.

533 COINFECTION WITH *SCHISTOSOMA MANSONI* AND INTESTINAL HELMINTHS IN BOA UNIAO, MINAS GERAIS, BRAZIL. Correa-Oliveira R, Viana IR, Addiss DG*, Prata A, Silveira A, Carvalho O, Colley DG, and Gazzinelli G. Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, Brazil; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Recommendations have been made that in areas where schistosomiasis is endemic, geohelminth control programs should include mass treatment for schistosomiasis. To determine the extent to which geohelminth and *Schistosoma mansoni* infections are associated, we collected 3 stool specimens from residents of Boa Uniao, a village of about 1500 persons in southeastern Minas Gerais, Brazil. Stool was examined microscopically for ova and parasites using the Kato-Katz method. Age-specific infection rates and geometric mean egg counts were calculated; differences in proportions were compared using Fisher's exact test and differences in distributions were compared using the Kruskal-Wallis test. Among 1120 residents tested, 583 (52%) had *S. mansoni* eggs detected in the stool; prevalence of infection was 58.8% for males and 45.9% for females ($p < 0.0001$). Among all persons with and without *S. mansoni* infection, respectively, the prevalence of *Ascaris* infection was 68.2% and 59.8% ($p = 0.003$), the prevalence of hookworm infection was 36.9% and 16.6% ($p < 0.0001$), and the prevalence of *Trichuris* infection was 20.4% and 9.1% ($p < 0.0001$). These differences were generally observed in all age groups. Intensity of infection, based on geometric mean egg counts (GMEC) for the 3 different geohelminths, was not associated with *S. mansoni* infection; however *S. mansoni* GMEC were 115 and 81 eggs/g stool in persons with and without hookworm infection, respectively ($p = 0.006$). In a multiple logistic regression model, age (> 10 years), male sex, and hookworm and *Trichuris* infection remained significantly associated with *S. mansoni* infection. Possible explanations for these findings, including familial, environmental, or spatial clustering as well as immunologic factors, are under investigation. These data suggest that coinfection with *S. mansoni* and intestinal helminths may occur more frequently than previously estimated. If these associations are supported by data from other areas, they would argue for integration of community-based programs to control schistosomiasis and intestinal helminths and would suggest that dual treatment may be a reasonable policy in certain settings.

534 THE IMPACT OF PASSIVE CHEMOTHERAPY ON *SCHISTOSOMA MANSONI* PREVALENCE AND INTENSITY OF INFECTION IN THE EGYPTIAN NILE DELTA. Miller FD*, El-Sayed MK, Farghaly A, El-Badway A, Soliman NK, Husein MH, and Barakat R. School of Public Health, University of Hawaii, Honolulu, HI; Faculty of Medicine, Suez Canal University, Ismailia, Egypt; Faculty of Medicine, Zagazig University, Zagazig, Egypt; High Institute of Public Health, Alexandria University,Alexandria, Egypt; and Faculty of Medicine, Cairo University, Cairo, Egypt.

We investigated the impact of the Egyptian Ministry of Health's (MOH) passive surveillance and treatment program on the prevalence and intensity of infection with *Schistosoma mansoni* in the northern Nile Delta, Egypt. The study was based on a large probability sample of the entire rural population of Kafr El Sheikh. Infection and egg counts were determined by examination of stools using the Kato technique. From a sample of 18,777 villagers, 15,017 provided stool specimens. A detailed history of treatment for schistosomiasis was obtained by interview using standardized questions in colloquial Arabic. A positive history of treatment for schistosomiasis was common, with over 40% of participating villagers reporting prior treatment. Treatment was further characterized by frequency, dates, type of treatment, and where the treatment had been received. In both male and female adults, a positive history of treatment with praziquantel was associated with significantly reduced prevalence of *S. mansoni* infection (For example, odds ratios for 20-24 year old males and femaleswho did not indicate prior treatment were 5.2 and 6.5, respectively, $p<0.001$). Geometric mean egg counts (GMEC) were significantly lower in both male and female adults by more than two fold ($p<0.001$). Age and sex specific prevalence and GMEC curves showed that young and middle aged adults had the largest differences in infection and GMEC. These results support strengthening programs for encouraging villagers to seek examination and treatment and suggest that the prevalence and intensity of infection would be even higher in absence of the MOH passive surveillance and treatment program.

535 TWO YEARS EPIDEMIOLOGICAL FOLLOW UP STUDIES ON *SCHISTOSOMA HAEMATOBIUM* INFECTION IN EL-MINIA GOVERNORATE, EL-MINIA, EGYPT. Gabr NS*, Hussein MH, Hammad TH, Aboel-Einin M, Orieby AO, Showky EM, and Saad A. Parasitology Department,Faculty of medicine, El-Minia University,El-Minia,Egypt; Department of Medicine, Faculty of Medicine, El-Minia University,El-Minia, Egypt; and Public Health Department, Faculty of Medicine, Cairo University, Egypt.

Individuals (9093) from the entire sample (12,111) and 1882 individuals from the subsample (2,180) selected for clinical and ultrasound examination were followed for two years with infection status ascertained. In the first year 1992, using the nucleopore technique for detection of *Schistosoma haematobium* eggs, the prevalence of infection was 8.4% and the mean intensity of infection was 10.9. The peak prevalence of infection in both males (23.4%) and females (11.5%) was in the age group 11-20 years and declined thereafter. The peak mean intensity of infection in both males (16.4) and females (12.7) was in the age group < 10 years. The prevalence and mean intensity of infection were higher in males than females among all age groups. The morbidity was increasing in a steady manner to reach a peak in the oldest age group (more than 50 years). In the second year (1993), after treatment with praziquantil (40mg/kg b.w.), the prevalence and mean intensity of infection were 4.6% and 8.2 with 3.8% incidence of infection. The incidence pattern followed closely the pattern of prevalence of infection in the first year among age and sex groups reaching the peak in the age group 11-20 years. Using the McNemar test, there were statistically significant (P-values ranged from 0.01 to 0.0001) overall drops in the levels of infection and morbidity in the second year than in the first year except for gall bladder ($P=0.17$) and urinary bladder ($P=0.11$) lesions.

536 GEOGRAPHIC INFORMATION SYSTEMS AND RISK OF SCHISTOSOMIASIS IN EGYPT. Malone JB*, Huh OK, Soliman MS, El Bahy MM, and Shafik M. School of Veterinary Medicine, Louisiana State University; Coastal Studies Institute, Louisiana State University; and Cairo University, Cairo, Egypt; Ministry of Health, Cairo, Egypt.

A 1990-1991 time series of eleven day-night pairs of AVHRR imagery of Egypt were acquired from US NOAA-NESDIS, Washington, DC global archives. Channel 4 thermal infrared day-night image pairs were calibrated, georeferenced, co-registered and used to create diurnal temperature difference (dT) images of Egypt using a TeraScan system. Vegetation index images from Ch 1 visible and Ch 2 near infrared spectral bands were also created. Analysis was done on Nile delta subsets of dT images to elucidate the relationship of earth surface thermal patterns to published 1935, 1983 and 1990 Schistosoma mansoni and S. haematobium surveys. Median dT values of a 5x5 pixel area (28km2) centered on 41 survey sites named in 1935, 1983 and 1990 surveys were inversely related to S. mansoni prevalence in Summer, Fall and Winter composite dT images. Vegetation index images for each date revealed no consistent relationship to thermal patterns. Results suggest that stable geologic features are reflected in dT patterns and that drainage, water table or other hydrologic factors determine the relative suitability of irrigation canals and drains for snail hosts and disease transmission. Preliminary analysis of Landsat images (30m2 pixel resolution) for the Northern Nile delta suggest similar patterns can be seen in AVHRR dT images (1.1km2 pixels). Thermal domains were used to select 19 field sites for current field studies on environmental determinants of schistosomiasis risk.

537 SEA-SPECIFIC ISOTYPE LEVELS DIFFER IN THE DISTINCT PATHOLOGIC SYNDROMES OF CHRONIC EXPERIMENTAL *SCHISTOSOMA MANSONI* INFECTIONS. Montesano MA*, Secor WE, and Colley DG.

Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Universidade Federal de Juiz de Fora, Minas Gerais, Brazil.

Inbred male CBA/J mice with chronic *Schistosoma mansoni* infections develop two distinct syndromes. Hypersplenomegaly syndrome (HSS) demonstrates pathologic similarities to the hepatosplenic form and moderate splenomegaly syndrome (MSS) resembles the asymptomatic "intestinal" form of chronic human schistosomiasis. Immunoaffinity-purified antibodies against *S. mansoni* soluble egg antigen (SEA) differ idiotypically according to MSS or HSS with the idiotypes (Ids) expressed in chronically infected mice being similar to those observed in patients with intestinal and hepatosplenic (respectively) disease. These idiotypic differences may have an important immunoregulatory role and help to determine clinical outcome of disease. The current study was done to investigate the immunoglobulin isotype expression of Id preparations from MSS and HSS mouse sera. In an anti-SEA ELISA, MSS and HSS Id preparations contained comparable levels of IgM and IgG1. However, the MSS Id preparation had higher levels of SEA-specific IgG2a and IgG2b than HSS Id. The possibility that distinct immunoregulatory environments may contribute to the development of MSS and HSS correlates with earlier hypotheses that hepatosplenism results, at least in part, from a lack of appropriate idiotype expression. These observations suggest that class switching might also be a marker for, or play a role in, disease regulation.

538 INDUCTION OF SPECIFIC *SCHISTOSOMA MANSONI* EGG ANTIGEN HYPORESPONSIVENESS IN UNSENSITIZED AND SENSITIZED MURINE MODELS. Hassanein HI*, Botros SS, Akl M, El-Ghorab N, and Doughty BL. Theodar Bilharz Research Institute, NAMRU-3, Cairo, Egypt; and Texas A&M University, College Station, TX.

The regulatory potential of i.v. injection of small doses of SEA on artificially induced pulmonary egg granulomas and on hepatic deposited egg granulomas in unsensitized and sensitized models was assessed. In unsensitized model, naive C57BL/6 mice were i.v. injected with SEA either as single dose (10 μg) or multiple doses (10 μg x 4) 7 days before i.v injection of viable eggs or cercarial infection. Sixteen days post egg injection or 8 and 16 weeks post cercarial infection, the mice were sacrificed. In sensitized model, C57BL/6 mice were i.v. injected with multiple doses (10 μg x 4) of SEA 7 days before cercarial infection. Eight weeks post cercarial infection the mice were i.v. injected with viable eggs. Ten weeks post infection (i.e. 2 weeks post egg injection) the mice were sacrificed. From all groups, lungs and livers were collected and processed for histologic examination and measurement of egg granuloma diameter. Splenic SEA-lymphoproliferative response and level of serum schistosomal antibodies were estimated. The data showed that in unsensitized model, single i.v. injection of the antigen reduced pulmonary granuloma diameter whereas the reduction of hepatic granulomas was obtained with multiple i.v. injection of the antigen. It was observed that the reduction in granuloma diameter was greater and amelioration of immunopathological changes was marked in unsensitized than in sensitized models. The results indicate that induction of SEA hyporesponsiveness is easier in unsensitized than sensitized models. Probably due to incrimination of different antigen presenting cells (APCs). Meanwhile, pulmonary induced egg granuloma is still recommended as an *in vivo* model for the preliminary screening of immunoregulatory potential of antigen.

539 ELEVATED INNATE PERIPHERAL BLOOD EOSINOPHILIA FAILS TO AUGMENT IRRADIATED CERCARIAL VACCINE-INDUCED RESISTANCE TO *SCHISTOSOMA MANSONI* IN IL-5 TRANSGENIC MICE. Freeman GL*, Tominaga A, Takatsu K, Secor WE, and Colley DG. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Kochi Medical School, Okoh-cho, Nankokushi, Kochi, Japan; and Department of Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Numerous factors contribute to host resistance to infection with *Schistosoma mansoni.* Although several studies have investigated the eosinophil as an effector cell of protective responses, its true role remains unclear. In vitro, human, but not mouse, eosinophils can kill schistosomula. Studies on schistosome infection susceptibility in naive or vaccinated eosinophil-deficient mice have yielded conflicting results. Transgenic IL-5 mice have been developed which exhibit very high peripheral blood eosinophil (PBE) levels. In our laboratory, mean PBE levels were 21,000 mm^3, while in naive C3H/HeN mice this value was 240mm^3. Using the gamma-irradiated cercariae (irr-cerc) model, we vaccinated IL-5 transgenic mice in parallel with background-matched controls (C3H/HeN) to examine whether innate eosinophilia contributes to increased protection from a challenge infection. In three separate experiments, both groups of vaccinated mice showed significant resistance to challenge infection. However, there was no significant difference in the percent worm reduction between transgenic IL-5 C3H mice (mean %protection = 44.3; range = 42%-45%) and the control C3H/HeN mice (mean %protection = 51.7; range = 41%-64%). Our findings indicate that high levels of innate PBE due to constitutive production of IL-5 does notaugment irr-cerc-stimulated immunity.

540 ANTISENSE OLIGONUCLEOTIDES AGAINST SCHISTOSOMIASIS: A PRELIMINARY STUDY. Tao LF*, Marx KA, Wongwit W, Jiang ZW, Agrawal S, and Coleman RM. Center for Tropical Diseases, University of Massachusetts-Lowell, Lowell, MA; Department of Chemistry, University of Massachusetts-Lowell, Lowell, MA; and Hybridon, Inc., Worcester, MA.

Antisense oligonucleotides have been shown to inhibit the replication of certain viruses and protozoan parasites, such as *Leishmania amazonensis* and *Plasmodium falciparum*, under *in vitro* or *in vivo* conditions. These particular oligonucleotides are thought to be able to enter cells of an organ is by receptor-mediated endocytosis, then interfere, in a sequence-specific manner, with RNA processing such as the translation or trans-splicing of mRNA into proteins. Recently we reported that a nonsequence-specific oligonucleotide phosphorothioate to which schistosome worms' had been exposed,was detected in both schistosomal cytosol/nuclei fractions and in the surface tegument. Since then several antisense oligonucleotides complementary to selected target sequences have been synthesized, and studied with *Schistosoma mansoni* worms. Their schistosomicidal activity and inhibition of schitosomal physiological functions have been recorded for their potential usage as anti-disease or therapeutic agents. So far those antisense oligonucleotides, complementary to nucleotide sequences chosen from *Schistosoma mansoni* glucose transporter protein (SGTP1), have been shown to kill schistosomes *in vitro*, and inhibit glucose uptake by adult worms *in vitro* and *ex vivo*. The most effective antisense oligonucleotide which has a length of 21 nucleotides, is complementary to the SGTP1 nucleotide sequence including the initiation region of mRNA translation. It was found to decease glucose uptake *in vitro* by as much as 50%, and kill all male worms within 24 hrs at a concentration of 4.0 mg/ml. A significant decrease up to 34% in glucose uptake was also noted when 100 mg/kg x 2 (with 2 hr interval) of the oligo was administered *ex vivo*. This is the first report demonstrating the inhibition of physiologic functions in metazoan parasites by antisense oligonucleotides.

541 *SCHISTOSOMA MANSONI* ALDOLASE: AN IMMUNODOMINANT T AND B CELL CANDIDATE VACCINE ANTIGEN IN HUMANS WITH SCHISTOSOMIASIS. Gamal El-Din N, Al-Sherbiny M*, and El Ridi R. Biomedical Research Center for Infetious Diseases, VACSERA, Cairo, Egypt.

A substantial majority of humans infected with Schistosomiasis were foundto respond by lymphoproliferation and release of serum antibodies to the SAWA band of 144 kDa. MoAbs to deglycosylated 144 kDa SAWA band were generated and used to prepare the target antigen in a native form by immunoaffinity chromatography. The anti-144 kDa MoAbs and rabbit antisera to the immunoaffinity-purified molecules showed strong and reproducible ADCC to 3 hr *in vitro* schistosomula, and mediated significant ($P< 0.05$) protection by passive transfer against challenge with *Schistosoma mansoni* cercariaein outbred Swiss mice. The immunoaffinity-purified molecules administeredto genetically disparate Swiss mice induced significant ($P<0.001$)protection against challenge cercariae, and elicited release of IL-4, a cytokine associated with anti-schistosomiasis resistance in humans, specifically from normal endemics and subjects resistant to *S. mansoni* infection after parasitological cure with praziquantel. Amino acid sequences of the amino end terminal of the immunoaffinity purified molecules electrotransferred onto PVDF membrane showed 85% homology to the mammalian aldolase and 40% to *S. mansoni* aldolase. Therefore, *S. mansoni* adlolase may be considered a candidate vaccine antigen based on its protective potential *in vitro* and *in vivo*, and its recognition by the T and B cells of a significant percentage of humans with schistosomiasis.

542 MULTIPLE EPITOPE CONFORMATIONS OF A SCHISTOSOME ANTIGEN. Bungiro RD*, Petzke MM, Goldberg M, Suri PK, McCray Jr. JW, and Knopf PM. Department of Molecular Microbiology & Immunology, Brown University, Providence, RI; and Department of Biology, Morehouse College, Altlanta, GA.

Gene GP22 encodes sm25, a *S. mansoni* tegumental glycoprotein identified as a candidate vaccine antigen. The gene also encodes an 18-22 kDa family of antigens present in biosynthetically labelled soluble worm extracts. The existence of multiple GP22 transcripts in 4- and 7-wk worms was originally detected by primer extension and has been confirmed by nuclease protection. Translation of these mRNAs would yield proteins of 140, 175, and 182aa, sharing a common carboxy terminal region. In vitro translation of 4- and 7-wk poly A+ RNA in a reticulocyte lysate system generates multiple products in the size range predicted for the GP22 gene products, including a subset of antigens precipitated by antibodies unique to protective rat antiserum. When antisera raised against recombinant proteins are used to precipitate translation products, three different bands are detected in the 18-22 kDa range, two by the rabbit anti-r4x47 and one by rabbit anti-FP40. r4x47 and FP40 each contain codons 117-163 of GP22 but in different context and copy number, apparently influencing immunogenicity. The epitope conformations of the *in vitro* synthesized proteins differ as a function of length, supporting our hypothesis that differential expression of GP22 could be utilized as a parasite strategy to manipulate the host immune system for concomitant immunity. Previous studies using anti-recombinant protein 140 (r140; codons 43-182) and anti-peptide antibodies favor this hypothesis.

543 A PUTATIVE CALCIUM-TRANSPORTING ATPASE GENE FROM *SCHISTOSOMA MANSONI.* De Mendonca RL*, Beck E, Rumjanek FD, and Goffeau A. Universite Catholique de Louvain, Unite de Biochimie Physiologique, Louvain-la-Neuve, Belgium; Der Justus Liebig Universitat, Biochemisches Inst., Giessen, Germany; and Universidade Federal do Rio de Janeiro, Department de Bioquimica Medica, ICB/CCS, Rio de Janeiro, Brazil.

Little information is available on cation-transporting ATPase systems of *Schistosoma mansoni* and their implication to the parasite's biology. This has prompted us to isolate a complementary DNA encoding a putative Ca^{2+}-transport ATPase (*SMA1*). The cDNA was isolated by a nested polymerase chain reaction based strategy. The oligonucleotides used were designed on the basis of conserved amino acid regions found in P-type ATPases. The complete nucleotide sequence was determined and the primary structure and topology of the enzyme were deduced. SMA1 has 1022 amino acids and a predicted molecular mass of 113 kDa. This protein is 67% identical and phylogenetically related to several sarco/endoplasmicreticulum Ca^{2+}ATPases. The membrane topology predicted for SMA1 is characteristic of the P-type ATPases. Sequences and residues involved in the catalytic function of the SER Ca^{2+}ATPases. are conserved in SMA1.These features suggest that the cloned gene is a Ca^{2}-transport ATPase from the SERCA family. In addition, three PCR products have been cloned which share homology with another SER Ca^{2}-ATPase, with the yeast secretory pathway Ca^{2}-ATPase PMR1 and its mammalian homologue, and with the α subunit of a Na^{+},K^{+}-ATPase. These results should contribute to the understanding of cation transport through parasite's membranes.

544 CHARACTERIZATION OF NON-CS PROTEIN REPEAT *PLASMODIUM VIVAX*-210 SPOROZOITE MONOCLONAL ANTIBODIES. Gonzalez-Ceron L*, Wirtz RA, Sina BJ, Hall T, Sattabongkot J, Tsutsumi V, and Rodriguez MH. Center for Malaria Research, Tapachula, Mexico; Walter Reed Army Institute of Research, Washington DC; University of Maryland, College Park, MD; US Army Medical Component, Bangkok, Thailand.; and Center for Research and Advance Studies, Mexico D.F.

The appearance of *Plasmodium vivax*-247 CS polymorph sporozoites has made the diagnosis of infected mosquitoes more complex. Therefore, monoclonal antibodies (Mabs) produced against *P. vivax*-210 (Sal I strain) sporozoites, that did not react with the repeat portion of the *P. vivax* -210 CS protein by ELISA were tested with *P. vivax* sporozoites by western blot and IFA to look for conserved epitopes. Sporozoites were obtained from *An. albimanus* mosquitoes fed with *P. vivax*-infected blood from patients in Chiapas, Mexico. *P. vivax*-247 CS-polymorph sporozoites were obtained from Thailand. Mabs 2G12.1 (IgM) and 2G1.1(IgG3) reacted with the CS protein of the 210 and 247 sporozoites, suggesting that they recognize conserved epitopes in the non-repeat regions. Mab 2G12.1 reacts only with the presumed CS-precursor band by western blot. The IFA patterns of these two mabs are similar to that of mabs known to bind to the CS protein repeat. Three other mabs showed an IFA staining pattern not previously observed with anti-CS mabs. Mab2E9.1 (IgM) produces non-homogeneous staining which varied among sporozoites and reacts with a 64Kd molecular weight band on western blots. Mab 2H11.1 (IgG1) reacts with a 68 Kd band and stains within sporozoites lightly and homogenously by IFA. Mab 2A10.1 (IgM) reacts with 98, 85, 62, 18, 10 and 8 Kd bands on western blots and displays a punctuated IFA pattern. IFA also showed that all these mabs react with *P. vivax*-247 sporozoites. None of these mabs reacted with *P. falciparum* sporozoites by IFA. The results show that the monoclonals tested recognize all isolates of *P. vivax* sporozoites analyzed to date and therefore offer potential tools for diagnosis.

545 MYOCARDIAL SEQUESTRATION IN RHESUS MONKEYS INFECTED WITH *PLASMODIUM FRAGILE.* Didier PJ*, Campeau RJ, Cogswell FB, Blanchard JL, Bohm RP, Ratterree MS, Peyman CA, Spurlock JP, Dupepe L, Krogstad FM, Collins WE, and Krogstad DJ. Tulane University, New Orleans, LA; Centers for Disease Control, Atlanta, GA; and Louisiana State University, New Orleans, LA.

The purpose of these studies was to use the rhesus monkey *(Macaca mulatta)/P. fragile* model of human cerebral malaria to study the effects of sequestration in the central nervous system on trans-capillary transport. We measured transport by the entry of a neutral, lipid-soluble molecule (^{99m}Tc-labelled HMPAO) that is thought to cross into the brain only at the capillary level. Two monkeys received intravenous injections of ~10^{6} parasitized red blood cells, and were followed with daily quantitative parasite counts. Both animals became parasitemic on day 6, and parasite counts rose slowly to 8-10% (~$3x10^{5}$/μl0. Single photon emission computed topography (SPECT) quantitative imaging of the brain using ^{99m}Tc-HMPAO clearly demonstrated cerebral hemispheres, cerebellum and midbrain, but revealed no changes consistent with blockage of trans-capillary transport. At necrospy, neither animal had histologic evidence of cerebral malaria, although both had substantial sequestration in the myocardium characterized by rows of parasitized erythrocytes in the microvasculature. These results demonstrate: 1) the

continuing need for a highly predictable model of human cerebral malaria, 2) the potential role of SPECT studies in cerebral malaria and 3) the potential importance of myocardial sequestration in human malaria.

546 *PLASMODIUM OVALE* IN THE SPLENECTOMIZED CHIMPANZEE. Morris CL*, Sullivan JS, McClure HM, Strobert EA, Richardson BB, Galland GG, Goldman IF, and Collins WE. Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Yerkes Regional Primate research Center, Emory University, Atlanta, GA.

Thirty-one infections with *Plasmodium ovale* were induced in splenectomized chimpanzees to produce parasites for biologic, immunologic, and entomologic studies, and for diagnostic development. Maximum parasite counts during 28 primary infections ranged from 1,240 to 127,224/µl (median 23,980/µl). Three animals with secondary infections with *P. ovale* had maximum parasite counts of 2,340, 4,560, and 11,500/µl, respectively. One infection was induced by the bites of *Anopheles gambiae* mosquitoes. Ninety-two of 228 lots containing 168,175 *An. stephensi, An. gambiae, An. freeborni, An. dirus, An. maculatus,* and *An. quadrimaculatus* mosquitoes were infected by membrane feeding on gametocytes from the chimpanzees. Attempts to transmit *P. ovale* infections from chimpanzees to *Aotus* or *Saimiri* monkeys via parasitized blood (11 times) or sporozoites (8 times) were unsuccessful, even though developing exoerythrocytic stages were demonstrated in liver tissue of *Saimiri boliviensis* monkeys.

547 MOLECULAR AND MORPHOLOGICAL RESOLUTON OF CRYPTIC SPECIES OF MALARIA VECTORS FROM SOUTH AMERICA. Lounibos LP*, Conn J, Hribar LJ, Wilkerson RC, and Fritz GN. Florida Medical Entomology Laboratory, University of Florida, Vero Beach FL; Department of Biology, University of Vermont, Burlington VT; Walter Reed Biosystematics Unit, Smithsonian Institution, Washington DC; and Department of Zoology, Eastern Illinois University, Charleston IL.

Human malaria is a health problem in many parts of South America where the vector species *Anopheles darlingi* is absent or uncommon. Identification of alternative vectors is confounded by anopheline species complexes and by the high species diversity of neotropical fauna. Traditional methods have failed to resolve many species boundaries in the vector subgenus *Nyssorhynchus* (=*Nys.*) because of unreliable morphological characters, polytene chromosomes that are less informative than those of other anophelines, and refractoriness of adults to hybridization tests. We present an example of the resolving power of combined morphological and molecular analyses to distinguish malaria vector species from non-vector relatives. Based on comparisons of only morphological characters from larvae and adults, *An. (Nys.) trinkae,* a known vector of human malaria on the eastern slopes of the Andes, was recently synonymized with *An. (Nys.) dunhami* from Amazonian Brazil, where this species is not a suspected vector. We have collected topotypic *An. dunhami* and *An. trinkae* from near its type locality in Ecuador, as well as from Bolivia, and determined that these two taxa are specifically distinct based on isoenzyme and RAPD patterns, nuclear gene (ITS2) sequences, polytene chromosomes, and morphological characters, especially in the egg stage. Isomorphic male genitalia led to previous confusion of the two species.

548 MALARIA IN INFANTS AND ITS RELATIONSHIP WITH MATERNAL MALARIA HISTORY. Luxemburger C*, Nosten F, Neminn K, McGready R, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Malaria during pregnancy reduces birthweight and induces maternal anaemia. Low birthweight and maternal anaemia are both associated with an increased risk of infant death. But the relationship between malaria during pregnancy and the malaria history in the infant is unknown. Our study site on the Thai-Burmese border is an area of low and unstable transmission. There is a unique source of antimalarials and we have established a surveillance system in which all confirmed malaria cases are recorded. The malaria history in pregnant women is therefore closely recorded, by passive case detection in the early pregnancy and by weekly active screening when the women are attending the ante-natal consultation (90% of them; starting around 4 months of pregnancy). We are then prospectively following their infants from birth to 1 year of age. They are seen monthly to assess physical and neurological development. Haematocrit is performed at each visit. Malaria cases are detected though a combination of passive detection and weekly visits at home. Blood smears are performed in all children with a history of fever. Malaria history, haematocrit, physical development and survival will be compared in children whose mother had a malaria episode during pregnancy and children born to mothers who did not suffer from malaria. To date, 419 children have been admitted in the cohort. The neonatal and infant mortality rates were 52.6 and 56.7 per 1000 live births respectively. The cumulative malaria incidence rates at the age of one year were 98/1000 for *P. falciparum* and 160/1000 for *P. vivax*. The median [range] age at the first *P. vivax* episode was 5.7 [1.0- 11.9] months and 7.6 [2.3- 11.4] months for *P. falciparum*.

549 CLINICAL PREDICTORS OF MALARIA DIAGNOSIS IN AN AREA OF LOW AND UNSTABLE TRANSMISSION OF RESISTANT *PLASMODIUM FALCIPARUM*. Luxemburger C*, Nosten F, Heh Wah T, Slight S, and White NJ. Shoklo Malaria Research Unit, Mae Sot, Thailand; and Wellcome Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The diagnosis of malaria in the absence of microscopy is notoriously difficult. The clinical predictors of malaria have been studied in an area of low and unstable transmission on the Thai-Burmese border. Previous epidemiological studies conducted in the area have shown that individuals across all age groups presented one malaria infection per person-year, most of them (87%) symptomatic. Over one third (34%) of the cases were due to *Plasmodium falciparum*, 56% to *P. vivax* and 10% were mixed infections. The symptomatic threshold parasitaemia was 200 parasites/mcl for *P. vivax* and 1,500 parasites/mcl for *P. falciparum*. Ten to 20% of the *P. falciparum* episodes could not be successfully treated. Diagnosis of malaria in this area is complicated by the coexistence of falciparum and vivax and the presence of the most drug resistant strains of *P. falciparum* in the world. Prior to a malaria vaccine trial, we prospectively followed 1,000 children aged between 2 and 15 years. They were referred to our clinic for each febrile episode. We have performed the same questionnaire and clinical examination at each consultation prior to a blood smear examination. Over 2,000 consultations were recorded over six months. Sensitivity and specificity of symptoms and clinical signs for the diagnosis of malaria and for the differential diagnosis by species are being analysed and will be presented.

550 POOLING STRATEGY FOR ESTIMATION OF SPOROZOITE RATES. Gu WD*. Department of Parasitology, Second Military Medical University, Shanghai, People's Republic of China.

Sporozoite rate of mosquitoes is a direct measurement of transmission intensity of malaria. The traditional approach of dissecting salivary glands for sporozoites is extremely cumbersome and labor intensive. Pooling sampled mosquitoes for sporozoite detection by immunoassays is an efficient and economic approach in situations of low vector infectivity. However, until now this approach hasn't been applied for estimation of mosquito infectivity, because if pool sizes are selected arbitrarily we have no knowledge of how many infected individuals in a positive pool. Based on statistic analysis of sampling procedures, in the present study a strategy was proposed to specify maximal pool sizes (MPS) for given sporozoite rates, so that the probability of more than one infected mosquitoes in any positive pool is less than a predetermined significance level, for example, if speculated sporozoite rates are 1% and 20%, MPS are 35 and 2, respectively. Thus the number of positive pool is an approximation of the number of infected mosquitoes in samples. The relationship between sporozoite rates and the proportion of positive pools is also revealed, and threshold proportions of positive pools for various pool sizes are calculated to judge the above estimation.

551 PATTERNS OF MALARIA EPIDEMICS IN THE HIGHLAND AREAS OF KENYA. Ouma JH*, Rapuoda B, and Beier JC. Division of Vector-Borne Diseases, Ministry of Health, Kenya; and Department of Tropical Medicine, Tulane University, New Orleans, LA.

Malaria is the leading cause of morbidity and mortality in Kenya and accounts for 30% of all illnesses. Apart from the Coast, Nyanza, andWestern Provinces where malaria is generally stable, the rest of the country experiences unstable malaria and epidemics occur during periods of rain. In the highland areas of Kenya, malaria was virtually unknown until the second decade of this century when periodic outbreaks occurred in Nandi District and surrounding areas. Thereafter, epidemics were experienced periodically at intervals of 3 to 4 years. Since 1988, malaria epidemics leading to considerable illness and deaths have occurred annually in Kisii, Kericho, Nandi, and Uasin Gishu Districts. This report discusses epidemiologic patterns of malaria epidemics over the past 4 years based on hospital records of morbidity and mortality. In general, high morbidity, and mortality coincided with periods toward the end of the long rainy season, usually in July.

552 OVERVIEW OF MALARIA IN NIGER. Mounkaila AB*. Ministäre de la Santé Publique, Niger.

Niger is a sub-Saharan, landlocked country, located between the 19-23th latitude and the 0-16th meridian. The total population was estimated to be 8,992,687 inhabitants in 1994, with a growth rate of 3.3%. Malaria risk exists throughout the year in the whole country. But, according to the ecological variations and the levels of endemicity and stability, there are three epidemiological strata: 1) hyperendemic area, with a mean rainfall of 600 mm, around the Niger river, the malaria infection prevalence is 80-100 % during the rainy season; 2) mesoendemic area, with a yearly rainfall of 200-500 mm, the prevalence is 60-90%; 3) hypoendemic area, interface with sahel and desert in the North, 20-30% of infection prevalence. The cumulative incidence aggregated by the national health information system for the whole country was 84.34 (66.92 - 215.94) per 1,000 inhabitants in 1993. The common species of

mosquitoes found are*Anopheles gambiae, An. funestus* and *An. nili. P. falciparum* is the predominant parasite (90-95%), then *P. malariae* (2-8%) and *P. ovale* (2%). The highest incidence of severe and complicated malaria, generally due to *P. falciparum*, occurs in October at the end of the rainy season as it does in the Gambia and Mali. However, the mechanism(s) responsible for the late seasonality of severe malaria is unknown. Resistance to chloroquine has been reported since 1989.

553 SEASONAL VARIATION OF ANTI-*PLASMODIUM FALCIPARUM* ANTIBODIES DIRECTED AGAINST GAMETOCYTE ANTIGEN PFS2400 IN THREE LOCALITIES IN AMAPA, BRAZIL. Marrelli MT, Malafronte RS, Nussenzweig RS, Kloetzel JK. Instituto de Medicina Tropical de Sao Paulo, Sao Paulo, Brazil; Department of Molecular and Biochemical Parasitology, New York University School of Medicine, New York, NY: and Department of Parasitology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

Antibodies (Ab) to the Pfs2400 gametocyte antigen(Ag) were shown to inhibit the development of *P. falciparum* (Pf) in Anophelines and therefore this Ag is a candidate for a transmission-blocking vaccine. To test seasonal variation of these Ab under field conditions, sera from 72 individuals were collected twice, first during the long-rainy season with low malaria transmission and then during the short-rainy season, when transmission is high. This study was conducted in various localities within the Jari Project, in Amapa, Brazil. All but 3 individuals had a positive IFA with asexual forms of Pf. Most of them did not report malaria attacks during the period between the 1st and 2nd sampling. Their sera were tested by IFA with Pf The overall positivity of this test did not vary between seasons, and was 45.8% (33/72) and 47.2% (34/72), respectively. Gametocyte reacting sera were tested by ELISA with the Pfs2400 repeat peptide. The positivity rate dropped from 63.6% (21/33) to 32.4% (11/34) ($p=0.02$) and the mean 0D from .652 to .354, when we compared the results of the long-rainy and short-rainy season. Soon after the 2nd sampling a malaria outbreak was reported in one of the localities. These results point toward a relatively short persistence of anti-Pfs2400 Ab, under natural field conditions. Administration of a gametocyte Ag to booster the Ab response before a high transmission period might contribute towards lowering malaria incidence.

554 MALARIA IN HAITI: EPIDEMIOLOGIC AND CLINICAL ASSESSMENTS. Nicolas E*, Jean-Francois V, Rogers HM, Bloland PB, Ruebush TK, and Nguyen-Dinh P. Ministry of Public Health and Population, Haiti; Pan American Health Organization, Haiti; Hopital Le Bon Samaritain, Limbe, Haiti; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

To assess the impact on malaria of the recent political and economic strife in Haiti, epidemiologic surveillance data were reviewed and parasitologic/clinical surveys were conducted in several geographically representative sites of the country. Findings to date indicate that: a) no major changes in the malaria situation have occurred compared to 5-10 years ago; b) several areas of the country (e.g. Léogane and Deschapelles) present seasonal transmission patterns, with peak incidence in October- December (slide positivity rate (SPR) up to 20%) alternating with low incidence periods (SPR 0-1%); c) other areas (e.g Limbé) present a high incidence (SPR 8-13%), with less tendency to seasonal fluctuations; d) *Plasmodium falciparum* remains the predominant species, and retains its susceptibility to chloroquine (25 mg/kg), as assessed in a simplified 7-day *in vivo* test. These findings suggest that a substantial impact might be achieved through intensified malaria control activities in Haiti, including improved case management with chloroquine and the testing of other measures, such as insecticide-impregnated bednets and curtains.

555 THE VILLAGE OF DIENGA AS A FIELD BASE FOR VACCINE TESTING IN EASTERN GABON-FREQUENCY OF MALARIA ASSOCIATED SYMPTOMS IN SCHOOLCHILDREN AND PARASITOLOGICAL SURVEY. Millet P*, Luty A, Reltien J, Renaut A, Tshipamba P, Lekoulou F, Mayombo J, DeLoron P, and Georges AJ. Centre International de Recherche Médicale de Franceville (CIRMF), Franceville, Gabon.

Many malaria vaccine research programs are reaching the stage of testing their vaccine candidate In the field for efficacy in various parts of the world, preferentially in children. In Gabon, located in the west equitorial part of Africa, malaria is holoendemic and transmission occurs intensely and perennially with little seasonal variations. Such permanent and stable contact between the population and the parasite allows the detection of protective efficacy by any control measures. The village, of Dienga (about 1200 individuals), in South Eastern Gabon, was selected as a CIRMF malaria vaccine testing base because 1) it had been left untouched by other research projects and 2) it is located more than an hour away from the last operating health center. No control measures had yet occurred in this area. Resistance to chloroquine is a major concern in Gabon (90% of *Plasmodium falciparum* strains were found resistant in Lambarené, Western Gabon). Several parasitological surveys performed from 1990 to present identified *P. falciparum* as the primary malarial agent present in South Eastern Gabon. Our cohort consisted of 230 schoolchildren 6 to 17 years of age. Parasitological prevalence surveys conducted in 1990 and 1992 in the same age

groups showed parasite rates consistently in the, range of 55 to 83% with higher rates during the rainy seasons (February to June and October to December). Between February and June 1995, axillary temperature was measured and recorded daily between 8 and 9 am. Subjects with fever (> 37.5 °C) were bled for thick and thin blood smaers, and for strain identification if parasitemia occurred. They received a curative trteatment with quinine and clindamycine for 3 days, chosen for its efficacy in Gabon. Parasitemia was monitored at day 4 and day 7. Every 2 weeks, approximately one sixth of the cohort was bled for HLA determination, and the remidnder had a finger prick for thin and thick blood smears. Entomological inoculation rate was derived from data collected by human bait night catches during the survey period. Within the first 2 months of the study, 234 fever cases were identified. Among them 119 (50.8%) were parasitemic. 105 received a full treatment and 93 were controlled at day 4. Treatment failure rate was 3.2%. No clinical can of severe malaria was seen. Among those 82% presented with one access, 16% with two and 2% with three. Data from the 4 month survey will be presented as the first part of a 1 year cohort study.

556 COMPARISON OF A PUTATIVE MULTI-DRUG RESISTANCE GENE IN SENSITIVE AND RESISTANT STRAINS OF *PLASMODIUM BERGHEI*. Gervais GW, Trujillo-Nevarez K, Serrano AE*. Department of Microbiology and Medical Zoology, University of Puerto Rico School of Medicine, San Juan, PR.

The human malaria *Plasmodium falciparum* expresses two genes (*pfmdr-1* and *pfmdr-2*) that share sequence homology with the multi-drug resistance (*mdr*) genes which are overexpressed in some human cancer cells. Amplifications or mutations in these genes have been associated with drug resistance in some malaria lines. The exact role of these genes in potentiating drug resistance in malaria parasites is still controversial, and we are currently developing an *in vivo* murine model of the MDR phenotype in order to better address the question. Several fragments were amplified from genomic DNA of the rodent malarias *P. berghei* and *P. yoelii* by the Polymerase Chain Reaction, using primers that recognize the ATP binding domain. Sequence analyses confirmed that a 580 bp PCR-product amplified from *P. berghei* genomic DNA shares 66% identity at the amino acid level with its *P. falciparum* homolog. Four different strains of *P. berghei* and four strains of *P. yoelii*, which display various levels of sensitivity to chloroquine, halofantrine and/or mefloquine, were karyotyped by Pulse Field Gel Electrophoresis. The *mdr*-like homolog of *P. berghei* was mapped to chromosome12. We are currently analyzing the expression of this gene in our collection of strains by quantitative Southern blots and chromosome mapping.

557 HEMOZOIN CATALYZED OXIDATION OF ARACHIDONIC ACID. Green MD*, Xiao L, Udhayakumar V, Lal AA, and Todd GD. Division of Parasitic Diseases, Centers for Disease Control and Prevention. Atlanta, GA.

The iron-containing heme moiety of hemozoin is an important source of non-enzymatic oxidizing potential during malaria infection. We wanted to determine if hemozoin can catalyze the oxidation of arachidonic acid (AA) to the hydroxyeicosatetraenoic acids (HETEs). Therefore we conducted an HPLC analysis of a mixture of AA and hemozoin. Two major chromatographic peaks with retention times similar for 5-HETE and 15-HETE authentic standards were detected. The catalytic effects of other heme derivatives such as hemin and hemoglobin on AA oxidation also resulted in 5-HETE formation, but the production of 15-HETE was specific to hemozoin. The significance of 15-HETE formation is that this compound has been reported to be a potent vasoconstrictor. This property may contribute to some of the pathological consequences of cerebral malaria. The effects of artemisinin (an antimalarial endoperoxide which has been reported to bind covalently to heme) and deferoxamine (a compound that forms complexes with iron) on hemozoin catalyzed HETE formation will also be presented.

558 HEMOZOIN (β-HEMATIN) INHIBITS CYTOKINE RELEASE AND ADHESION MOLECULE EXPRESSION OF STIMULATED HUMAN ENDOTHELIAL CELLS. Taramelli D*, Basilico N, Saresella M, Pagani E, Ferrante P, Chione M, Olliaro P. Institute of Medical Microbiology, University of Milan, Milan, Italy; Laboratory of Biology, Don Gnocchi Foundation, Milan, Italy; Sieroterapico, Soc Coop., Milan, Italy; and UNDP/World Bank/WHO Special Program for Research and Training (TDR), Geneva, Switzerland.

Multiple endothelial receptors (ICAM, CD36, thrombospondin, etc) and counter-receptors on RBCs are involved in cytoadherence and sequestration of *Plasmodium falciparum*-infected red blood cells. Host cytokines and parasite products may play a crucial role by interfering with the expression of adhesion molecules and/or production of soluble mediators, respectively. Isolates may differ in their binding capacity to ICAM-1, and upregulation of ICAM-1 expression appears to be critical in the pathogenesis of severe malaria. The aim of the present study was to investigate whether human endothelial cells (EC) exposed to synthetic malaria pigment, β-hematin (BH), retained their ability to produce cytokines, namely interleukin-6 (IL6) and Nitric Oxide (NO) and to modulate ICAM-1 (CD-54) expression after stimulation with TNF or LPS. Pre-treatment with BH (10 - 100 g/ml) for 18 hours inhibited in a dose-dependent manner the production of NO and IL6 by human umbilical vein endothelial cells (EC) stimulated

by LPS (0.1 - 10 g/ml) or IFN-γ (50 - 100 U/ml) for 48 hours. In contrast, IL6 production remained unchanged when BH treatment occurred 24 hours after stimulation. Cell viability, as measured by trypan blue exclusion and reduction of metabolic dye (MTT), was not affected, whilst EC appeared engulfed with the black pigment. Moreover, it was observed by flow cytometric analysis the TNF-mediated upregulation of ICAM-1 expression in EC was inhibited by pre-treating ECs with BH in a dose-related manner. ICAM-1 expression, however, was not reduced by BH when treatment was administered to already activated ECs. It appears thus that BH-fed ECs (as would be the case in repeat exposure to malaria parasites) behave differently to normal ECs, in that they show a reduced capacity to produce pro-inflammatory mediators and to up-regulate the expression of adhesion molecules.

559 CLONING AND SEQUENCING OF A PROTO-ONCOPROTEIN INVOLVED IN SIGNAL TRANSDUCTION IN *PLASMODIUM FALCIPARUM*. Hatin I*, Hernandez-Rivas, Hernandez-Rivas R, and Jaureguiberry G. Institut National de la Santé et de la Recherche Médicale, Paris, France.

Approaches to drug design have become refocused in response to our rapidly emerging understanding of the role of signaling pathways in health and disease. The proto-oncogenes Ras, Raf and MAPK kinases are indispensable sequential elements in the transduction of growth and differenciation signals initiated by receptor and non receptor tyrosine kinase. The determination of transduction targets can be used as an approach to drug development. In this goal, we screened a cDNA library of *P. falciparum* asexual stages, by using a probe obtained by PCR amplification with degenerated oligonucleotides corresponding to consensus sequences from protein tyrosine kinases genes. Different clones were obtained and their sequencing is under process.

560 CYTOADHERENCE CHARACTERISTICS OF *PLASMODIUM FALCIPARUM* ISOLATES IN THAILAND: EVIDENCE FOR CHONDROITIN SULFATE A AS A CYTOADHERENCE RECEPTOR. Chaiyaroj SC*, Buranakiti A, Angkasekwinai P, Looareesuwan S, Rogerson SJ, and Brown GV. Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; and Immunoparasitology Unit, The Walter and Eliza Hall Institute, Victoria, Australia.

The association between cytoadherence and antigenic expression of *Plasmodium falciparum* infected erythrocytes and the pathogenesis of malaria has been evaulated. In this study, we investigate agglutinating phenotypes and adherence to C32 melanoma cells, CD36, intracellular adhesion molecule 1(ICAM-1), thrombospondin(TSP), E-selectin, vascular cell adhesion molecule 1(VCAM-1) and chondroitin sulfate A (CSA) of 50 *P. falciparum* isolates from Thai patients. The degree of adherence to purified adhesion molecules varied greatly between different parasite isolates. Most isolates except for 1 adhered to CD36. No adherence beyond that of controls was observed to chinese hamster ovary cells transfected with E-selectin and VCAM-1. Only a few *P. falciparum* isolates adhered to ICAM-1 and CSA, a newly identified receptor for adherence. We saw no consistant correlation between *in vitro* binding of any one ligand to the patients' conditions. In addition, a wide range of variant antigenic types was also observed by the comparison of agglutinating phenotypes to convalescent sera and a panel of adult immune sera. We also investigate further the charactreristics of adherence to CSA and demonstrate its role in natural infection of *P. falciparum*.

561 STUDIES ON THE MECHANISMS OF CYTOADHERENCE AND ANTIGENIC VARIATION IN *PLASMODIUM FALCIPARUM*. Reeder JC*, Rogerson SJ, Davern KM, and Brown GV. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

The cytoadherence of *Plasmodium falciparum* infected erythrocytes to venous endothelium is thought to be an important mechanism in the avoidance of splenic clearance and to be a prerequisite for cerebral malaria. In response to *P. falciparum* infections, humans develop antibody to parasite derived neoantigens on the erythrocyte surface and clonal variation of these antigens is believed to be an important factor in immune evasion for the parasite. In this laboratory we have previously demonstrated a direct association between cytoadherence phenotype and modulation of variant antigenic type, presumptively through alterations in *P. falciparum* erythrocyte membrane protein-1 (PfEMP1). More recently this work has led to the discovery of co expression of multiple cytoadherence ligands, including a novel trypsin resistant ligand, and the demonstration of Chondroitin Sulfate-A as a potentially important, novel receptor for *P. falciparum*. Here we will discuss the most recent findings in these investigations, along with a report on our progress in unravelling the molecular mechanisms which underlie these phenomena.

562 ANALYSIS OF VARIABILITY IN THE MEROZOITE SURFACE PROTEIN OF *PLASMODIUM BERGHEI*. Jennings GJ*, van Belkum A, van Doorn LJ, and Wiser MF. Department of Tropical Medicine, Tulane University Medical Center, New Orleans LA; and TNO Primate Center, Rijswijk, The Netherlands.

Due to surface exposure and a possible role in invasion, the merozoite surface protein-1 (MSP-1) is currently considered a promising candidate in malaria vaccine development. Despite the value of rodent models forthe testing and characterization of vaccines, relatively little work hasbeen carried out on the molecular biology of MSP-1 from rodent malaria species. The screening of a lambda gtll cDNA library with mAbs specificfor PbMSP-l resulted in the cloning of a 462 bp fragment containing an open-reading frame which aligns with conserved block 3 and variable block 4 of PfMSP-1. The 462 bp fragment was used as a probe in the subsequent screening of gDNA libraries for the *Plasmodium berghei* MSP-1 gene.Two overlapping clones (a 4.2 kb HindIII fragment and a 2.5 kbEcoRI/SpeI fragment) encompassing the entire gene were cloned and sequenced. Overall the PbMSP-1 sequence is similar to MSP-1 sequences from other *Plasmodium* species in terms of the distribution of conserved and variable blocks. In order to examine intraspecies diversity within PbMSP-1, PCR was used to amplify the variable block 4 sequences from several *P. berghei* strains which exhibit size heterogeneity at the protein level. The nucleotide sequences of all strains are identical indicating that block 4 does not contribute to the size heterogeneity between *P. berghei* strains. The sequence of MSP-1from other species will contribute to our understanding of the structureof MSP-1 whereas the cloned gene can be used in the development of recombinant vaccines.

563 CHARACTERIZATION OF TWO CLONES EXPRESSING FUSION PROTEINS RECOGNIZED BY MAB RAISED AGAINST A GLU-RICH PROTEIN ASSOCIATED WITH THE ERYTHROCYTE MEMBRANE. Giraldo LE*, Jennings G, Schmitt-Wrede HP, Wunderlich F, and Wiser MF. Department of Tropical Medicine, School of Public Health & Tropical Medicine, Tulane University, New Orleans, LA; and Division of Molecular Parasitology, Universitat Dusseldorf, Dusseldorf, Germany.

During the blood cycle of malaria, the parasite alters the red blood cell (RBC) membrane. Some of the alterations include the transportation of malarial proteins to the RBC membrane. Pc(em)93, a 93 kDa *Plasmodium chabaudi* protein, is synthesized and transported to the RBC membrane very early in the ring stage (MBP 27: 11). The deduced amino acid sequence reveals tandem repeats rich in glutamate residues. Several *Plasmodium falciparum* antigens like RESA, GLURP, Pfs230, Pf320 and MESA also contain glu-rich tandem repeats have been characterized. Some of these antigens are proposed to be vaccine candidates or involved inimmunity against malaria. Little is known about glu-rich proteins in rodent malaria, limiting the possibility of testing these glu-richproteins as vaccines and their correlation to immunity. In the present study we describe the antigenicity of Pc(em)93. Two distinct lambda gt11clones (P26-R6 and Pc90-1) which expressed fusion proteins recognized by the same mAb (13.5) raised against Pc(em)93 have been obtained. Both clones were subclone into pGEX and expressed as GST fusion proteins. Disrupted bacteria expressing the fusion proteins were then used to immunize mice. Pc90-1 fusion protein induced antibodies that reactedwith the native Pc(em)93, whereas P26-R6 did not. The antibody levels induced by Pc90-1 fusion protein were similar to those produced during *Plasmodium chabaudi* infection. The high antibody titer did not provide protection against challenge infection. These results suggest that glu-rich proteins may not confer protective immunity despite their high antigenicity and that their used as predictors of immunity must be interpreted with caution. Its interesting to note that immunization with the erythrocyte membrane fraction did have a protective effect (I&I56: 3326), suggesting that another less immunogenic protein is responsible for protection.

564 SICKLE CELL TRAIT AND PLACENTAL MALARIA INFECTION IN TWO AREAS OF KENYA WITH DEFFERENT TRANSMISSION PRESSURE. Nahlen BL*, Udhayakumar V, Parise ME, Ayisi JG, Bloland PB, Oloo AJ, and Lal AA. Division of Parasitic Diseases, NCID, CDC, Atlanta, GA; Vector Biology and Control Research Center, KEMRI, Kisumu, Kenya.

Several studies have shown that sickle cell trait (HbAS) provides protection against severe malaria, but whether it provides protection against placental malaria infection is unknown. We studied women at delivery during 1992-94 in a rural area of western Kenya with high malaria transmission (>100 infected bites per year) and during the 1994 rainy season in a maternity ward in coastal Kenya where malaria transmission is lower (<10 infected bites per year). Among women in the high transmission area (Group 1; N=492), the prevalence of HbAS was 21.3% and the incidence of placental parasitemia was 28.3%; among women in the low transmission area (Group 2: N=157), 9.4% had HbAS and 18.4% had placental malaria infection. Primigravidae were significantly more likely to have placentalparasitemia in both Group 1 (OR 2.61, 95% CI 1.42-4.79) and Group 2 (OR2.52, 95% CI 1.03-6.26). In both groups women with HbAS were as likely to have placental malaria infection as women without sickle cell trait(HbAA). When stratified by gravidity, there were no significant differences among women with HbAS compared to theose with HbAA in mean density of placental parasitemia, the prevalence or density of maternal peripheral parasitemia, mean maternal hemoglobin, or infant birthweight. These results suggest that an innate

resistance mechanism such as HbAS which provides protection against one form of malarial disease (cerebral malaria or severe anemia) may not provide protection against another form of the disease (placental malaria).

565 THE NOD/LTSZ-*SCID* MOUSE MODEL FOR *PLASMODIUM FALCIPARUM* INFECTION: POTENTIAL AND LIMITATIONS. Moore JM*, Schultz LD, and Rajan TV. Department of Pathology, University of Connecticut Health Center, CT; and Jackson Laboratory, Bar Harbor, ME.

We have previously reported on an immunodeficient mouse model for *Plasmodium falciparum* using NOD/LtSz-scid mice. We have now investigated (a) conditions to establish long-term human red blood cell (hRBC) chimerism in mice; (b) whether infection of fresh, uninfected hRBC occurs in the murine host; and (c) if such reinfection occurs in the peritoneal cavity or in circulation, or both. Using a protocol of appropriately spaced intraperitoneal injections of hRBCs, we are now able to maintain hRBC chimerism up to 60% in mice for up to two months. Using appropriately stained hRBCs, we can demonstrate by two color flow cytometry that reinfection of uninfected hRBCs does occur in the murine host, both in the peritoneal cavity and in circulation. While these data support the potential of this model as a small animal laboratory model for *P. falciparum*, there are also serious limitations. The most significant problem is that there is great variability between individual animals in terms of the longevity of parasitemia. In addition, the lifetime of an infected hRBC in circulation, in contrast to uninfected hRBCs, appears to be short and finite, suggesting that that the reinfection in the peritoneal cavity is solely responsible for the maintenance of infection. We are currently investigating the reasons for the individual variability and are attempting to develop methods for prolonging the longevity of individual parasitized red blood cells in circulation.

566 DEVELOPMENT OF AN *IN VITRO* MODEL FOR INVASION OF MOSQUITO SALIVARY GLANDS BY MALARIA SPOROZOITES. Suwanabun N*, Sattabongkot J, Linthicum KJ, and Rosenberg RM. Department of Entomology, U.S. Army Medical Component, AFRIMS, Bangkok, Thailand; and Department of Entomology, Walter Reed Army Institute of Research, Washington DC.

The development of an *in vitro* model for the invasion of mosquito salivary glands by malaria sporozoites is needed to better understand the mechanisms of sporozoite invasion and identify vulnerable points in the parasite development. Salivary glands dissected from female *Ae. aegypti* (Bangkok colony) were cultured in standing drops of modified Reddy's medium pH 7.4. *Plasmodium gallinaceum* sporozoites ($2x10^6$) harvested from either mature oocysts or infected salivary glands of *Ae. aegypti* were added to the gland cultures. After incubation at 25°C in sealed humidified chambers, the glands were washed in fresh medium and were examined with a phase contrast microscope for invasion by sporozoites. Observations on the adhesion of the sporozoites at the gland surfaces and invasion of salivary gland cells by the sporozoites after incubation for 12 hours were made. Sporozoites harvested from infected glands were found to adhere at the gland surfaces and about 10% of the glands were invaded by the sporozoites. Only adhesion but no invasion of the glands by sporozoites prepared from oocysts was observed. The differential invasion we observed may be caused by the greater number of immature sporozoites in the oocyst preparations. Efforts are underway to increase the number of sporozoites invading the cells, and improve the simplicity and consistency of the model.

567 PCR-BASED NON-ISOTOPIC LIQUID HYBRIDIZATION VERSUS MICROSCOPY IN MONITORING SPOROZOITE-INDUCED *PLASMODIUM VIVAX* INFECTIONS IN *SAIMIRI* MONKEYS. Grady KK*, Oliveira DA, Sullivan JS, Lal AA, and Collins WE. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

Experimental sporozoite-induced infections with *Plasmodium vivax* Salvador I (Sal I) strain in *Saimiri boliviensis* monkeys are often characterized by very low density parasitemia, especially during early stages of infection. In vaccine trials designed to measure efficacy of anti-sporozoite vaccines, development of parasitemia as well as the prepatent period must be determined. After challenge with 10,000 sporozoites of *P. vivax* Sal I, thirty-three *S. boliviensis* monkeys were sampled twice weekly. A PCR-based non-isotopic liquid hybridization test was utilized to determine the presence of parasites. These results were compared with those obtained by examination of Giemsa-stained thick blood films. We found that parasitemia was detected earlier by the hybridization method, and in some cases, animals that were never parasitemic by blood-film examination had short periods of subpatent parasitemia as determined by hybridization. The findings may have significance in the testing of sporozoite-based vaccines.

568 EXPRESSION OF THE *PLASMODIUM FALCIPARUM* NUTRIENT-PERMEABLE CHANNEL ON OOCYTES. Desai SA*. Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, NC.

The rapid growth of the human malaria parasite, *Plasmodium falciparum*, within the red blood cell requires access to large amounts of nutrients. A 140-pS channel recently identified on the parasitophorous vacuole membrane of the intraerythrocytic parasite may facilitate this nutrient acquisition. This channel is present at high density, is open more than 98% of the time at the membrane resting potential, is permeable to both cations and anions, and can readily transport amino acids andmonosaccharides. Here, the channel has been expressed on *Xenopus laevis* oocytes to allow further characterization. Harvested oocytes were defolliculated and stored in 94 mM NaCl, 1.5 mM KCl, 1.2 mM $CaCl_2$, 2.0mM $MgCl_2$, 10 mM HEPES, 50 mg/l gentamicin, pH 7.6 before microinjection of 36 nl of a 1 mg/ml suspension of *P. falciparum* trophozoite mRNA inDEPC-treated H_2O. Oocytes were then stored in the same medium at 19°C for 8 days. Outside-out patch clamp recordings on these oocytes (n=4) revealed the expression of a channel resembling the nutrient channel: expressed channels had a high slope conductance, small negative reversal potentials with two different pipette solutions, transitions consistent with subconductances, and were not seen on uninjected oocytes (n=3). Expression of the nutrient channel on oocytes may facilitate searches for specific inhibitors and eventual channel cloning.

569 HIGH RESOLUTION X-RAY MICROSCOPY, A NEW TOOL TO INVESTIGATE INTACT PARASITES. Meyer-Ilse W*, Magowan CC, and Moronne MM. Center for X-ray Optics, Lawrence Berkeley Laboratory, Berkeley, CA.

A new x-ray microscope (XM-1) has been installed at the Advanced Light Source in Berkeley. X-ray microscopy does not compete with techniques such as electron microscopy in terms of resolution, but rather offers unique advantages, including the ability to take images of samples in an aqueous environment, with a resolution exceeding the resolution of a visible light microscope by about a factor of five. X-ray microscopy yields information from thick samples that cannot be obtained by any other technique, including such methods as atomic force microscopy or near field optical microscopy, which are essentially surface techniques. XM-1 has been used to image *Plasmodium falciparum* in intact human red blood cells and red cell ghosts. The technical design of XM-1 includes visible light microscopes to view and select interesting objects before x-radiation. Typical exposure times range from a few seconds to about half a minute, depending on required signal to noise and sample thickness. Convenient sample change mechanisms allow us to investigate hundreds of cells per day in an aqueous environment, thus filling a gap between visible light microscopy and electron microscopy, which requires fixation and sectioning of the sample.

571 OPTIMIZATION OF ELECTROPORATION PARAMETERS FOR *IN VITRO* TRANSFECTION OF *PLASMODIUM FALCIPARUM*. Bell CA*, Mbachem WF, Miller RE, Kesler JC, Glassic MA, Wirth DF, and Nuzum EO. Walter Reed Army Institute of Research, Department of Parasitology, Division of Experimental Therapeutics, Washington, DC; and Harvard School of Public Health, Department of Tropical Public Health, Boston, MA.

Transfection of sexual stage *Plasmodium gallinaceum* and asexual *P. falciparum* parasites has been reported. Transient transfection of the sexual stage *P. gallinaceum* results in 10-40% of the ookinetes expressing high levels of the reporter genes. In contrast, transient transfection of the asexual stages of *P. falciparum* appears to be much less efficient. The goal of this work is to maximize the efficiency of transfection of the asexual blood stages of *P. falciparum*. The routine transfection of blood-stage *P. falciparum in vitro* remains technically challenging. This is due, at least in part, to the poorly described effects of various electroporation conditions on parasite survival. Using a BTX 600 Electroporator and a highly automated ^{3}H-hypoxanthine uptake assay, many parameters were evaluated. These parameters include temperature, media, voltage, resistance, DNA concentration, and parasite stage. Parasite stage may be important to transfection efficiency and may explain differences between stable and transient systems. The data presented will delineate those conditions that should produce optimal transfection conditions. Transfection of *P. falciparum in vitro* is a potentially powerful molecular tool that is essential to malaria gene function and mechanistic drug action/resistance studies. By increasing our understanding of electroporation and the biological implications for the malaria parasite, this study will hasten the day when malaria transfection is a commonly used laboratory procedure.

572 IDENTIFICATION OF PUTATIVE 3' GENE FLANKING ELEMENTS NECESSARY FOR GENE EXPRESSION IN MALARIA. Golightly LM*, Budge E, Mbacham WF, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The worldwide spread of drug resistant malaria mandates an understanding of the molecular mechanisms responsible. The development of a stable gene transfection system would permit the functional analysis of genes purported to be mediators of drug resistance and greatly enhance our ability to formulate novel chemotherapies.

We previously reported the development of a transient transfection system for the chicken malaria species *Plasmodium gallinaceum*. We are utilizing this system to understand mechanisms of gene regulatory control in malaria which are currently not defined. Once identified the essential elements for gene expression may be utilized in the construction of plasmids for the stable transfection of the human pathogen *Plasmodium falciparum*. The 3' gene flanking region is potentially important due to its role in RNA polyadenylation and stability. The 3' flanking region of the pgs28 gene was utilized in the construction of our previously reported malaria expression vectors pgs28.1GUS and pgs28.1LUC. To determine the role of the 3' flanking region in gene expression, deletion mutants of the 3' flanking region of these plasmids have been constructed. Cells transfected with plasmids lacking the complete 3' flanking region do not express enzyme activity. To delineate the essential regions of the 3' UTR necessary for gene expression nested deletion mutants have been generated for transfection analysis. Results of these experiments will be discussed.

573 ANALYSIS OF MUTATIONS IN *pfmdr1* ASSOCIATED WITH DRUG RESISTANT PARASITES EXPRESSED IN *SACCHAROMYCES CEREVISIAE*. Volkman SK*, Woodcock S, Cowman AF, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

Drug resistant *Plasmodium falciparum*, which causes human malaria, is a critical health concern. A candidate drug resistance gene, *pfmdr1*, has been identified and *pfmdr1* gene expression is strongly correlated with mefloquine resistance. We have developed a functional assay system in the yeast *Saccharomyces cerevisiae* to examine the nature of the *pfmdr1* gene product, Pgh1, as a transport molecule. We have previously demonstrated that expression of *pfmdr1* in yeast containing a null mutation in *ste6*, can complement STE6 function and restore mating to a level 1000-fold over background. Furthermore, we have demonstrated that expression of the *pfmdr1* gene containing two mutations associated with chloroquine resistance in the wild, abolish this mating phenotype, suggesting that these mutations are important for the transport function of Pgh1. We now extend this analysis to demonstrated that another mutation, resulting in a tyrosine to phenylalanine change at amino acid position 184 which has been identified in a mefloquine resistant parasite, failed to restore mating in this heterologous system. Both of these mutations have also been associated with dramatic changes in drug resistance profiles mediated by mammalian P-glycoprotein molecules. Further extension of this work involves the use of this functional expression system to test various drugs which may interact with Pgh1. Drug studies using these yeast transformants with various compounds indicate that the different forms of Pgh1 identified in parasites either sensitive or resistant to drugs have distinct sensitivity profiles, suggesting a role for these mutations in the mediation of drug resistance.

574 HEMOZOIN CATALYZED OXIDATION OF ARACHIDONIC ACID. Green MD*, Xiao L, Udhayakumar V, Lal AA, and Todd GD. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA.

The iron-containing heme moiety of hemozoin is an important source of non-enzymatic oxidizing potential during malaria infection. We wanted to determine if hemozoin can catalyze the oxidation of arachidonic acid (AA) to the hydroxyeicosatetraenoic acids (HETEs). Therefore we conducted an HPLC analysis of a mixture of AA and hemozoin. Two major chromatographic peaks with retention times similar for 5-HETE and 15-HETE authentic standards were detected. The catalytic effects of other heme derivatives such as hemin and hemoglobin on AA oxidation also resulted in 5-HETE formation, but the production of 15-HETE was specific to hemozoin. The significance of 15-HETE formation is that this compound has been reported to be a potent vasoconstrictor. This property may contribute to some of the pathological consequences of cerebral malaria. The effects of artemisinin (an antimalarial endoperoxide which has been reported to bind covalently to heme) and deferoxamine (a compound that forms complexes with iron) on hemozoin catalyzed HETE formation will also be presented.

575 ASSESSMENT OF THE EFFECT OF THE ORAL IRON CHELATOR DEFERIPRONE ON MILD *PLASMODIUM FALCIPARUM* PARASITEMIA IN HUMANS. Thuma PE*, Olivieri NF, Mabeza GF, Biemba G, Parry D, Zulu S, Fassos FF, Koren G, Brittenham GM, and Gordeuk VR. Division of General Pediatrics, Pennsylvania State University College of Medicine, Hershey, PA; Division of Haematology/Oncology, The Hospital for Sick Children and University of Toronto, Toronto, Canada; Macha Mission Hospital, Choma, Zambia; Division of Clinical Pharmacology, The Hospital for Sick Children and University of Toronto, Toronto, Canada; Division of Hematology/Oncology, MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH; and Division of Hematology/Oncology, The George Washington University Medical Center, Washington, DC.

While the parenteral iron chelating agent desferrioxamine B has anti-malarial activity in humans, the usefulness of an orally active chelator for this indication has not been investigated previously *in vivo*. We conducted a

prospective, double-blind, placebo-controlled cross-overtrial of deferiprone (L1; CP20; 1,2-dimethyl-3-hydroxypyridin-4-one) in 25 adult Zambians with asymptomatic *Plasmodium falciparum* parasitemia. Deferiprone was administered daily for three or four days in divided doses of 75 or 100 mg/kg body weight. Pharmacokinetic studies on five subjects revealed a mean peak plasma concentration of deferiprone of 108.2 ± 24.9 μmol/L, which is within the range demonstrated to inhibit the growth of *P. falciparum in vitro.* No reduction in asexual intra-erythrocytic parasites was observed during deferiprone treatment. No evidence of deferiprone-associated hematological toxicity was noted in these subjects with normal body iron stores. We conclude that, in the doses and schedules examined in this study, the oral iron chelator deferiprone has no clinically detectable anti-malarial effect in humans.

576 EFFECT OF ORALLY ACTIVE IRON CHELATORS IN COMBINATION WITH ANTIMALARIALS ON THE GROWTH *IN VITRO* OF *PLASMODIUM FALCIPARUM.* Pattanapanyasat K*, Kotipun K, Yongvnithchit K, Hider RC, Kyle DE, and Heppner DG. Department of Immunology and Parasitology, AFRIMS, Bangkok, Thailand; Department of Pharmacy, King's College, University of London, UK; Division of Experimental Therapeutics, WRAIR, Washington, D.C.; Department of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The resurgence of drug-resistant malaria makes urgent the evaluation of new antimalarial combinations. The parenteral iron chelator desferrioxamine (DFO) has suppressed parasitemia in preclinical & clinical trials and, as adjunct therapy, shortened recovery from cerebral malaria in a pilot study. In contrast to DFO, the ketohydroxy-pyridine derivatives (KHP's) are cheap, stable, and orally active iron chelators. KHP's are already in clinical use and have been shown to suppress *Plasmodium falciparum in vitro.* Both DFO and the KHP's form inert iron-chelator complexes, and exert their antimalarial effect by depriving parasities of iron. We examined their *in vitro* growth inhibitory effects in combination with multiple antimalarials (quinine, mefloquine, tetracycline, artesunate, atovaquone and 5-flouro-orotate) against TM267, a chloroquine-sensitive *P. falciparum* isolate. Growth inhibition was measured by tritiated hypoxanthine uptake, and by flow cytometric methods. Results of the two methods were highly correlated (R^2=0.96). TM267 IC50's and IC90's for the KHP's CP20, CP38, & CP110 were in the range of 60-70 Êm and 90-110 Êm respectively, 2-4 times higher than reported for DFO. Isobolograms were constructed for each KHP in combination with each antimalarial drug; classic additive interactions were observed although mefloquine:KHP was slightly antagonistic [Sigma FIC>2<4]. *In vivo* assessment of KHP's is supported by the absence of classical antagonism and by their potential to modify disease, perhaps by limiting iron-mediated free radical damage or by augmenting γ- interferon induced cytotoxicity.

577 CHARACTERIZATION OF CHLOROQUINE-FERRIPROTOPORPHYRIN-IX BINDING USING ISOTHERMAL TITRATION MICROCALORIMETRY. Vippagunta SR*, and Vennerstrom JL. College of Pharmacy, University of Nebraska Medical Center, Omaha, NE.

The precise mechanism of action of chloroquine (CQ), the most widely used drug in the chemotherapy of *Plasmodium falciparum* malaria, remains undefined. *Plasmodia* infect red blood cells of humans and digest hemoglobin, releasing globin and ferriprotoporphyrin IX (FP). Most of this toxic FP, in turn, is converted to hemozoin, a nontoxic form of FP. Previous studies have suggested that FP is the CQ receptor which mediates its antimalarial activity. This hypothesis was tested using isothermal titration microcalorimetry (ITC) to provide a thermodynamic characterization of CQ-FP binding as function of pH, and ionic strength. At pH 6.0-8.0, binding constants (Ka) of 4-10 x 10^5 were observed for enthalpy driven CQ-FP binding. Stoichiometry of CQ-FP binding was 1:3 at pH 7.0-8.0 and 1:20 at pH 6.0 which suggests that pH-dependent FP aggregation may be important with respect to the mechanism of action of CQ. Analogs of both FP and CQ were studied to elucidate structural features important to their binding. Correlations between FP-binding, heme polymerase inhibition, and intrinsic antimalarial potency will be presented for CQ, amodiaquine, quinacrine, quinine, mefloquine, piperaquine, and WR 268668.

578 THE CRYSTAL STRUCTURE OF THE TRIAZINE ANTIMALARIAL AGENT WR 99,210 AS A MODEL FOR ITS BIOLOGICAL INTERACTION. Karle JM*. Department of Pharmacology, Walter Reed Army Institute of Research, Washington, DC.

The crystal structure of the potent antimalarial agent WR 99,210 as a carboxylate salt shows that WR 99,210 possesses an elongated structure and that the hydrogen atom from the salt molecule attaches to the ring nitrogen atom adjacent to the methylated carbon atom. WR 99,210, 4,6-diamino-1,2-dihydro- 2,2-dimethyl-1-[3-(2',3',5'-trichlorophenoxy)propyloxy]-1,3,5-triazine, a cycloguanil analog, is one of the most potent compounds against *Plasmodium falciparum in vitro* in the Walter Reed inventory with IC50's as low as 0.001 ng/ml. The crystal structure, determined by x-ray crystallography, demonstrates the preferential site of hydrogen attachment.

Calculation of point charges for the neutral molecule results in the ring nitrogen atom which attracts the hydrogen atom from the salt being the most negative nitrogen atom. Also noteworthy is the bond lengths of the triazine portion of the molecule. The C-N bonds associated with the methylated carbon atom are consistent with single bonds. However, all of the other C-N bond lengths in the molecule range from 1.30 to 1.38 Angstroms and are consistent with partial double bonds. All of the amine hydrogen atoms are co-planar with the triazine ring. The triazine ring structure in WR 99,210 salt is nearly identical to the crystal structure of cycloguanil hydrochloride. In WR 99,210, both of the amine groups attached to the triazine ring as well as the hydrogen atom from the salt molecule participate in hydrogen bonding. The aliphatic portion of the molecule between the rings resides in a zigzag conformation except for the oxygen atom attached to the triazine ring which turns out of the zigzag plane. These structural features indicate the likely sites and geometry of the interaction of WR 99,210 with biological molecules.

579 ERADICATION OF *PLASMODIUM VIVAX* LIVER-STAGE PARASITES BY ANTIMALARIAL DRUGS IN A TISSUE CULTURE MODEL. Karnasuta C*, Aikawa M, Fujioka H, Chantakulkij S, and Watt G. Department of Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Institute of Pathology, Case Western Reserve University, Cleveland, OH.

Plasmodium vivax liver-stage parasites cultured in HepG2 liver cells were studied to: 1) compare the antimalarial effects of a series of new and old drugs with primaquine, 2) determine the stage-specificity of the antimalarial drugs, and 3) to measure the potentiating effects of a reversing agent on the different drugs. Equimolar amounts of primaquine, mefloquine, WR238605, halofantrine, sodium artesunate, azithromycin and doxycycline were used alone and with verapamil. Liver parasites were counted after a single day of drug treatment (either day 0, day 1, day 2,or day 5), after a week of treatment (either the first or second week), and after 14 days of treatment. The numbers of Giemsa-stained drug-exposed parasites counted by direct microscopy were compared to the numbers of untreated controls. The most effective drugs when given for a single day were primaquine, mefloquine and WR238605. All drugs except doxycycline eradicated more than two-thirds of liver parasites when given during the first week and for two weeks. Several drugs inhibited liver parasite development by 95% or more. Verapamil potentiated the antimalarial effets of all drugs tested. Attempts to determine parasite viability using electron microscopy are underway. Perhaps some parasites which look intact under light microscopy are damaged and non-viable.

580 MANNICH BASES WITH GREATER ANTIMALARIAL ACTIVITY THAN PYRONARIDINE. Kotecka BM*, Barlin GB, and Rieckmann KH. Army Malaria Research Unit, Sydney, Australia; John Curtin School of Medical Research, Canberra, Australia.

Pyronaridine (PRN) is a Mannich base which has been used in China for the treatment of chloroquine (CQ) resistant malaria. We have compared the *ex vivo* activity of PRN with that of 13 other Mannich bases with an *in vitro* MIC of < 100 nM against the CQ-resistant K1 isolate of *Plasmodium falciparum*. Each compound was administered orally (30 mg/kg) to 3 or more uninfected *Saimiri* monkeys and serum samples were collected 1 to 7 days after dosing to determine their antimalarial activity against variousisolates of *P. falciparum*. Serum antimalarial activity was determined by measuring the maximum serum dilution at which the maturation of parasites was inhibited *in vitro*. The maximum inhibitory dilution (MID) of serum following administration of 7 of the 13 drugs was much greater than that observed after PRN administration. In serum collected 1 day after dosing, MID values (against K1) were 4 to 16 for PRN and 32 to 128 for the 7 drugs. In serum collected 3 days after PRN administration, no antimalarial activity could be detected in 2 of 5 treated monkeys, whereas 22 of 24 serum samples collected at this time from monkeys receiving the 7 drugs had MID values of 8 or above. In fact, monkeys given TN 109 had MID values of 64 and 16 in serum samples collected at 3 and 7 days, respectively. Four of the most potent of these drugs had comparable activity against 4 other CQ-resistant and 2 CQ-sensitive isolates of *P. falciparum*. Further evaluation of the most promising of these drugs is indicated because they have a very marked and prolonged antimalarial activity and no adverse reactions have been observed following their administration.

581 THE EFFECTS OF BISTRATENE A ON THE DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN CULTURE. Mann VH*, Law MH, Watters D, and Saul A. Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

The effects of the marine ascidian compound bistratene A on *in vitro* cultures of *Plasmodium falciparum* were assessed. Concentrations from 0.0 to 1.5 μg/ml of the compound were tested. The parasitaemia in asynchronous cultures treated with bistratene A increased normally over the first 40 hours, then decreased leaving only gametocytes. When synchronized cultures were treated with a constant dose of 50 ng/ml, gametocytes developed more rapidly than they did in control cultures. In addition, gametocytes developed in drug treated cultures of *P.*

falciparum that normally do not produce gametocytes. Bistratene A appears to inhibit merozoite invasion as well as inducing gametocytogenesis.

582 EVALUATION OF ANTIMALARIAL ACTIVITY OF MEDICINAL HERBS. Obih*. College of Pharmacy, Xavier University of Louisiana, New Orleans, LA.

Each year, at least one million people die from malaria infection. This situation has worsened with the emergence of *Plasmodium* strains resistant to currently used antimalarials such as chloroquine. With the ever-increasing trend in the international travels, there is danger of importing these resistant strains into areas where they never existed before. The aim of this study is to obtain information necessary to develop more potent and specific therapeutic agents from plant sources for the treatment of malaria. The antimalarial activities of medicinal herbs: *Morinda lucida, Azadirachta indica, Cymbopogun citratus* and *Offor* were investigated. Antimalarial activity was assessed on chloroquine sensitive strains of*Plasmodium falciparum in vitro*. [G-^{3}H]hypoxanthine was used as a radioactive precursor of nucleic acids to assess antimalarial activity by measuring uptake of the precursor in human erythrocytes infected with *Plasmodium falciparum*. The schizontocidal activity of these extracts was also evaluated on *Plasmodium berghei* in mice. Among all the extracts screened, *Azadirachta indica* demonstrated the most potent antimalarial activity.

583 STRUCTURE-BASED DEVELOPMENT OF NOVEL MALARIA CYSTEINE PROTEASE INHIBITORS. Miller R*, Nuzum E, Li R, Chen X, Gong B, Dominguez J, Rosenthal P, Kenyon G, Kuntz I, and Cohen F. Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA.

A cysteine protease from *Plasmodium falciparum* malaria has been chosen as a target for developing novel antimalarials because peptide inhibitors of this enzyme cure malaria-infected mice. A computer model of the three dimensional structure of this protease, based on known cysteine protease sequences from other organisms, was used to search the Available Chemical Directory (ACD) database for potential inhibitors. A lead compound was identified by the computer program DOCK and was found to inhibit malaria growth *in vitro* and an *in vitro* enzyme assay at an Inhibitory Concentration (IC_{50}) of approximately 10 μM. Based on this lead compound, we used simple synthetic chemistry to develop many derivatives of two classes of novel inhibitors. Several of these compounds have *in vitro* IC_{50}s of less than 1μM against both chloroquine-resistant and chloroquine-sensitive strains of malaria. The data suggest that these novel cysteine protease inhibitors are not cross-resistant with chloroquine and represent a novel strategy for developing new antimalarial agents.

584 MOLECULAR MODELING STUDIES OF POLYCYCLIC PEROXIDES AND THE SEARCH FOR DESCRIPTORS OF ANTIMALARIAL ACTIVITY. Jefford CW*, Weber J, Grigorov M, Thomson C, Suter HU, and Maric D. Departments of Organic and Physical Chemistry, University of Geneva, Geneva; Department of Chemistry, University of St. Andrews, St. Andrews, Scotland; and Swiss Scientific Computer Center, Manno, Switzerland.

The geometries and certain properties of qinghaosu (= artemisinin), yingzhaosu and five related synthetic peroxides have been calculated with the goal of uncovering descriptors for antimalarial activity. Semi-empirical and ab initio self-consistent field methods were performed to evaluate the respective importance of key molecular properties involved in the activation of the drug by heme released within the intraerythrocytic parasite. In essence, the rate determining step was assumed to be the complexation of ferrous ion in heme with the peroxide moiety and the concomitant transfer of a single electron to the lowest unoccupied molecular orbital of the oxygen-oxygen sigma bond. Consequently, an attempt was made to correlate antiparasitic activity with molecular electrostatic potentials, frontier orbital energies, and electron densities at the peroxide linkage. The degree of success of this approach and its relevance to the design of new antimalarial drugs will be discussed.

586 ARTEMISININ DERIVATIVES AS INDUCERS OF CELL DIFFERENTIATION. Wongpanich V*, Suh N, Angerhofer CK, El-Feraly FS, and Pezzuto JM. Program for Collaborative Research in the Pharmaceutical Sciences, Department Medicinal Chemistry and Pharmacognosy; and Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

One strategy for inhibiting the rapid proliferation of cancer cells is treatment with an agent that can induce terminal differentiation. Retinoic acid and vitamin D3, for example, are well known to induce such differentiation, and additional compounds are being actively sought in our laboratory as potential anti-cancer agents. An *in vitro* assay

system based on the induction of biochemical markers of differentiation in cultured HL-60 (human promyelocytic) cells has been utilized in this search. In light of the possibility that such induction could also represent a potential antimalarial mechanism, a number of antimalarial compounds including chloroquine, quinine, mefloquine and a series of natural and synthetic artemisinin derivatives were subjected to the HL-60 assay. While none of the quinoline-based drugs were active in this screen, all of the artemisinin derivatives we tested induced specific monocytic differentiation of HL-60 cells as measured by their ability to hydrolyze the substrate, a-naphthyl acetate. In contrast to the qualitative similarity in activity, the potency of these compounds as differentiating agents was found to vary significantly with relatively minor changes in structure. However, the antimalarial activities of the artemisinin derivatives were not well-correlated with their potential to induce differentiation in mammalian HL-60 cells, therefore, it is uncertain whether this mechanism plays a role in their activity against protozoal organisms.

587 METABOLISM OF ARTEETHER TO DIHYDROQINGHAOSU BY ISOZYMES OF HUMAN AND RAT CYTOCHROME P-450. Grace JM*, Peggins JO, Aguilar AJ, and Brewer TG. Division of Experimental Therapeutics, WRAIR, Washington, DC; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

In an effort to elucidate which form(s) of human cytochrome P-450 catalyzed the conversion of arteether(AE) to dihydroqinghaosu (DQHS), AE was incubated with CYP1A1, 1A2, 2A6, 2D6, 2C9 and 3A4. The specific isozymes were obtained from Gentest Corp as microsomal suspensions containing the human recombinant form of each enzyme. Incubations were run for three hours at 37°C, in the presence of an NADPH regenerating system, 2μg AE and 50μg microsomal protein in a volume of 1 ml. After incubation the reactions were stopped by the addition of 2x5 ml 90: 10, n-butylchloride: ethylacetate. The organic phases were combined and evaporated under a stream of Nitrogen. The residues were reconstituted with 500 μl of 50: 50 ethanol: water and 100 μl analyzed for AE and DQHS using reversed phase HPLC with reductive electrochemical detection. CYP1A2 and CYP3A4 were the only isozymes which significantly metabolized AE to DQHS. CYP3A4 converted AE to DQHS at a rate approximately 5x that of CYP1A2. Reactions were time and protein dependent. CYP3A4 catalyzed the conversion of AE to DQHS with a Km of approximately 43 μM and a Vmax of 0.52 nmol DQHS formed/min/mg protein. In rat hepatic microsomes, AE inhibited the metabolism of phenacetin a probe for CYP1A2 to acetaminophen in a dose dependent manner with an apparent IC50 of 37μM. These results indicate that AE is metabolized to DQHS by CYP1A2 in rats and humans, and by CYP3A4 in humans. Any drugs which aresubstrates for these isozymes and given in combination with AE may influence the efficacy and/or the toxicity of AE, since DQHS is known to be more toxic than AE *in vitro* and *in vivo* in animal models.

588 ARTEETHER PHARMACOKINETICS IN RATS AFTER 7 DAILY INTRAMUSCULAR DOSES. Li QG*, Brueckner RP, Peggins JO, Masonic KJ, Trotman KM, and Brewer TG. Department of Pharmacology, Division of Experimental TherapeuticsWalter Reed Army Institute of Research, Washington, DC.

Multiple doses of arteether (AE) at 25 mg/kg cause CNS toxicity in rats. This dose was used to study the pharmacokinetics (PK) of AE after multiple i.m. injections. Animals were administered AE in sesame oil, i.m. daily for 7 days. Blood samples were collected using destructive sampling for up to 192 hr, and assayed by EC-HPLC. Other rats were administered a single 25 mg/kg dose i.v. The body weights of rats were reduced after 3 doses. Analysis of the data revealed a marked change in the PK parameter estimates after day 1. The central compartment volume of distribution (Vc) during days 2-7 (2.9 L) was 36% of the day 1 Vc (7.9 L). Clearance (CL) was reduced to 25% of the day 1 value, from 5.3 L/h to 1.3 L/h on days 2-7. The $t_{1/2}$ of AE elimination was also extended from 4.4 hr on day 1 to 12.1 hr during days 2-7. This suggests that the effects of AE administration may have resulted in an alteration of its own distribution or metabolism. Analysis of the injection sites showed that 38% of the AE dose was still left in the muscle after 24 hr. Fast and slow absorption phases were demonstrated with $t_{1/2}$ s of 3.1 hr and 22.3 hr, respectively. Although the elimination $t_{1/2}$ of AE after IV administration was only 1 hr, after i.m. dosing the plasma AE levels demonstrated significant accumulation during the 7 days of dosing due to the prolonged absorption phase. These findings suggest that alterations in AE distribution may occur after multiple i.m. administrations.

589 ANORECTIC TOXICITY OF DIHYDROARTEMISININ, ARTEMETHER AND ARTEETHER IN RATS FOLLOWING MULTIPLE INTRAMUSCULAR DOSES. Peggins JO*, Li QG, and Brewer TG. Division of Experimental Therapeutics, WRAIR, Washington, DC; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

During studies of Arteether (AE), Artemether (AM) and Dihydroqinghaosu (DQHS) neuro-toxicity, the effect of 7 daily i.m. doses on gastro-intestinal function was also examined in rats. A modified Nichols' method was used to measure daily food and water consumption. To determine gastric retention, the tied-off stomach pouch was removed and the contents weighted 24 hours after the last dose or when the rat became moribund. AM and AE

dose solutions were prepared using sesame oil while 50% DMAC/sesame oil was used for DQHS. The results show that after dosing with 50 mg/kg for 7 days, 50% inhibition of food consumption (ID50) occurred at 1.9 days for DQHS, 3.9 days for AM, and 4.1 days for AE. Similar data was observed for water intake. After 100 mg/kg dosing, the ID50 for food and water consumption decreased to 2.8 - 2.9 days for AM, and 3.1-3.7 days for AE. Due to decreased food consumption, body weights were decreased following all three treatments. In addition, the weight of the stomach contents was 16.6 g for DQHS, 3.2 g with AE and 1.3 g for AM following 50 mg/kg dosing. When the 100 mg/kg dose was administered, the weight of the stomach contents was doubled for AE (6.4 g) and AM (2.4 g). These data indicate differences in stomach emptying time between the two drugs and demonstrate that after multiple doses of DQHS, AM and AE, food consumption and gastric emptying were decreased, resulting in significant body weight reduction. The gastrointestinal toxicity of DQHS, AM and AE may result from decreased gastric motility due to decreased vagal stimulation secondary to the CNS neurotoxicity since the CNS toxicity was observed in same experiment dosing.

590 THE STATE OF PYRIMETHAMINE-RESISTANT *PLASMODIUM FALCIPARUM* PARASITES AMONG TANZANIAN CHILDREN; A FACILITY-BASED STUDY USING PCR. Edoh D*, Mshinda H, Kitua A, Jenkins J, Burger M, and Tanner M. Ifakara Centre, Ifakara, Tanzania; Swiss Tropical Institute, Basel, Switzerland; Friedrich Miescher-Institut, Basel, Switzerland.

A sensitive mutation-specific PCR assay was used to determine the proportion of pyrimethamine-resistant parasites in children reporting sick at Clinic in Ifakara, a town in Tanzania. The assay is based on the observation that a point mutation (Asn-108) in the dihydrofolate reductase (DHFR) gene confers resistance to pyrimethamine. Twenty-eight percent of the examined children had pyrimethamine-resistant parasites, 65% had pyrimethamine-sensitive parasites with wild-type Ser-108 codon and 9% had both alleles, suggesting a mixed infection. Correlating molecular, clinical and parasitological results, from the same patients, revealed a strong association only between proportion of pyrimethamine-resistant parasites and malaria parasite density: they were inversely related. Interestingly, none of those who had malaria episode, based on a case definition, had pyrimethamine-resistant parasties. Currently, sulphadoxine-pyrimethamine is considered as a potential candidate first line drug for malaria treatment in most African countries. We suggest that although sulphadoxine-pyrimethamine could still be effective against chloroquine-resistant malaria in this area, its judicious use is more important. The implications of these finding to health policy-making is further discussed.

591 SUSTAINED EFFICACY OF SULFADOXINE-PYRIMETHAMINE AS FIRST-LINE TREATMENT FOR MALARIA IN UNDER FIVE MALAWIAN CHILDREN. Ziba C*, Nwanyanwu OC, Kazembe PN, Wirima, and Redd SC. Ministry of Health, Lilongwe, Malawi; College of Medicine, University of Malawi, Blantyre, Malawi; and Centers for Disease Control and Prevention, Atlanta, GA.

In March 1993, sulfadoxine-pyrimethamine (SP) replaced chloroquine as the first line drug for malaria treatment in Malawi. We assessed the efficacy of SP treatment for malaria in children after the policy change. Studies were conducted during the 1994 rainy season at Mangochi and Karonga District Hospitals and during the 1995 rainy season at the Dwanga Sugar Corporation clinics. Children under 5 years old attending these outpatient clinics who had an axillary temperature ≥38°C and parasite density ≥ 2,000/ L and whose parents consented were enrolled. Children were treated with SP and followed for 28 days or until clinical failure. At Mangochi, 69 of 82 children enrolled and completed follow-up, compared with 76 of 77 at Karonga and 117 of 117 at Dwangwa. RI resistance ranged from 3% at Karonga to 14% at Dwangwa; RII resistance was 0% at Mangochi and Karonga and 3% at Dwagwa; and RIII resistance was 1% at Karonga and Dwangwa and 3% at Mangochi. Over 92% of children had improved clinically by day 3 and 99% by day 7. In addition, mothers reported improvement in 94% of the children by day 3 and 77% were reported as being active by day 3. No deaths occurred. These data suggest that SP has remained highly efficacious in the treatment of malaria after introduction and widespread use; resistance in *Plasmodium falciparum* remains low. Countries with high levels of chloroquine resistant *P. falciparum* should consider changing to SP as first line treatment.

592 A SYSTEMATIC REVIEW OF PUBLISHED AND UNPUBLISHED DATA ON ORAL AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA. Olliaro P*, LeBras J, Nevill C, Ringwald P, Mussano P, Garner P, and Brasseur P. WHO/TDR, Geneva, Switzerland; Hopital Bichat-C. Bernard, Paris, France; AMREF, Nairobi, Kenya; OCEAC, Yaounde, Cameroon; Cochrane Parasitic Diseases Group, Geneva, Switzerland; Cochrane Parasitic Diseases Group, Liverpool School of Tropical Medicine, UK; and Hopital C. Nicolle, Rouen, France.

Opinions and policies with regard to the use of amodiaquine (ADQ) for the treatment of malaria vary, with debate concerning its comparative effectiveness with respect to chloroquine (CLQ) and the possibility of serious adverse

reactions (ADRs). To inform policy, we conducted a systematic review of randomized or pseudramdomized trials concerned with ADQ to examine effectiveness and tolerability. Of the trials identified, 27 were deemed eligible (14 published and 6 unpublished studies, 17 were as raw data). Observational reports were also reviewed to assess evidence of fatal ADRs. A total of 1011 uncomplicated falciparum malaria patients have reportedly received ADQ in controlled trials vs. CLQ (n=1045) at 22 sites (21 from Africa). Considering the patients seen on day 7 (ADQ n=941; CLQ n=957), the parasitological success rates (PSRs) were 87 and 63% respectively. The OR (95% CI) of parasitological clearance for ADQ vs CLQ were 6.22 (4.94 - 7.94) on d7 and 6.32 (4.48 - 8.71) on d14. Time to parasite clearance was shorter with ADQ, as calculated on 428 ADQ and 424 CLQ recipients with 4 data points (399 and 256 successes, respectively, log-rank p=0.0001). An S/RI response was reported in 595/681 ADQ recipients, compared to 366/682 CLQ patients [OR (95% CI) - 5.97 (4.51 - 7.91)]. Fever clearance times (FCT) were similar in the two groups. An additional 455 asymptomatic *Plasmodium falciparum*-infected subjects received ADQ (compared to 451 CLQ controls) [OR (95% CI) of PSR on d7 - 3.49 (2.38 - 5.18)]. Sulfadoxine/pyrimethamine (S/P) was used as a comparator drug at 10 sites (9 from Africa) involving 597 S/P and 599 ADQ recipients. The PSR were similar on d7 (ADQ 92.2%; S/P 93.8%) [OR (95% CI) - 0.78 (0.5 - 1.22)]: a marginal benefit on d14 [0.61 (0.37 - 0.99)], and a significantly better response on day 28 were apparent in favour of S/P, although these latter results were based on only 3 studies. Patients on ADQ had significantly shorter FCT than those on S/P. Tolerability was assessed for both comparative and non-comparative trials. Overall, 466 ADQ, 389 CQ and 333 S/P recipients were available for tolerability analysis in comparative trials. The rates of ADRs were 11.2, 8, and 15.3%, respectively. AEs were reportedly mild or moderate. Clinical laboratory parameters were assessed in only a few studies, there appeared to be no obvious toxicity concern. No life-threatening ADR nor any significant shift in the laboratory parameters tested were reported.

593 VARIABLE PROGUANIL METABOLISM IN DIFFERENT ETHNIC GROUPS HAS CLINICAL IMPLICATIONS FOR PROPHYLAXIS OR TREATMENT OF MALARIA. Edstein MD*, Looareesuwan S, Kyle DE, Canfield CJ, Hutchinson DB, and Rieckmann KH. Army Malaria Research Unit, Sydney, Australia; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Experimental Therapeutics, WRAIR, Washington, DC; Pharmaceutical Systems Inc, Gaithersburg, MD; Wellcome Research Laboratories, Beckenham, UK.

Proguanil (PROG) has been used in combination with either chloroquine or asulfone for malaria prophylaxis. Recently, PROG in combination with atovaquone has been shown to be highly effective in the treatment of falciparum malaria. PROG, a pro-drug, is metabolised to cycloguanil (CYC) and exhibits oxidative polymorphism with poor metabolisers (PMs) having lower plasma CYC concentrations than extensive metabolisers (EMs). The clinical implication for those individuals with low CYC concentrations ist hat they are less likely to be protected against malaria and the sub-inhibitory levels of CYC may promote the development of parasite resistance. The purpose of this study was to compare plasma CYC levels in 12 healthy Thai volunteers following a prophylactic dose of PROG (200 mg) with those in 11 Thai patients given a high dose of PROG (500 mg bid for 3days). Despite receiving 2.5 times more drug, Thai patients, after the first dose of PROG, had similar mean peak CYC levels (EMs: 69 ng/ml; PMs: 18 ng/ml) to those observed in healthy volunteers (EMs: 52 ng/ml; PMs 15ng/ml). Although it is unknown whether malaria affects the kinetics and metabolism of PROG, Thais appear to have a limited capacity to form CYC. When comparing CYC levels between healthy Thais and other ethnic groups (eg., Caucasian and African populations) Thais, irrespective of their phenotypic status, produce considerably less plasma CYC. Thus, knowledge of the ability of different ethnic groups to produce CYC is of clinical importance when PROG is used in combination with other drugs for the prophylaxis or treatment of malaria.

594 *IN VIVO* SAFETY AND ANTIPLASMODIAL ACTIVITY OF CHLOROQUINE (CQ) ANALOGS ACTIVE AGAINST CQ-RESISTANT *PLASMODIUM FALCIPARUM IN VITRO*. Cogswell FB*, Preslan J, Spurlock JP, Mack PA, Krogstad FM, De D, George WJ, and Krogstad DJ. Tulane University, New Orleans, LA.

We have recently found that modification of the isopentyl diaminoalkane side chain of CQ obviates CQ resistance. This strategy has now yielded three CQ analogs with IC_{50}s similar to CQ against CQ-susceptible *Plasmodium falciparum* (5-15 nM), and against CQ-resistant, mefloquine-resistant and multiply-resistant *P. falciparum in vitro*. The studies reported here were performed to test for potential *in vivo* toxicities of these analogs in mice and monkeys, and for *in vivo* activity against *P. cynomolgi* in the rhesus monkey model of human infection with *P. vivax*. In the mouse studies, no untoward toxicities were observed with doses of 5 mg base/kg SC x 4 days (which are 3 times the CQ ED_{90} for *P. berghei*). Similarly, in the rhesus monkey, no detectable adverse effects were observed with doses of 7 mg base/kg IM x 5 days, the standard curative regimen for *P. cynomolgi* infection in rhesus macaques. Using gas chromatography-mass spectroscopy (GC-MS), plasma levels of CQ and the three CQ analogs were examined 0, 4, 8, and 24 hours after IM drug administration during Phase I testing, and again during Phase II studies of the treatment of *P. cynomolgi* infection.

595 *IN VITRO* SENSITIVITY OF *PLASMODIUM FALCIPARUM* ISOLATES IN THAILAND, 1990-1994. Wongsrichanalai C*, Wimonwattrawatee T, Sookto P, Laoboonchai A, Heppner DG, and Wernsdorfer WH. Department of Immunology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Institute of Specific Prophylaxis and Tropical Medicine, University of Vienna, Vienna, Austria.

As part of our continued effort to monitor the *in vitro* sensitivity of *Plasmodium falciparum* isolates in Thailand to various antimalarial drugs, we present the results for 1990-1994. We used a semi-automated, ^{3}H-hypoxanthine incorporation, microdilution technique to measure metabolic activity of the malaria parasites that were subject to different concentrations of antimalarials. Scintillation counter results (CPM or counts per minute) were analyzed by log-probit analysis. 50% and 90% inhibitory concentrations (IC_{50}s, IC_{90}s), slopes and potency ratio estimates will be reported for mefloquine, halofantrine, chloroquine, quinine and artemisinin for isolates collected from the Thai-Cambodian border, Thai-Myanmar border and Southern Thailand. As a preliminary result, the Table depicts the 1990- 1993 sensitivity pattern of mefloquine for isolates from the southeastern border of Thailand with Cambodia. There was a decrease in the slope of the regression lines, an increase in the IC_{50}s, but the IC_{90}s were relatively unchanged. The potency ratio estimate for the comparison between 1992 vs 1993 regression lines was not statistically significant. These findings demonstrate that although there was a continuing loss of mefloquine *in vitro* sensitivity on the Thai-Cambodian border, the degree of deterioration was not as prominent as in the earlier period following its introduction for operational use in Thailand (1985- 1989), during which time there was a large influx of non-immune and semi-immune migrant gem miners to the area.

Year	N	Slope	IC50	IC90
1990	31	1.6	14.2 (9.9 - 20.2)	89.4 (46.9 - 170.7)
1991	18	1.8	17.8 (11.8 - 26.9)	91.1 (45.5 - 182.5)
1992	18	2.6	28.4 (20.2 - 39.8)	90.3 (56.6 - 144.3)

ng/ml (95% CI)

596 CHLOROQUINE-SENSITIVE FALCIPARUM MALARIA IN LAOS. Watt G*, Jongsakul K, Chum, and Karnasuta C. Department of Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Medical Director, Ministry of Public Health, Khammouang Province, Laos.

Thai strains of *Plasmodium falciparum* are among the most drug resistant in the world and are virtually unaffected by chloroquine. However, anecdotal reports suggested that chloroquine was still used for malaria chemotherapy adjacent to the Thai border in Thakhek, Laos. We performed *in vitro* and *in vivo* studies to attempt to document chloroquine sensitivity. Seven isolates of *P. falciparum* from Thakhek patients were tested against chloroquine and other antimalarials by incorporation of radiolabelled hypoxanthine. All seven isolates were sensitive to chloroquine. The median IC_{50} was 4.9 ng/ml (range 3.8 - 6.6 ng/ml). Despite abscence of drug pressure, 4 of the 7 isolates were resistant to mefloquine and 3 of the 7 were resistant to halofantrine. Nine patients admitted to Khammouane Provincial Hospital with uncomplicated falciparum malaria were then treated with a standard course of chloroquine (2,500 mg given over 3 days) and hospitalized for 14 days. All patients recovered rapidly, there were no deaths nor severe complications. Parasites disappeared from the peripheral blood in a median time of 58h (range 28-86h) and did not reappear within 14 days in any patient. This focus of chloroquine sensitive falciparum malaria in Southeast Asia illustrates that recommendations for malaria prevention and treatment should be based on local data whenever possible.

597 ANTIMALARIAL CHEMOPROPHYLAXIS IN AFRICA: EXTRAPOLATION FROM *IN VITRO* ACTIVITY OF CHLOROQUINE, CYCLOGUANIL, AND MEFLOQUINE. Basco LK*, Ringwald P, Thor R, Doury JC, and Le Bras J. Laboratoire de Parasitologie, Hopital Bichat-Claude Bernard, Paris; and Institut de Médecine Tropicale du Service de Santé des Armées, Le Pharo, Marseille, France.

At present, there is no consensus on the antimalarial chemoprophylaxis, especially for travel to west and central Africa, where low-level chloroquine resistance is present. An indirect approach to evaluate the efficacy of chloroquine plus proguanil versus mefloquine is the determination of their *in vitro* activity against fresh clinical isolates of *Plasmodium falciparum*. We have analysed the drug sensitivity patterns of more than 300 recent isolates obtained from malaria-infected travelers returning from Africa (with or without antimalarial chemoprophylaxis).

51%, 33%, and 5% were resistant to chloroquine (IC_{50} >100 nM), cycloguanil (biologically active metabolite of proguanil; IC_{50} >50 nM), and mefloquine (IC_{50} >30 nM), respectively. 20 % of the isolates were resistant to both chloroquine and cycloguanil. Our study suggests that cycloguanil alone has a high *in vitro* activity against the intraerythrocytic asexual stages of African isolates, that there are a few isolates that are resistant *in vitro* to both chloroquine and cycloguanil, and that mefloquine-resistant isolates are rare in Africa. Despite the uncontestably high activity *in vitro* and proven clinical efficacy of mefloquine, chloroquine and cycloguanil are active *in vitro* against a considerable proportion of African isolates and still have a place in the chemoprophylaxis of travelers visiting Africa.

598 EVALUATION OF SELECTED ANTIMALARIAL DRUGS IN THE *BABESIA MICROTI*-HAMSTER MODEL. Marley SE*, Eberhard ML, Stuerer FJ, Ellis WY, McGreevy PB, and Ruebush TK. Parasitic Diseases Division, Centers for Disease Control, Atlanta, GA; Zoology Department, University of Georgia, Athens, GA; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Babesiosis is an intraerythrocytic protozoan parasite that is morphologically similar to malarial parasites. The number of human infections with this parasite appear to be increasing, especially in several non-traditional areas such as the midwest and western states. Babesiosis is of public health significance due to the potential severity of the infection, the apparent spread in geographical range, and also because *Babesia microti* is no longer recognized as the only species which results in human infection. An increasing number of human Babesia infections have proven difficult to treat and have not responded to conventional chemotherapy and a number of deaths related to *Babesia* infections have been recently reported. The currently used combination of drugs, quinine and clindamycin, do not seem to result in parasitological cures in many instances. We undertook a limited screen of newer antimalarial drugs which have either recently been released for human use, or were in experimental stages of development in the WRAIR drug screening system. The drugs were screened in the hamster-*B. microti* system. At least two classes of the drugs, 8-aminoquinolines and pyrroloquinazolines, showed promising results and were studied further at various doses and schedules. These results suggest that several of these compounds merit further attention in other screening systems and may prove useful in treating human infections.

599 SHORT-TERM CULTURE OF *PNEUMOCYSTIS CARINII* FROM INOCULATED MOUSE. Shaw MM*, Bartlett MS, Durant PJ, Queener SF, and Smith JW. Department of Pathology and Laboratory Medicine, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

Inocula from *Pneumocystis carinii* infected rats has been used in short-term cultures for over 16 years to test drugs for inhibition of *P. carinii* proliferation. Inability to culture organisms from other hosts prevented comparisons of drug susceptibilities for various strains or species of *P. carinii*. By mechanically breaking up clumps of *P. carinii* in mouse lung homogenates, we have successfully cultured this strain using the same human embryonic lung fibroblasts and conditions used for culture of *P. carinii* from rat. In four separate experiments the number of trophozoites per 1000X microscopic field increased in cell cultures over 7 days with either mouse or rat *P. carinii*. Trimethoprim/sulfamethoxazole at 50/250 μg/ml inhibited proliferation of *P. carinii* in culture from both hosts: at day 7 in a typical experiment, the numbers of *P. carinii* per 1000X microscopic fields were 1.6 ± 0.3 for mouse and 2.9 ± 0.1 for rat. The ability to use mouse *P. carinii* in short-term culture expands research capabilities both in study of effects of drugs and study of inherent strain differences.

600 A BACTERIOPHAGE P1 GENOMIC LIBRARY OF *PNEUMOCYSTIS CARINII*. CONSTRUCTION AND CHARACTERIZATION. Metcheva IS*, and Buck GA. Medical College of Virginia, Virginia Commonwealth University, Richmond,VA.

Pneumocystis carinii is an important opportunistic pathogen in immunocompromised host. It causes a severe pneumonia that is a leading cause for morbidity and mortality among AIDS patients. A few genes of *P. carinii* have been cloned and analyzed due to the lack of reliable system for *in vitro* cultivation, and the failure of this organism to grow in cell culture is a major obstacle for development of new drugs against *P. carinii* pneumonia. Using the bacteriophage P1 cloning system we have constructed high molecular weight genomic library from rat derived *P. carinii*. The library consists of approximately 4800 clones with an average size of about 55 kb arrayed in fifty 96-well microtiter plates. Screening by colony hybridization for several unique *P. carinii* genes detected on average 3 to 4 positive clones for each. The stability of number of P1 clones was examined by comparing the restriction pattern after repeated subculturing. Riboprobes from the insert ends of several clones were hybridized to Southern blots of Pulsed-field gel separated *P. carinii* chromosomes and no chimerism was detected. Thus, we have constructed a stable, large insert arrayed *P. carinii* genomic library, representing 3 to 4 fold coverage of ~10 mb *P. carinii* genome. This library will provide ready access to genetic material from this important pathogen and will be a valuable tool in physical mapping and characterization of the *P. carinii* genome.

601 HUMAN BABESIOSIS IN TAIWAN: FIRST CASE OF BABESIAL INFECTION IN A NATIVE TAIWANESE. Shih CM*, and Chung WC. Department of Parasitology and Tropical Medicine, National Defense Medical Center, Taipei, Taiwan, R. O. C.; and Department of Parasitology, Taipei Medical College, Taipei, Taiwan, R. O. C.

We reported here a case of human babesial infection in Taiwan. The patient was a 51-year-old Taiwanese woman who had lived in the Southwestern Taiwan and not traveled abroad during the past ten years. The chief complaints were tiredness, dull pain and fullness in the upper abdomen, and occasionally mild fever with chill during the last nine months. She had been diagnosed as gallstone with slightly enlargement of spleen by the local hospital and transferred to the Medical Center one month thereafter. Babesial parasites were observed in the red blood cells of Giemsa-stained thin smears. Hamster inoculation and serologic tests for various babesial parasites were performed to amplify the infection and identify the strain. The patient was treated with quinine sulfate and clindamycin for ten days. The source of infection as well as the vector responsible for the transmission need to be further investigated.

602 CYCLOSPORIASIS IN ONTARIO - A LABORATORY REVIEW AND ESTIMATE OF PREVALENCE, AND CLINICAL DESCRIPTION OF CASES. Keystone JS*, MacPherson DW, and Palmer J. Tropical Disease Unit, The Toronto Hospital (General Division), University of Toronto, Toronto, ON; Regional Parasitology Lab, St. Joseph's Hospital, McMaster University, Hamilton, ON; and Technical Services, MDS Laboratories, Etobicoke, ON.

The objective of this study was to survey three parasitology laboratories to estimate the prevalence of *Cyclospora cayetanensis* infection in Ontario, and to describe the clinical presentation of these cases. A review of laboratory records for the period June 1993 to February 1994, and chart review of available cases was conducted. 39 individuals were diagnosed by these three laboratories in this time period. Two patient specimens had been referred from outside Ontario (one each from Saskatchewan and Quebec). An estimated prevalence was calculated to be: 0.5 cases per 1000 stool examinations or 0.25 per 1000 patients examined. Nine case reports were reviewed: 6 female:3 males, average age 36 years. One female with no travel history outside of Ontario. The others had travelled: Mexico (3), and El Salvador, Columbia, Dominican Republic, India and Korea (one each). The average duration of illness was 32 days (range 8-60 days). One patient was treated with SMX/TMP and resolved completely in 8 days with clearance of the parasite from his stool. Cyclosporiasis is a rare enteric finding in Ontario. It was usually found in travellers. One patient had not travelled and one patient may have responded to treatment with SMX/TMP.

603 IMPROVED RECOVERY OF *CRYPTOSPORIDIUM* OOCYSTS FROM DRINKING WATER USING VORTEX-FLOW FILTRATION COMBINED WITH IMMUNOMAGNETIC SEPARATION. Fricker CR, Turner NB, Rolchigo PM, Margolin AB, and Crabb JH*. Thames Water Utilities, Reading, UK; ImmuCell Corporation, Portland, ME; Membrex Incorporated, Fairfield, NJ; Department of Microbiology, University of New Hampshire, Durham, NH.

Current methods for the detection of protozoan parasites in water rely on concentration of large volumes (10-400 liters) by filtration, elution from the filtration media, centrifugations, and visualization using epifluorescence microscopy. The most widely used method of concentration (ASTM) employing wound cartridge filters often results in overall recoveries < 10%, and has a high degree of variability. We have combined a vortex-flow ultra/microfiltration (VFF) system together with immunomagnetic separation (IMS) technology to develop a method that optimizes the recovery of *Cryptosporidium* oocysts from a wide variety of water qualities. VFF was optimized by examining throughput, membrane pore size, system configuration and oocyst integrity. Overall oocyst recovery efficiencies were measured using the ASTM detection method, direct enumeration by IFA/contrast microscopy, and flow cytometry. Spike and recovery experiments were performed using VFF on 10-100 liters of varying quality water (1-30 NTU), and resulted in high recoveries of oocysts (approx. 70%). IMS performed on VFF concentrates demonstrated equally high recoveries. Epifluorescence microscopy demonstrated that the combination of VFF/IMS can achieve a 6 log concentration of environmental samples with overall *Cryptosporidium* oocyst recovery efficiencies > 50%. By comparison, the ASTM detection method using the VFF concentrates showed dramatically lower recovery rates. The VFF/IMS method demonstrated recoveries comparable to those seen with flocculation/flow cytometry, with advantages of higher throughput capacity and significantly lower capital costs (compared to cytometry).

604 USE OF ELISA-BASED ASSAYS TO STUDY *CRYPTOSPORIDIUM PARVUM*-HOST CELL INTERACTION *IN VITRO*. Ward HD*, Joe A, Kelley MA, Verdon R, Tzipori S, Pereira ME, and Keusch GT. Division of

Geographic Medicine and Infectious Diseases, Tufts University-New England Medical Center; Division of Infectious Diseases, Tufts University School of Veterinary Medicine.

Cryptosporidium parvum infection is initiated by attachment of excysted sporozoites to host cells followed by invasion and intracellular development. In order to study these initial processes in a quantitative manner, ELISA-based attachment and infection assays were developed and standardized with respect to parasite dose, time of infection and detection system. These assays were compared to assays developed earlier which utilized immunofluorescence and counting of parasites for quantitation. For the attachment assay confluent monolayers of Caco-2A cells grown in 96-well plates were fixed with glutaraldehyde in order to prevent invasion. Sporozoites were added to test wells, incubated for 1 h at 37°C, and the wells washed to remove unbound parasites. Adherent sporozoites were fixed with methanol and quantified using a polyclonal antibody to *C. parvum* and a biotinylated second antibody, followed by an avidin/biotin-alkaline phosphatase detection system. For the infection assay confluent monolayers were incubated with hypochlorite-treated oocysts for 2 hours at 37°C. Wells were washed to remove residual parasites, replaced with fresh medium and incubated for a further 24 or 48 hours. Monolayers were washed and fixed with methanol. Infection was quantified using the same detection system as for the attachment assay. For both assays, in order to determine the effect of various inhibitors on attachment and infection parasites were pre-incubated with the inhibitor for 30-60 min at 37°C prior to addition to Caco-2A cells. In both assays attachment and infection occured in a dose dependent manner. There was good correlation between the results obtained with the ELISA-based assays and those obtained with the immunofluorescence-based assays. These assays are a fast, reproducible and quantitative means of studying *C. parvum*-host cell interaction and should be of use in assessing the effect of various immuno and chemotherapeutic agents on infection *in vitro*.

605 EFFECTS OF GAMMA IRRADIATION ON VIABILITY AND IMMUNOGENICITY OF *CRYPTOSPORIDIUM* OOCYSTS. McLain SD, and Crabb JH*. ImmuCell Corporation, Portland ME.

The increased recognition of the importance of *Cryptosporidium parvum* as a human health threat has led to expanded research and development of novel vaccines and immunotherapeutic approaches to control this parasite. Vaccine studies are commonly conducted with purified oocysts or sporozoites administered in combination with adjuvant(s) as a test preparation or as a control for immunogenicity in the test host. For safety reasons, it is important to use organisms that are known to be non-infectious for vaccination studies. We have studied the effects of gamma irradiation of purified oocysts in regards to immunogenicity, infectivity, and excystation rate (surrogate marker for infectivity). Vaccination of mice with oocysts irradiated at levels up to 1kGy resulted in immunogenicity equivalent to vaccination with viable oocysts, whereas irradiation with 10 kGy abolished immunogenicity. Gamma irradiation of oocysts revealed an interesting phenomenon with respect to excystation rate and infectivity. Firstly, irradiation with 100-1000 Gy (10-100 krad) rendered the oocysts non-infectious (≥ 3 logs inactivation) when tested in a neonatal mouse infectivity assay. Secondly, these levels of irradiation permit up to 30% excystation of the oocysts after 8 weeks. Thus, irradiation uncoupled the oocyst's ability to excyst from the ability to infect. This finding may have implications for the interpretation of "viability testing" results with *Cryptosporidium* spp. detected in drinking water samples.

606 EVALUATION OF COMMERCIAL TEST KITS FOR DETECTION OF *CRYPTOSPORIDIUM* OOCYSTS OF SPECIES OTHER THAN *CRYPTOSPORIDIUM PARVUM*. Graczyk TK*, Cranfield CR, and Fayer R. Department of Molecular Microbiology and Immunology, The Johns Hopkins University, SHPH, Baltimore, MD; Division of Comparative Medicine, The Johns Hopkins University, Baltimore, MD; and Parasite Immunobiology Laboratory, LPSI, ARS, U.S. Department of Agriculture, Beltsville, MD.

Commercial enzyme immunoassay (EIA) ProSpect® and immunofluorescence antibody (IFA) *MERIFLUOR*™ *Cryptosporidium/Giardia* kits were evaluated for detection of *Cryptosporidium* oocyst isolates from camel, guinea pig, chicken, turkey, lizard and snake feces, which represent a low public health risk, and for *C. parvum* oocyst isolates from human and bovine feces which represent a high public health risk. All 9 snake oocyst isolates (*C. serpentis*), 1 of 6 lizard isolates, 2 avian isolates (*C. meleagridis*) from turkeys, 1 *C. wrairi* isolate from guinea pigs, and 2 *C. muris* isolates from a Bactrian camel were positive by IFA; 6 of these 15 isolates were EIA positive. The EIA showed less cross-reactivity with the non-*C. parvum* isolates (29%) than the IFA (71%), and was less sensitive to those isolates (23%) than IFA (58%). A simulated sampling model for high and low public health risk *Cryptosporidium* isolates showed that the low risk isolates may constitute up to 33% of all positive environmental samples by IFA determination, and up to 11% of all EIA positive samples. This study indicates a superiority of both tests for screening of fecal specimens, whereas testing of environmental samples may lead to misidentification of medically important isolates. The results demonstrated that the EIA test kit can more accurately than IFA identify environmental samples containing oocysts pathogenic for humans. The specificity of commercially available diagnostic kits to *C. parvum* should be critically examined for cross-species identification before they are recommended or adopted for use in testing environmental samples.

607 EFFECT OF VITAMIN A AND VITAMIN A DEFICIENCY ON EXPERIMENTAL CRYPTOPORIDIOSIS. Leitch GJ*, He Q, Russell SA, and Rawls RA. Department of Physiology, Morehouse School of Medicine, Atlanta, GA.

Adult athymic nude mice were chronically infected with *Cryptosporidium parvum* and 3 or more months later the animals were placed on a radiation-sterilized control diet (CD) or vitamin A deficient diet (VDD). A greater number of animals on the VDD died within a 50 day observation period. No animal on the CD lost more than 20% of its body weight before dying, while all animals on the VDD lost in excess of 25% of their body weight before death. No weight losses were detected in either uninfected animals or chronically infected animals that did not die regardless of their diet. The weekly i.m. injection of 1 mg all-trans retinol failed to reduce the death rate or prevent the normal weight loss associated with death in animals on the CD. Adult athymic animals maintained on the VDD for 50 days developed parasitosis faster after oral oocyst administration than did animals maintained on the CD as judged by the shedding of fecal oocysts. Adult BALB/c mice were immunosuppressed by the daily s.q. injection of 1.5 mg prednisolone for 4 days with or without the i.m. injection of 1 mg all-trans retinol prior to being infected with *C. parvum* oocysts. Animals receiving the vitamin A shed significantly fewer oocysts over the following 4 days than did animals receiving vehicle control injections. These results are consistent with the vitamin A playing two roles in experimental cryptosporidiosis, one related to its deficiency exacerbating the effect of the malabsorption associated with the infection in the immunodeficient animals, and the other related to its stimulating the immune response in the steroid immunosuppressed animals.

608 *CRYPTOSPORIDIUM PARVUM*IN CHILDREN WITH DIARRHEA IN ZULIA STATE, VENEZUELA. Chacín-Bonilla L*, Bonilla MC, Soto de Torres L, Rios de Cándida Y, Sardiña M, Enmanuels C, Parra AM, and Sánchez-Chávez Y. Instituto de Investigaciones Clínicas, Universidad del Zulia, Maracaibo, Venezuela.

Previous studies of pediatric populations in developing countries have shown a 2-31.5% prevalence of *Cryptosporidium parvum* in diarrheic children. The aim of this study was to know the prevalence of this coccidium in children with diarrhea from Zulia State, Venezuela. Single stool specimens were collected from 310 children with acute diarrheal disease, less than five years of age, admitted to one of three Public Hospitals and from 150 comparable control children without gastrointestinal symptoms who were seen as outpatients. Modified Ziehl-Neelsen carbol-fuschin staining of formalin-preserved stool was used to identify *C. parvum* oocysts. Direct wet mounts, iron-hematoxylin-stain smears and formalin-ether concentrates were examined for the presence of other intestinal parasites. *C. parvum* oocysts were identified in 35 of 310 (11.2%) children with diarrhea andthe coccidium was the single detectable pathogen in only 12 (34.2%) of them. Other pathogenic parasites were present in most of the patients shedding oocysts (21 of 35, 60%). Oocysts were identified in 9 of 150 (6%) non-diarrheic control children. Although this study demonstrates that *C. parvum* may be an important pathogen associated with diarrhea, the data suggest that it may not play a consistently pathogenic role in acute childhood diarrhea in the region and that the proportion of cases of gastroenteritis due to the coccidium might be much less than it is thought.

609 COMPARISON OF THREE STAINING METHODS FOR DETECTING MICROSPORIDIA IN FLUIDS. Didier ES*, Orenstein JM, Aldras AM, Bertucci DB, Rogers LB, and Janney FA. Department of Microbiology, Tulane Regional Primate Research Center, Covington, LA; Department of Pathology, George Washington Univ. Med. Center, Washington, DC; and Department of Pathology, Louisiana State Univ., New Orleans, LA.

Calcofluor white, modified trichrome blue (MTB), and immunofluorescent antibody (IFA) staining methods were evaluated and compared for detecting microsporidia in fluids. Serial ten-fold dilutions of *Encephalitozoon intestinalis* were prepared in TBS or formalinized stool, and stained. The calcofluor stain was most sensitive, required approximately 15 min to perform, but did generate some false positive results due to similarly-staining small yeasts. The MTB stain was nearly as sensitive and allowed for distinction between microsporidia and small yeasts, but required nearly 60 min to complete. The IFA stain with polyclonal antiserum was least sensitive and required 130 min to perform. The lower limit ofdetection was at a concentration of 500 organisms in 10 µl using the calcofluor or MTB stains. Reliability was assayed using 74 TEM-confirmed specimens. All TEM-positive specimens were detected by calcofluor and MTB staining but ten positive specimens were missed by IFA staining. An additional seven specimens were read positive by calcofluor staining, and of those, five also were read positive by MTB staining. The resulting diagnostic paradigm was to screen specimens for microsporidia by calcofluor staining and to confirm the presence of microsporidia by MTB staining. IFA was least sensitive but may become useful for species identification as specific antibodies become available

610 CULTURE AND ANTIGENIC ANALYSIS OF FIVE ISOLATES OF *ENCEPHALITOZOON (SEPTATA) INTESTINALIS* ORIGINATING FROM SPUTUM AND URINE OF 3 AIDS PATIENTS. del Aguila C*, Croppo GP, Moss DM, Leitch GJ, Wallace S, and Visvesvara GS. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; and Department of Physiology, Morehouse School of Medicine, Atlanta, GA.

Five isolates of microsporidia from 3 AIDS patients were cultured on E6 monolayers. TEM revealed that all stages of the microsporidia developed within septated parasitophorous vacuoles characterisitic of *Septata intestinalis* now reclassified as *Encephalitozoon intestinalis*. In the IIF test all 5 isolates reacted with anti- *E. intestinalis* serum and produced a titer of > 1,280. They also reacted with the anti-*E. cuniculi* and anti-*E. hellem* sera and produced a titier of < 200. In the immunoblot all 5 isolates reacted with the anti-*E. intestinalis* serum and produced bands ranging from 190 to 14 kDa. The banding pattern was identical with that of *E. intestinalis* (CDC: V297) strain indicating that these isolates are conspecific with *E. intestinalis*. These isolates reacted minimally with anti-*E. hellem* and anti-*E. cuniculi* sera. The 5 isolates also reacted with sera of the 3 patients and produced bands ranging from 190 to 14 kDa. However, *E. hellem* and *E. cuniculi* antigens also reacted with the 3 patients sera and produced several week bands particularly in the region between 43 and 20 kDa indicating that all species of *Encephalitozoon* share certain cross reacting antigens.

611 RECOVERY OF MICROSPORIDIA IN SAF-FIXED FECAL SAMPLES BY LIGHT MICROSCOPY AND CORRELATION WITH ELECTRON MICROSCOPY. Carter PL*, MacPherson DW, McKenzie RA. Regional Parasitology, St. Joseph's Hospital, Hamilton, Ontario, Canada; Parasitology, St.Joseph's Hospital and Pathology, McMaster University, Hamilton, Ontario, Canada; and Electron Microscopy, McMaster University, Hamilton, Ontario, Canada.

The objective of this study was to examine direct and centrifuged fecal samples to determine the rate of recovery of microsporidia spores from simulated heavy to lightly infected individuals. The results of the light microscopy (LM) were then correlated with Transmission Electron Microscopy (TEM) findings. A confirmed positive SAF-fixed fecal sample containing numerous microsporidia spores was diluted in 10-fold serial dilutions (10^0 to 10^{-6}), and variably centrifuged. Smears were prepared in duplicate and stained by Weber's Modified Trichrome (WMT) and Giemsa (GS) stains. A second aliquot of the material was pretreated with 50% v/v of 10% KOH and similarly prepared. Both sets of smears were randomized and examined blindly by LM at 600X and 1250X magnification. A portion of the dilutions after centrifugation were fixed for TEM. WMT was rated higher than GS as it was easier to interpret, and required less examination time at a lower magnification. The number of positive smears and the quantity of spores detected were significantly higher following pretreatment of sample with KOH. Those specimens fixed and processed by TEM were positive only if the number of spores present was high, and therefore considered less sensitive than LM. Pre treatment of SAF-fixed fecal samples with 10% KOH followed by a 5 minute centrifugation time and staining with WMT provides excellent recovery of microsporidia in the routine Parasitology Laboratory. TEM was determined to be less sensitive than LM where the number of spores were low, but quality of photomicrographs was superior after pretreatment with KOH.

612 IDENTIFICATION OF *NEOSPORA CANINUM* cDNA CLONES EXPRESSING RECOMBINANT ANTIGENS USEFUL FOR THE DIAGNOSIS OF BOVINE NEOSPOROSIS. Lally NC*, Jenkins MC, and Dubey JP. USDA, ARS, Parasite Immunbiology Laboratory, Beltsville, MD; and USDA, ARS, Parasite Biology and Epidemiology Laboratory, Beltsville MD.

Neospora caninum is a recently described apicomplexan parasite first identified causing neurological disease in dogs. *Neospora* parasites are also an important cause of abortion and neonatal morbidity and mortality in cattle. *Neospora* is closely related to the human pathogen *Toxoplasma gondii*, however the complete life cycle and definitive host of *Neospora* are unknown. One of the factors hindering research into the biology and epidemiology of this recently recognized disease is the inadequacy of currently available diagnostic tests. The aim of this project is to develop a sensitive and specific serodiagnostic test for neosporosis using recombinant antigens. A *Neospora caninum* tachyzoite cDNA library was constructed in the expression vector UNI-ZAP XR and screened using serum from experimentally infected cattle. Several immunopositive clones were identified and subclones prepared from these were analysed by western blotting using a panel of nine sera from experimentally infected cattle. None of the clones was recognized by all nine sera, indicating that two or more antigens maybe required for diagnosis of all infected animals. On the basis of lack of reactivity with prebleed sera, and recognition by a higher proportion of the experimental sera, two clones were selected for further study.

613 POLYMERASE CHAIN REACTION FOR THE DETECTION OF *TOXOPLASMA GONDII* IN FORMALIN FIXED, PARAFFIN EMBEDDED TISSUE FROM AN INFECTED CAT. Soleimani Z*, Luinstra K, Mahony JB,

and MacPherson DW. Department of Parasitology, Iran University of Medical Sciences, Tehran, Iran; and Medical Microbiology, St. Joseph's Hospital, McMaster University, Hamilton, Ontario, Canada.

The objective of this study was to extract *Toxoplasma gondii* DNA from formalin fixed, paraffin embedded tissue derived from a histologically proven infected cat, and to compare PCR results to histology. The paraffin was extracted using xylol and alcohol washes. The total DNA was extracted using phenol-chloroform extraction, followed by ethanol-salt precipitation. PCR amplification of the product was done using primers for the B1 gene. PCR was compared to histological sections taken from the same part of the tissue block. Results: DNA could be sucessfully extracted from tissues previously fixed in formalin and embedded in paraffin. *T. gondii* DNA was detected from tissue samples. PCR performed as well or better than histological interpretation of infected tissues from various sites. The apparent increased sensitivity of PCR compared to the "gold standard" of tissue diagnosis for toxoplasmosis will need to be examined to determine its clinical significance and diagnostic utility.

614 EPIDEMIOLOGY OF BRUCELLOSIS AT A UNIVERSITY HOSPITAL IN SOUTH TEXAS, 1985-1995. Dib JC*, Miller D, and Patterson JE. Department of Med. Infectious Diseases, University of Texas Health Science Center at San Antonio, San Antonio TX.

From 1985-1995 a total of 31 laboratory confirmed cases of human brucellosis were reported in our 600 bed university teaching hospital in South Texas. Retrospective chart review of cases was done to determine the epidemiology of brucellosis in our institution. The annual number of cases ranged from 1 in 1986 to 6 in 1991. Distribution by gender was equal. Cases ranged in age from 4 to 41 years with 65% between the ages of 20 and 39 years. *Brucella melitensis* infections accounted 80% of cases. Infections with *B. canis* was identified in 2 patients, *B. abortus* in 2 patients and *B. suis* in 2 patients. Annual incidence rates were higher in Hispanics in each age and gender group. Exposure to cattle, swine or dog was reported for 20% of the cases while ingestion of unpasteurized milk products was reported for 80% of the cases. The major features on presentation, irrespective of the course of the disease, were fever, weakness, headache, nausea and abdominal pain. Splenomegaly was present in 20% of the cases. The hematologic findings were not specific and hepatic dysfunction (by liver enzyme abnormalities) was common. No significant differences in outcome were noted for regimens using doxycycline/gentamicin compared to doxycycline/rifampin. Two pregnant patients received rifampin alone without relapse. There was a long duration of disease(>4 weeks) prior to diagnosis in 70% of the patients. Brucellosis is not an uncommon disease in our geographic area but the index of suspicion for this disease remains low in most patients. A carefully elicited history of risk factors may lead to earlier diagnosis.

615 A NEW MONOCLONAL ANTIBODY AGAINST *TOXOPLASMA GONDII* FOR DIAGNOSTIC USE WITH TISSUE SECTIONS. Sundermann CA*, Estridge BH, Branton M, and Lindsay DS. Department of Zoology & Wildlife Science, Auburn University, AL; Department of Biology, Stillman College, Tuscaloosa, AL; and Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL.

A new monoclonal antibody (MAB) against the RH strain of *Toxoplasma gondii* was characterized and compared with commercially available antibodies for specificity and ease of use with paraffin-embedded tissue. When used with an avidin-biotin horseradish peroxidase system, new MAB 3F5 labeled intracellular tachyzoites and the margin of the parasitophorous vacuole of the RH strain; portions of tissue cysts of the ME-49 strain were also stained. Unlike commercially available antibodies, pretreatment of tissue with enzymes was not necessary for reaction with MAB 3F5. High specificity was demonstrated in that MAB 3F5 did not cross-react with developmental stages of *Caryospora bigenetica, Sarcocystis cruzi, Hammondia hammondi,* or *Neospora caninum;* a commercial antibody didcross-react with *N. caninum.* MAB 3F5 also labeled *T. gondii* RH strain tachyzoites with indirect immunofluorescence microscopy. At the ultrastructural level with the immunogold application, solid and spongy rhoptries were decorated with gold-labeled MAB 3F5. Analysis of western blots of RH tachyzoites revealed that MAB 3F5 reacts with two bands of apparent molecular weights 38 and 82 kD. Reactivity without enzyme pretreatment and lack of cross-reactivity with related apicomplexans make MAB 3F5 useful for precise identification of zoites of *T. gondii* in tissue.

616 INTERACTION OF *ACANTHAMOEBA* SPP. WITH MACROPHAGES. Toney DM, Cabral GA, and Marciano-Cabral F. Department Microbiology & Immunology, VA Commonwealth University, Medical College of VA, Richmond, VA.

Acanthamoeba spp. are opportunistic protozoa which cause granulomatous amoebic encephalitis in immunocompromised individuals including AIDS patients. There is little information concerning the mechanisms of host immunity against *Acanthamoeba*, although macrophages (Mø) have been shown to be important effector cells in defense against amoebae. In the present study the interaction of *Acanthamoeba* spp. with

primary Mø and Mø-like cells was investigated. RAW264.7 Mø-like cells activated with IFN-γ and LPS had no apparent effect on *Acanthamoeba*. Scanning EM demonstrated that amoebae targeted the Mø. Amoebae extended finger-like projections which contacted the Mø and effected cell damage. Also, Acanthamoeba ingested Mø using "food cup" surface structures. The interaction of*Acanthamoeba* with Mø obtained from $B_6C_3F_1$ mice was investigated to establish whether effects on Mø-like cells were operative also on primary Mø. Peritoneal Mø activated by *Proprionibacterium acnes in vivo* effected damage on *Acanthamoeba* based on the observation of numerous surface punctate lesions. In contrast, Mø obtained from mice treated with δ-9-tetrahydrocannabinol (THC), the major immunosuppressive component in marijuana, did not elicit damage toward *Acanthamoeba*. These data suggest that THC immunosuppression in mice correlates with increased susceptibility to *Acanthamoeba* infection and impairment of Mø function.

617 AN H^+-ATPase REGULATES CYTOPLASMIC pH IN PNEUMOCYSTIS TROPHOZOITES. McLaughlin G, Shaw M, Durant P, Bartlett MS, VanDerHeyden N, Moreno SNJ, and Docampo R. Indiana University School of Medicine, Indianapolis, IN; and Univerisy of Illinois, Urbana, IL.

Since cells are very sensitive to concentrations of intracellular ions including hydrogen ions, we have begun to investigate the mechanisms of pH regulation in a lung pathogen, *Pneumocystis carinii*. In eukaryotic cells, internal pH (pHi) is regulated by activiites of several distinct types of extrusion and uptake mechanisms, including: Na/H antiports that exchange external Na+ for internal H+ using the inward sodium gradient; pumps that exchange the weak base HCO^{3-} for Cl^-; and a third type found to date in yeast plasma membranes and intracellularly in some cell types such as macrophages, proton ATPases. Short-term-cultured (3-7 day) rat *P. carinii* trophozoites were loaded with a pH-sensitive fluorescent dye and pHi's were measured through time under acid loads as a function of the ionic composition of the medium, and in the presence of inhibitors. The mean base-line pHi was 7.4 ± 0.1 (n=4 independent experiments). Recovery from acidic pH's persisted without extracellular sodium or potassium, in the absence of HCO_3^-, and the presence of disulfonic stilbenes (inhibitors of Cl^-/HCO_3 exchangers). Acid extrusion was blocked by ATPase inhibitors N-ethylmaleimide, DCCD, DES, and omprazole; the latter acidified cells in a dose-dependent manner. These results are all consistent with an H+ATPase pump like those found in fungi, so we postulate that this type of pump helps to regulate pHi in *P. carinii*.

618 INTESTINAL OPPORTUNISTIC PARASITES AMONG DIFFERENT GROUPS OF IMMUNOCOMPROMISE D HOSTS. Abaza SM*, Makhlouf LM, El-Shewy KA, and El-Moamly AA. Parasitology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

The present study was designed to determine the prevalence of intestinal opportunistic parasites in different groups of immunocompromised hosts: patients suffering from malignancy with or without immuno-suppressive drugs, or with chronic renal failure, or diabetes mellitus, or patients receiving corticosteroid therapy. Examination of stool samples collected from 427 immunocompromised hosts detected intestinal opportunistic parasites in 98 samples, with a prevalence of 23%. Infection with opportunistic parasites was higher in males than in females (32.6% versus 12.9%). Infection with two or more parasites occurred in 16 cases, 11 of which represented infection with *G. lamblia* and *Cryptosporidium*. The group with the highest prevalence were patients receiving corticosteroid therapy (31.7%), followed by patients suffering from renal failure (28.8%), malignancy (25.7%), and diabetic patients (8%). The most prevalent parasite was *G. lamblia* (10.3%, followed by *E. histolytica* (7%), *C. parvum* (6.3%), *Microsporidia* (2.3%) and *Stronglyoides* (0.7%). No cases of *Isospora bello* infection were detected. Associations between the occurrence of intestinal opportunistic parasites and various risk factors will be discussed.

619 A SIMPLE METHOD FOR SCREENING DRUGS AGAINST *CRYPTOSPORIDIUM PARVUM IN VITRO*. Meloni BP*, and Thompson RC. WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary Studies, Murdoch University, Western Australia.

Cryptosporidium is a protozoan parasite with a worldwide distribution. *C. parvum* is the species found infecting a range of domestic and wild animals and humans. The parasite primarily invades the epithelium of the gastrointestinal tract of infected hosts causing asymptomatic infections or an acute or chronic cholera-like diarrheal disease, which can be life-threatening in immunocompromised and neonatal individuals. The serious nature of cryptosporidiosis in high risk groups is compounded by the lack of curative treatment strategies. A major limiting factor in the search for effective anti-cryptosporidial agents has been the difficulty in obtaining oocysts and the lack of a cultivation system for the systematic assessment of potential anti-cryptosporidial chemotherapeutic agents. In this study we used a new born mouse model and a simple purification method for obtaining large quantities of oocysts suitable for *in vitro* cultivation. We have optimized growth of *Cryptosporidium* in a humanileocecal adenocarinoma cell line, HCT8, and using this system have developed a simple growth assay for assessing drug

activity against the parasite. We report the results of over 50 compounds tested against *Cryptosporidium* using this system.

620 DEVELOPMENT OF PCR PRIMERS FOR THE DIAGNOSIS OF *ENTEROCYTOZOON BIENEUSI* INFECTIONS, BASED ON REGION CODING FOR THE SSU-RRNA. Da Silva AJ*, Slemenda SB, Visvesvara GS, Moura H, Schwartz DA, Wilcox CM, and Pieniazek NP. Division of Parasitic Diseases NCID, Centers for Disease Control and Prevention, Atlanta, Georgia; Estate University of Rio de Janeiro, Rio de Janeiro, Brazil; and Grady Memorial Hospital, Emory University School of Medicine, Atlanta, GA.

Microsporidia have been increasingly reported as opportunistic infectious agents in patients with acquired immunodeficiency syndrome (AIDS). Among them, *Enterocytozoon bieneusi* is the most common agent of chronic diarrhea, with prevalence estimated between 7% and 50%. This wide range may be due to the lack of reliable tools for diagnosis of infections caused by this microorganism. We have developed specific PCR primers for identification of *E. bieneusi* in clinical specimens. The diagnostic pair of primers was based on the SSU-rRNA coding region, cloned and sequenced from an *E. bieneusi* isolate. Using these primers, we could specifically amplify a fragment of 607 base pairs from clinical samples, as well as from an *E. bieneusi* short-term cultured sample. No cross amplification was observed when these primers were tested on templates of other known microsporidia species. These primers can be reliably used for the diagnostic of *E. bieneusi* infection and useful tools to ascertain its prevalence.

621 THE USE OF RAPD ANALYSIS IN THE DEVELOPMENT OF DIAGNOSTIC PCR PRIMERS FOR *CRYPTOSPORIDIUM*. Morgan UM*, O'Brien PA, and Thompson RC. School of Veterinary Studies and Biotechnology Programme, Murdoch University, Western Australia.

Diagnostic PCR primers were developed for *Cryptosporidium parvum* using the random amplified polymorphic DNA (RAPD) assay. The primers produced were highly specific for *Cryptosporidium* and could detect as little as one oocyst. The primers were also used to reproducibly amplify *Cryptosporidium* directly from faeces without any prior purification. Over 38 different isolates of *C. parvum* of both bovine and human origin from diverse geographic locations were screened. All produced the desired amplification product, indicating that the sequence is conserved amongst isolates. The RAPD primers described here could be used both in the diagnosis of *C. parvum* from faecal samples and also in environmental monitoring. Given the inadequacies of current diagnostic methods for this parasite and the increasing importance of environmental and clinical monitoring of *Cryptosporidium*, particularly amongst AIDS patients, A PCR-based assay should greatly improve the detection and diagnosis of *Cryptosporidium* and the clinical management of the disease in the future.

622 ASSESSMENT OF ANTI-*CRYPTOSPORIDIUM* ANTIBODIES IN VOLUNTEERS BEFORE AND AFTER EXPERIMENTAL EXPOSURE TO OOCYSTS. Moss DM*, Lammie PJ, Hightower AW, Arrowood MJ, Chappell CL, and DuPont HL. Parasitic Diseases Division, Centers for Disease Control and Prevention, Atlanta, GA; and Center for Infectious Diseases, School of Public Health, University of Texas at Houston, Houston, TX.

Understanding the evolution of anti-*Cryptosporidium* antibodies is critical for the evaluation of cross-sectional serologic data. We performed immunoblots on serum samples from volunteers receiving oocysts and monitored changes in antibody reactivity to the 27- (IgM, IgG), 17-(IgG, IgA), and 15-kDa (IgG) diagnostic antigen groups. Changes in reactivity to at least two of these groups were detected for 13/18 oocyst-positive volunteers and 5/11 oocyst-negative volunteers. All five oocyst-positive volunteers who had no change in antibody reactivity demonstrated reactivity at day 0 post inoculation (PI) to at least two antigen groups. When data from all volunteers were analyzed, the presence of antibody at day 0 PI was not significantly related to the magnitude of the net response nor was the net response associated with the dose. The oocyst-positive volunteers who developed symptoms generally mounted the greatest antibody responses but this was statistically significant onlyfor IgM to the 27-kDa antigens ($P = 0.023$). Rates of increase in antibody reactivity were not significantly influenced by infection outcome or by pre-existing antibody and were similar for all three isotypes. In contrast, IgM and IgA antibodies were cleared more rapidly than IgG. Although immunoblot-based surveys should provide information on prevalence and incidence of infection, further studies are needed to understand factors which influence the development and persistence of anti-*C. parvum* antibodies.

623 MUCOSAL IMMUNOTHERAPY OF CRYPTOSPORIDIOSIS USING IgA MABS TO NEUTRALIZING ANTIGENS. Enriquez FJ*, Riggs MW, Palting J, and Hensel J. Department of Veterinary Science, University of Arizona, Tucson, AZ.

Dimeric IgA monoclonal antibodies (MAbs) were developed to previously defined neutralizing antigens of *Cryptosporidium parvum* sporozoites. Mice weresubjected to different intestinal mucosal immunization protocols including oral, intraperitoneal, intraluminal, and surgical intra-Peyer's patches,using excysted sporozoites and purified neutralizing sporozoite antigens (GP23, GP25-200, and CPS-500). Batteries of IgA MAbs generated showeddifferent patterns of reactivity to sporozoites and merozoites by immunofluorescence assay. When introduced parenterally (i.p., i.v., or back-pack tumors) in BALB/c, nude, or SCID mice, the IgA MAbs were foundin significant concentrations in bile and in intestinal mucus. Protective studies of intestinal and chronic hepatobiliary cryptosporidiosis *in vivo* using immunodeficient mouse models will be presented and discussed. When compared to non-specific IgA MAb-treated groups, defined anti-sporozoite mucosal IgA showed variable levels of efficacy against infection. We conclude that IgA may have a functional role during cryptosporidiosis and that IgA immunotherapy of intestinal and extraintestinal cryptosporidiosis might be a feasible therapeutic avenue in the future.

624 IMMUNOLOCALIZATION AND CHARACTERIZATION OF A *CRYPTOSPORIDIUM PARVUM* SECRETORY GLYCOPROTEIN ASSOCIATED WITH SPOROZOITE. El-Shewy KA*, Kilani RT, Sherburne R, and Wenman WM. Departments of Pediatrics and Medical Microbiology, Division of Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada.

Infection with the protozoan parasite *Cryptosporidium parvum* causes important disease in humans and calves for which there is no effective treatment or prevention. We have previously identified a 20 kDa copro-antigen associated with *C. parvum* infection in both humans and calves. In the present study we have used immunoelectron microscopy and a monospecific antibody directed against this 20 kDa copro-antigen to localize it on the parasite. Gold labeling appeared as an anterior extrusion of excysted and released sporozites, as well as at the apical end and trilaminar pellicle. Nonexcysted or excysted non-releasedsporozoites did not label. affinity blotting, with biotinylated jacalin, demonstrated the O-linked oligosaccharide moiety of this glycoprotein. The potential role of this protein in the host cell invasion and/or gliding motility remains unelucidated. However, its location and secretory nature suggest that it may be a target for neutralization or inhibition of invasion of *Cryptosporidium.*

AUTHOR INDEX

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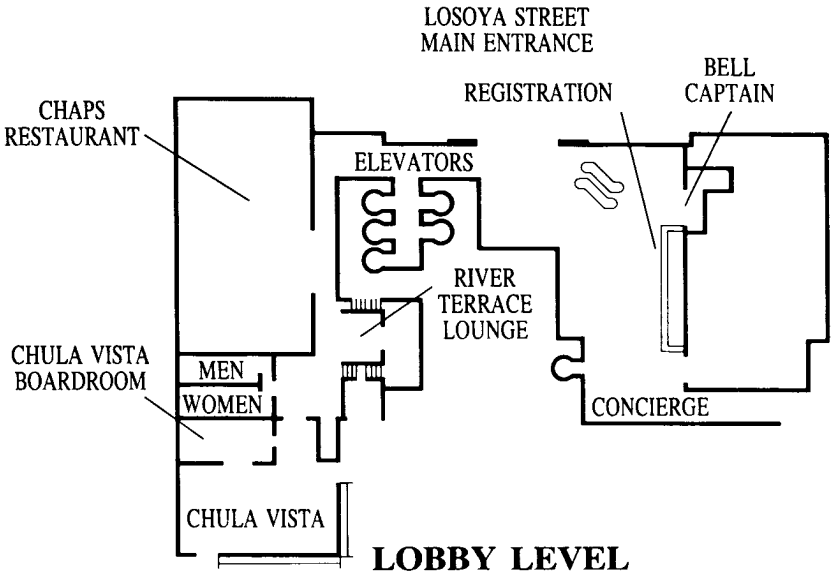

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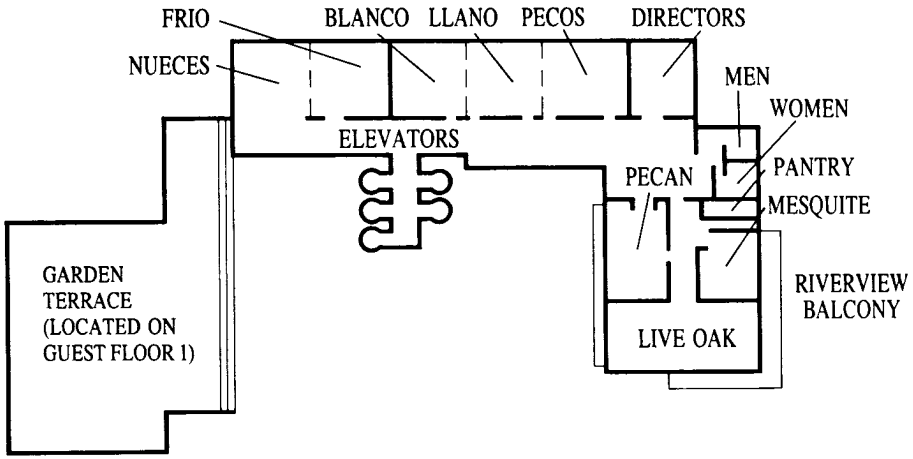

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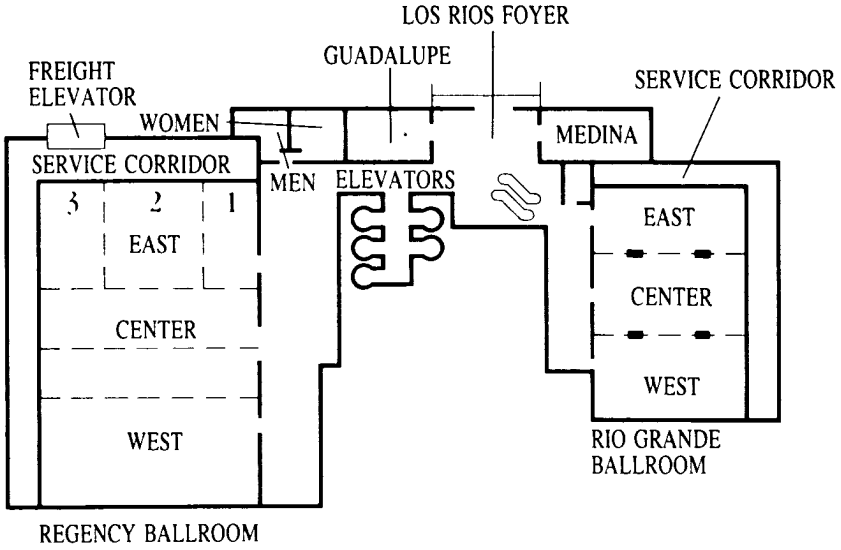

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