(2)

AD_____

ARMY PROJECT ORDER NO: 91PP1801

TITLE: MAINTENANCE SUPPORT OF A FIELD STATION IN SIERRA LEONE, WEST AFRICA

PRINCIPAL INVESTIGATOR: Brian W. J. Mahy, Ph.D.

CONTRACTING ORGANIZATION: Centers for Disease Control
Department of Health & Human Services
Atlanta, GA 30333

REPORT DATE: May 1, 1992

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

REPORT DOCUMENTATION

Public reporting burden for this collection of information is estimated to average ... hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY

May 1, 1992 Final Report (6/14/91 - 4/14/92)

4. TITLE AND SUBTITLE

Maintenance Support of a Field Station in Sierra Leone, West Africa

Army Project Order No. 91PP1801

6. AUTHOR(S)

Brian W.J. Mahy, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Centers for Disease Control
Department of Health & Human Services
Atlanta, GA 30333

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick
Frederick, Maryland 21702-5012

11. SUPPLEMENTARY NOTES

12a DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. ABSTRACT (Maximum 200 words)

The Lassa Fever Research Project has been operating in Sierra Leone, West Africa, since 1976 as a collaborative project of the Sierra Leone Ministry of Health, Centers for Disease Control, and the United States Army Medical R&D Command, USAMRIID. The Project has studied Lassa fever and the epidemiology of the virus in the Eastern Province with study sites in three locations. During this funding period, two Hospitals have been the focus of clinical activity, Nixon Memorial Hospital in Segbwema and later, Panguma Catholic Hospital in Panguma.

14. SUBJECT TERMS

BD; RA I; Lab animals; volunteers; project order

17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT

Unclassified | Unclassified | Unclassified | Unlimited

FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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 Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Final Report

Memorandum of Understanding
(Army Project Order 91PP1801)

MAINTENANCE SUPPORT OF A FIELD STATION
IN SIERRA LEONE, WEST AFRICA
FOR LASSA FEVER INVESTIGATIONS

Field Station Status Update: The Lassa Fever Research Project has been operating in Sierra Leone, West Africa since 1976 as a collaborative project of the Sierra Leone Ministry of Health, Centers for Disease Control, and the United States Army Medical R&D Command, USAMRIID. The Project has studied Lassa fever and the epidemiology of the virus in the Eastern Province with study sites in three locations. During this funding period, two Hospitals have been the focus of clinical activity, Nixon Memorial Hospital in Segbwema and later, Panguma Catholic Hospital in Panguma.

In April 1991, the Liberian rebels invaded the Eastern Province and caused the evacuation of the research team from the main study site in Segbwema to Panguma. The work in Panguma, which had been shut down due to decreased funding levels, was reinstated and work continued despite rebel activity in the area and the displacement of the local population. The upheaval had a significant negative impact on patient numbers. A sizable influx of refugees from Liberia's ongoing war has also affected the area.

Funding from this grant has supported the Sierra Leone Field Station with laboratory supplies and personnel during the FY91 funding period. In addition to maintenance, the grant was used to purchase specific equipment items needed to provide complete clinical laboratory diagnostic capability for a second hospital located in Panguma at the center of the Lassa endemic area. As stated above this hospital now serves as the major center of project activity. The grant also purchased a Toyota Landcruiser 4x4 which will provide the project with reliable transportation for several years into the future.

The Project has been unable to recruit a Clinical Director during this period due to the uncertainty of project funding and the internal upheaval. This has severely limited the scientific progress during the funding year. The following is a report of the current status of the ongoing research:

1 RESEARCH ON CLINICAL FEATURES, IMMUNE RESPONSES, AND PATHOPHYSIOLOGY:

From April 11, 1991 to February 25, 1992, a total of 96 patients were studied with respect to clinical and laboratory parameters. Of the 96, 47 were confirmed as Lassa fever by seroconversion to immunofluorescent antibody, 24 were suspect Lassa or unknown, and 25 were designated non-Lassa. Clinical signs and symptoms were recorded daily on the patient chart. Laboratory specimens were collected on admission, at 24, 48, and 72 hours, then on day 5, 7, 10, 12, 14, 18 during hospitalization, and on

discharge. Tests include Lassa virus antibody titer, hemoglobin/hematocrit, white blood count, platelet counts, SGOT, and urinalysis (on admission only). (See Specimen collection protocol, Attachment 1) Follow-up specimens were collected two weeks to one month after discharge for antibody titer.

See attached spread sheet and codes (Attachment 2) for specimen dates, type and antibody titer results. These specimens (which arrived at CDC March 17, 1992) have not yet been confirmed by virus isolation or processed for other viral agents.

Peripheral blood leukocytes were not collected due to the disruption and the lack of a clinical director. Facilities have been established to carry out this collection.

2 RESEARCH ON PERSON TO PERSON AND HOUSEHOLD TRANSMISSION:

It was impossible to execute this aspect of the research due to the lack of an on-site medical director.

3 RESEARCH ON IMPROVED METHODS FOR EARLY RAPID DIAGNOSIS:

a. Polymerase chain reaction

The attached manuscript abstract (Attachment 3) describes progress made to date on this aspect of the project. In addition, we have now examined the samples described in the PCR manuscript that were culture positive and PCR negative. Cultured virus from these patients yielded PCR products with the primers used in the study, suggesting that the original failure to detect viral RNA was due to the extraction of the sera or some factor other than a marked difference in genome sequence.

b. Antigen Capture ELISA:

Preparation of reagents for the antigen detection ELISA are underway. Monoclonal antibodies were prepared under a previous contract and have been screened for capture ability. Polyclonal mouse hyperimmune ascitic fluid is nearing completion. Rabbit hyperimmune is awaiting delivery of rabbits for immunization. We have made antigens for the immunization of both species. Lassa virus (Josiah) has been grown in suckling mouse brains for immunization of mice, and also in a rabbit cell line (RK13) with rabbit serum in the maintenance medium. All of the arenaviruses attempted so far have grown in RK13 cells.

Once reagent preparation is completed, variations of capture and detection antibodies will be evaluated with killed tissue culture materials, and the best combination chosen for trials on clinical materials. Longer term goals would include the labeling of the detection antibodies to reduce the number of steps necessary to complete the assay.

c. Serological Techniques:

An IgG ELISA, based on preparing detergent extracts of Lassa (Josiah) infected vero cells has proven to be useful for detection IgG in patients who had been infected some time ago. We tested the sera of one of the 1st documented cases of Lassa fever, and found that he was still clearly positive (OD values ~1.0 @ 1:100 dilution of serum) and the same is true of another serum from a Peace Corps volunteer with documented infections more than 10 years ago. These same persons had IgG IFA titers of 1:8 and 1:32.

However, in looking at acute (admission) and convalescent (discharge) sera taken from patients with Lassa fever, the acquisition of IgG is more delayed than the IFA test. In fact, a number of sera were not yet positive by the IgG ELISA by the time they were released from the hospital. It is worth noting, however, that the IFA IgG test is not as rapid in rising in this viral infection as it is in many others. The relatively late IgG ELISA response is a definite disadvantage for acute diagnosis using IgG test results (rising titers in serial sera). However, when combined with an IgM ELISA, may actually offer increased discrimination of very acute cases from previous infections.

The assembly of an IgM capture ELISA test is also in progress but awaiting the completion of some of the polyclonal hyperimmune sera mentioned above. It is hoped that this assay will have the same advantage over the IFA IgM test that we have observed in other viral hemorrhagic fevers and that the combination of the antigen detection ELISA (and/or PCR), IgM-capture ELISA will allow improved acute laboratory diagnosis and that the IgG ELISA will allow improved efficacy in survey use.

4 DIAGNOSIS OF OTHER ETIOLOGIES OF LASSA FEVER LIKE ILLNESS IN SIERRA LEONE:

a. Exclusion of other viral hemorrhagic fevers

All specimens which are not confirmed Lassa by virus isolation (or seroconversion) will be tested at CDC for other hemorrhagic fevers, CCHF, LCM, Rift Valley fever, Ebola, and yellow fever by florescent antibody technique and positives will be confirmed by virus isolation when possible.

Table 1 below outlines the testing and techniques which will be utilized on samples from all patients screened for Lassa fever and found negative:

Table 1: LASSA PATIENT SPECIMEN TESTING

Agent	Virus Isolation	Cells	Supernatant	Serology
Lassa	+	IFA	Antigen Detection	IFA Elisa (G/M)
Rift Valley Fever	+	IFA	Antigen Detection	Elisa (G/M)
Congo Crimean HF	+	IFA		Elisa (G/M)
Hantaan	0	IFA		Elisa (G/M)
Filovirus	+	IFA	Antigen Detection	Elisa (G/M)
Yellow fever	+	IFA	Antigen Detection	Elisa (G/M)
Rickettsia	0		PCR?	IFA
Hepatitis	0			Elisa ?
Typhoid				patient chart
Malaria				patient chart

b. Rickettsial and leptospirosis serology

The CDC Rickettsial diagnostics section will perform rickettsial serology on paired sera from patients identified as non-Lassa.

Specimens are available for Leptospirosis testing and will be made available on request to USAMRDC.

c. Screening for arbovirus antibodies

Aliquots of non-Lassa sera are available to USAMRDC to perform serology for the detection of arboviral antibodies as foreseen in the original proposal.

d. Isolation of identified non-Lassa agents

Acute sera are available for isolation attempts when USAMRDC requests aliquots.

5 REDEFINITION OF EPIDEMIC CHARACTERISTICS OF LASSA VIRUS INFECTIONS IN SIERRA LEONE:

a. Prospective survey of village populations

This study will be implemented when an on site clinical director is identified and in place in Sierra Leone.

b. Epidemiology in other regions

Guinea:

The attached draft manuscript (Attachment 4) by Mr. Austin Demby describes the seroprevalence study conducted in 1980 by the Lassa Fever Research Project in collaboration with the Government of Guinea Ministry of Health. The survey found most evidence of Lassa virus in forest and savannah regions, most particularly along the north east border of Sierra Leone's Eastern Province where Lassa in endemic. One region had a seroprevalence of 29% percent with others ranging from 0 to 14 %.

In February 1992, the Assistant Director for the LFRP returned to Guinea to renew collaboration and determine the status of the ongoing surveillance by the Government of Guinea. Dr. Lukeshevich in collaboration with the Guinea Ministry of Health has just completed a country wide seroprevalence survey which identifies high seroprevalence areas for Lassa which correlate with the 1980 study. This survey utilizes a different antibody test and found much higher levels. Until further work is done, it is impossible to say whether this is due to increase in Lassa virus activity or may be attributable to testing differences.

Liberia:

Specimens collected in Liberia and sent to the Lassa Fever Research Project identify several acute cases in one region of Liberia (previously unstudied for Lassa fever). A total of 25 patients with paired sera were tested, 9 have strong serological evidence of recent infection, 7 provisional positives, 7 with no evidence in the pairs that were provided and 2 with somewhat questionable results. Many of the convalescent sera are drawn relatively early, and there is not sufficient time for antibody to have developed and to declare that these patients are negative for evidence of Lassa infection.

Further investigation of this outbreak is prevented by the current instability of the Liberian government. CDC is in direct contact with World Health Organization and prepared to give assistance as needed.

Other regions of Sierra Leone:

Seroprevalence studies of the Northern and Western Regions of Sierra Leone have been hampered by the political situation, however these surveys are planned for the near future.

c. Rodent survey

Due to the political insecurity of various areas, no live rodent trapping was carried out until January 1992. In January, the field team began live trapping in Segbwema, Eastern Province, a town of approximately 8,000 persons. Segbwema was the major project site until April 1991 when it was evacuated due to the rebel incursions. The abrupt displacement of inhabitants and the influx of Liberian refugees led to some concern about the disturbance of the rodent population and the possible occurrence of a Lassa fever outbreak in the displaced communities.

Sherman and Tomahawk live traps were used to capture rodents in households food storage areas, and nearby vegetation. Each site was trapped for three consecutive days or until rodent capture ceased. Traps were set out in the evening hours baited with dried fish, peanut butter, palm oil and quaker oats. The traps were inspected each morning and fresh traps set at capture sites.

Captured rodents were then transported to the laboratory for identification, numeration and processing. A complete Field Data Sheet was completed on each Mastomys natalensis recording age and sex. From January through March all Mastomys captures were processed for blood, liver, spleen and kidney samples. Liver touch preparations were made and fixed on microscope slides. Specimens are stored in a -20 degree gas freezer in Segbwema, then transferred to Kenema laboratory for testing for Lassa antibody. As of May 1, 1992, non Mastomys rodent species are being completely processed as well.

Table 2: Trapping in Segbwema and Surrounding Villages

Location	No. Of Traps	No. of Rodents Captured	Capture Rate
Segbwema	7,632	604	7.9%
Kormah Village	264	22	8.3%
Moa Barracks (Daru)	528	39	7.4%
Pelewahun Village	253	22	8.7%
TOTAL	8,677	687	Average 7.9%

Table 3: RODENTS CAPTURED BY MONTH AND SPECIES

Month	Ratus	Mastomys	Mus	Crocidura	Total
January	72	102	68	8	250
February	85	37	87	5	214
March	45	95	16	1	157
April	37	45	17	2	101
Total	239	279	188	16	722

Laboratory Test Results

Of the 279 Mastomys samples, testing has been done on 212. 77 of 212 (36%) are IFA Lassa antibody positive tested with IgG antimouse conjugate.

6 FINAL ANALYSIS

The samples collected under the Memorandum of Understanding span almost one year, but clearly cannot be tested in time to meet the deadlines for reporting. This is a consequence of the need to accumulate samples on site in Sierra Leone for secure shipment to Atlanta. A major shipment has just been received at CDC, Atlanta and is being processed. This will result in data on the following:

1. Non-Lassa causes of diseases (Table 1).

2. Use of ELISA IgM and Antigen capture in diagnosis of acute Lassa fever.

3. Infection and antibody data on rodent populations.

Although the time span of the Memorandum of Understanding is now completed, we will finish these analysis and provided the results to USAMRDC as a supplemental report. This report will require 6-9 months for the laboratory testing and data analysis.

ATTACHMENT 1

LASSA FEVER RESEARCH - TREATMENT STUDY - SPECIMEN COLLECTION PROTOCOL

Name FATMATA MANSARAY Acc. No. 87 048125 Hospital Segbwema

Treatment Rx: IV Ribavirin High Dose #302 Plasma Donor No. _____

8/11/89

Date	Admission	Pre RX 7/11/89	24 Hours Post RX	48 Hours 9/11/89	72 Hours 10/11/89	5 Days 12/11/89	7 Days 14/11/89	10 Days 17/11/89	12 Days	14 Days	18 Days	D/C
Day of Illness		12	13	14	15	17	19	22				
Setum sbec. Series		01	02	03	04	05	06	07				
Urine		XXX	XXX	XXX	XXX		XXX	XXX	XXX		XXX	
Throat		XXX	XXX	XXX	XXX		XXX	XXX	XXX		XXX	XXX
HCG		XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX
Ab Titer		<16	<16	<16	<16	<16	64	128				
Hgb/Hct		30%	30%	30%	28%	28%	27%	24%				
WBC		XXX 6250	6250	XXX 2750	7500	5000	XXX 6250	6000	XXX		XXX	
Platelet		256×10^3	275×10^3	XXX 104×10^3	163×10^3	140×10^3	XXX 168×10^3	198×10^3	XXX		XXX	
SGOT		184	227	261	166	87	39	67				
SGPT												
Other Chemistry												
UA Prot (ALB) WBC. RBC Casts HP	Note: D/c 18-11-89 Ribavirin discontinued due to shortage 14/11/89											

CDCP 412

CDC 55.6
10/8L

Code Sheet for Lassa Fever Patient Specimens - Attachment 2

SPECIMEN TYPE COL.20-21	METHOD: COLUMNS 28-29	AGENT: COLUMNS 30-31	RESULTS: COLUMNS 32-33	DIAGNOSIS: COLUMNS 61-62
01 SERUM	VIRUS ISOLATION:	01 UNTYPED ARENAVIRUS	ANTIGEN:	01 - LASSA - CDC
02 URINE	01 VERO CELLS	02 LASSA	01 NO AGENT ISOLATED	02 - LASSA - SL
03 EYE SCRAPING	11 REISOLATION VERO	03 MOZAMBIQUE	02 AGENT ISOLATED, NOT TITRATED	05 - NON-LASSA - CDC
04 C.S.F.		04 LCM	03 AGENT ISOLATED, 0.3 DEX UNITS	06 - NON-LASSA - SL
05 BREAST MILK	ANTIGEN:	05 MACHUPO	04 - 09 AGENT ISOLATED	99 - UNKNOWN - CDC
06 BUFFY COAT	20 IFA - CDC	06 JUNIN	0.4 - 0.9 DEX UNITS/ML	11 - LASSA - PLASMA
07 TEARS	21 IFA - SL	07 TACARIBE	10-19 AGENT ISOLATED,	12 - LASSA - PREG.PLASMA
08 PLEURAL FLUID	22 DIRECT FA - CDC	08 AMAPARI	1.0 - 1.9 DEX UNITS/ML	13 - LASSA - DRUG
09 FETUS	23 DIRECT FA - SL	09 PICHINDE	20-97 AGENT ISOLATED	14 - LASSA - CONTROL
10 SPUTUM	24 - EM FIXED	10 PARANA	2.0 - 9.7 DEX UNITS/ML	15 - NONLASSA - PLASMA
11 CITRATED PLASMA	25 EM NEG. CONTRAST	11 TAMIAMI	98 QNS	16 - NONLASSA - PREG.PLA
12 SERUM FOR FDP		12 LATINO	99 CONTAMINATED	17 - NONLASSA - DRUG
13 URINE FOR FDP	ANTIBODY:	13 BEAN 293022 (BRAZIL)		18 - NONLASSA - CONTROL
14 THROAT SWAB	40 IFA POLYVALENT - CDC	20 CONGO	ANTIBODY:	19 - UNKNOWN - PLASMA
15 NURSING INFANT SERUM	41 IFA POLYVALENT - SL	21 HAZARA	20 TEST NEGATIVE - UNDILUTE	20 - UNKNOWN - PREG.PLA
16 JOINT ASPIRATE	42 IFA - IgG - CDC	25 MARBURG	21 TEST NEGATIVE - 1:2 DILUTION	23 - AB+LASSA - ORAL DRUG
17 CORD BLOOD	43 IFA - IgG - SL	26 EBOLA, ZAIRE	22 TEST NEGATIVE - 1:4 DILUTION	24 - AB+NONLASSA-ORAL DRUG
18 FP BLOOD	44 IFA - IgM - CDC	30 YELLOW FEVER	23 TEST NEGATIVE - 1:8 DILUTION	25 - AB+LASSA - IV DRUG
19 NASAL WASHING	45 IFA - IgM - SL	31 RIFT VALLEY FEVER	24 TEST NEGATIVE - 1:16 DIL	26 - AB+LASSA - COMBINED
20 BONE MARROW	50 CF - CRUDE - CDC	32 EBOLA, SUDAN	25 TEST NEGATIVE - 1:32 DIL	27 - AB+NONLASSA - IV DRUG
21 PLASMA, 1st UNIT	51 CF - CRUDE - SL	40 CHICKUNGUNYA	26 TEST NEGATIVE - 1:64 DIL	28 - AB+NONLASSA - COMBINED
22 PLASMA, 2nd UNIT		50 HERPESVIRUS		29 - WAS 11
23 SEMEN	COAGULATION:	51 ADENOVIRUS	30 TEST POSITIVE UNDILUTE	30 - AB NEG, LASSA - IV DRUG
24 WHOLE BLOOD/CLOT	80 PROTHROMBIN TIME (PT)	99 UNIDENTIFIED	31 TEST POSITIVE 1:2 OR >	31 - AB NEG, LASSA - COMBINE
25 ANTERIOR EYE FLUID	81 PARTIAL THROMB. TIME		32 TEST POSITIVE 1:4 OR >	32 - WAS 15
26 ASCITIC/PERITONEAL FL	82 FIBRINOGEN		33 TEST POSITIVE 1:8 OR >	33 - NONLASSA - IV DRUG
27 VESICLE FLUID	83 FIBRINOGEN, DEGRAD.PR		34 TEST POSITIVE 1:16 OR >	34 - NONLASSA - COMBINED
28 RECTAL SWAB		STORAGE: COLUMNS 22-25	35 TEST POSITIVE 1:32 OR >	35 - UNKNOWN - 2 UNITS PLASM
29 SERUM DRUG CONC. PRE	BIOCHEMISTRY:	BOX NUMBERS	36 TEST POSITIVE 1:64 OR >	36 - UNKNOWN - IV DRUG
30 TREATMENT PLASMA	60 AMYLASE SU/DL	8888 USED UP	37 TEST POSITIVE 1:128 OR >	37 - UNKNOWN - COMBINED
31 SERUM DRUG CONC. 1 HR	61 BUN MG/DL		38 TEST POSITIVE 1:256 OR >	OND=ORAL NORMAL DOSE
32 SERUM DRUG CONC. 24HR	62 CPK MG/DL		39 TEST POSITIVE 1:512 OR >	OHD=ORAL HALF DOSE
33 LIVER TOUCH PREP	63 GLUCOSE MG/DL	COMPARTMENT: COL'S 26-27	40 TEST POSITIVE 1:1024 OR >	IND=IV NORMAL DOSE
34 PLACENTA TOUCH PREP	64 GOT MG/DL	COMPT. NUMBERS	41 TEST POSITIVE 1:2048 OR >	IHD=IV HALF DOSE
	65 GPT MG/DL		42 TEST POSITIVE 1:4096 OR >	38 - AB+ LASSA OND
39 FETUS OTHER				39 - AB+ LASSA OHD
40 FETUS KIDNEY (WHOLE)	70 PRP		50 TEST POSITIVE UNDILUTE	40 - AB+ LASSA IND
41 FETUS LIVER	71 HEPANOSTICA		51 TEST POSITIVE, TITER IS 1:2	41 - AB+ LASSA IHD
42 FETUS MUSCLE			52 TEST POSITIVE, TITER IS 1:4	42 - AB+ NON-LASSA OND
43 FETUS SPLEEN	BIOCHEM. RESULTS →		53 TEST POSITIVE, TITER IS 1:8	43 - AB+ NON-LASSA OHD
44 FETUS BRAIN	USE METHOD + AGENT		54 TEST POSITIVE, TITER IS 1:16	44 - AB+ NON-LASSA IND
45 FETUS LUNG	SPACES (4 DIGITS)		55 TEST POSITIVE, TITER IS 1:32	45 - AB+ NON-LASSA IHD
46 FETUS HEART			56 TEST POSITIVE, TITER IS 1:64	46 - AB+ UNKNOWN OND
47 FETUS PANCREAS			57 TEST POSITIVE, TITER IS 1:128	47 - AB+ UNKNOWN OHD
			58 TEST POSITIVE, TITER IS 1:256	48 - AB+ UNKNOWN IND

MEMORANDUM OF AGREEMENT - FINAL REPORT

ATTACHMENT 2:

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048392	1	1	7	4/16/91	24	63
87048392	2	1	8	4/17/91	24	63
87048392	3	1	9	4/18/91	56	63
87048392	4	1	10	4/19/91	58	63
87048392	5	1	12	4/21/91	40	63
87048392	6	1	14	4/23/91	40	63
87048392	7	1	17	4/26/91	40	63
87048393	1	1	21	4/17/91	•54	41
87048393	2	1	22	4/18/91	57	41
87048393	3	1	23	4/19/91	40	41
87048393	4	1	24	4/20/91	40	41
87048393	5	1	26	4/22/91	40	41
87048393	6	1	28	4/24/91	40	41
87048393	7	1	31	4/27/91	40	41
87048394	1	1	10	4/18/91	24	67
87048394	2	1	11	4/19/91	24	67
87048394	3	1	12	4/20/91	24	67
87048395	1	1	5	4/20/91	24	1
87048395	2	1	6	4/21/91	24	1
87048395	3	1	2	4/22/91	24	1
87048396	1	1	7	4/22/91	24	41
87048396	2	1	8	4/23/91	24	41
87048396	3	1	9	4/24/91	24	41
87048396	4	1	10	4/25/91	24	41
87048396	5	1	12	4/27/91	24	41
87048396	6	1	14	4/29/91	24	41
87048396	7	1	17	5/02/91	24	41
87048396	8	1	44	5/29/91	40	41
87048397	1	1	7	4/23/91	24	56
87048397	2	1	8	4/24/91	24	56
87048397	3	1	9	4/25/91	24	56
87048397	4	1	10	4/26/91	24	56
87048397	5	1	12	4/28/91	24	56
87048397	6	1	14	4/30/91	24	56

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048397	7	1	199	4/11/91	24	56
87048398	1	1	7	4/25/91	24	61
87048398	2	1	8	4/26/91	24	61
87048398	3	1	8	4/27/91	24	61
87048398	4	1	10	4/28/91	24	61
87048398	5	1	12	4/30/91	24	61
87048398	6	1	14	5/02/91	24	61
87048398	7	1	17	5/05/91	24	61
87048399	1	1	9	4/30/91	24	61
87048399	2	1	10	5/01/91	24	61
87048399	3	1	11	5/02/91	24	61
87048399	4	1	12	5/03/91	24	61
87048399	5	1	14	5/05/91	24	61
87048399	6	1	16	5/07/91	24	61
87048400	1	1	7	4/30/91	24	41
87048400	2	1	8	5/01/91	24	41
87048400	3	1	9	5/02/91	24	41
87048400	4	1	10	5/03/91	56	41
87048400	5	1	11	5/04/91	58	41
87048400	6	1	13	5/06/91	40	41
87048400	7	1	15	5/08/91	40	41
87048400	8	1	18	5/11/91	40	41
87048401	1	1	10	4/28/91	24	2
87048401	2	1	11	4/29/91	24	2
87048401	3	1	12	4/30/91	24	2
87048401	4	1	13	5/01/91	57	2
87048401	5	1	15	5/03/91	40	2
87048401	6	1	17	5/05/91	40	2
87048402	1	1	7	5/04/91	24	41
87048402	2	1	8	5/05/91	24	41
87048402	3	1	9	5/06/91	24	41
87048402	4	1	10	5/07/91	24	41
87048402	5	1	12	5/09/91	56	41
87048402	6	1	14	5/11/91	58	41
87048402	7	1	17	5/14/91	40	41

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048403	1	1	3	5/04/91	24	63
87048403	2	1	4	5/05/91	24	63
87048403	3	1	5	6/05/91	24	63
87048403	4	1	6	7/05/91	56	63
87048403	5	1	8	9/05/91	59	63
87048403	6	1	10	5/11/91	40	63
87048404	1	1	10	5/05/91	24	65
87048404	2	1	11	5/05/91	24	65
87048404	3	1	12	5/06/91	24	65
87048404	4	51	13	5/07/91		65
87048404	5	33	13	5/07/91		65
87048405	1	1	5	5/06/91	24	57
87048405	2	1	6	5/07/91	24	57
87048405	3	1	7	8/05/91	24	57
87048405	4	1	8	5/09/91	24	57
87048405	5	1	10	5/11/91	24	57
87048405	6	1	12	5/13/91	24	57
87048405	7	1	15	5/16/91	24	57
87048405	8	1	17	5/28/91	24	57
87048406	1	1	7	5/07/91	54	41
87048406	2	1	8	5/08/91	55	41
87048406	3	1	9	5/09/91	56	41
87048406	4	1	10	5/10/91	57	41
87048406	5	1	12	5/12/91	58	41
87048406	6	1	14	5/14/91	40	41
87048406	7	1	17	5/17/91	40	41
87048407	1	1	7	5/16/91	24	41
87048407	2	1	8	5/17/91	24	41
87048407	3	1	9	5/18/91	24	41
87048407	4	1	10	5/19/91	55	41
87048407	5	1	12	5/21/91	58	41
87048407	6	1	14	5/23/91	40	41
87048407	7	1	17	5/26/91	40	41

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048408	1	1	14	5/26/91	24	41
87048408	2	1	15	5/27/91	24	41
87048408	3	1	16	5/28/91	24	41
87048408	4	1	17	5/29/91	24	41
87048408	5	1	19	5/31/91	56	41
87048408	6	51	20	6/01/91		41
87048408	7	33	20	6/01/91		41
87048409	1	1	10	5/31/91	24	63
87048409	2	51	10	5/31/91		63
87048409	3	33	10	5/31/91		63
87048410	1	1	14	6/01/91	24	57
87048410	2	1	15	6/02/91	24	57
87048410	3	1	16	6/03/91	24	57
87048410	4	1	17	6/04/91	24	57
87048410	5	1	19	6/06/91	24	57
87048410	6	1	21	6/08/91	24	57
87048410	7	1	24	6/11/91	24	57
87048410	8	1	38	6/25/91	24	57
87048411	1	1	5	6/04/91	24	6
87048411	2	51	5	6/04/91		6
87048411	3	33	5	6/04/91		6
87048412	1	1	14	6/07/91	24	70
87048412	2	1	15	6/08/91	24	70
87048412	3	1	16	6/09/91	24	70
87048412	4	1	17	6/10/91	24	70
87048412	5	1	19	6/12/91	24	70
87048412	6	1	21	6/14/91	57	70
87048412	7	1	24	6/17/91	59	70
87048412	8	1	226	1/09/92	40	70
87048413	1	1	8	6/08/91	24	53
87048413	2	51	10	6/10/91		53
87048413	3	33	10	6/10/91		53
87048414	1	1	6	6/12/91	24	63

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048414	2	1	7	6/13/91	24	63
87048414	3	1	8	6/14/91	24	63
87048414	4	1	9	6/15/91	24	63
87048414	5	1	11	6/17/91	24	63
87048414	6	1	13	6/19/91	56	63
87048414	7	1	16	6/22/91	40	63
87048414	8	1	18	6/24/91	40	63
87048415	1	1	14	6/17/91	24	53
87048415	2	1	15	6/18/91	24	53
87048415	3	51	15	6/18/91		53
87048415	4	33	15	6/18/91		53
87048416	1	1	7	6/17/91	24	63
87048416	2	51	8	6/18/91		63
87048416	3	33	8	6/18/91		63
87048417	1	1	10	6/19/91	24	73
87048417	2	51	10	6/19/91		73
87048417	3	33	10	6/19/91		73
87048418	1	1	7	6/19/91	24	57
87048418	2	1	8	6/20/91	24	57
87048418	3	1	9	6/21/91	24	57
87048418	4	1	10	6/22/91	24	57
87048418	5	1	12	6/24/91	24	57
87048418	6	1	14	6/26/91	24	57
87048418	7	1	17	6/29/91	24	57
87048418	8	1	141	11/02/91	24	57
87048419	1	1	7	6/19/91	24	57
87048419	2	1	8	6/20/91	24	57
87048419	3	1	9	6/21/91	24	57
87048419	4	1	10	6/22/91	24	57
87048419	5	1	11	6/23/91	24	57
87048419	6	1	13	6/25/91	24	57
87048419	7	1	15	6/27/91	24	57
87048419	8	1	18	6/30/91	24	57
87048419	9	1	33	7/15/91	24	57

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048420	1	1	10	6/20/91	24	41
87048420	2	1	11	6/21/91	24	41
87048420	3	1	12	6/22/91	54	41
87048420	4	1	13	6/23/91	56	41
87048420	5	51	14	6/24/91		41
87048420	6	71	14	6/24/91		41
87048420	7	33	14	6/24/91		41
87048421	1	1		6/20/91	24	72
87048421	2	1		6/21/91	24	72
87048421	3	1		6/22/91	24	72
87048421	4	1		6/23/91	24	72
87048421	5	1		6/25/91	55	72
87048421	6	1		6/26/91	58	72
87048421	7	1		6/27/91	40	72
87048421	8	1		6/28/91	40	72
87048421	9	1		6/30/91	40	72
87048422	1	1	8	6/22/91	24	65
87048422	2	1	9	6/23/91	24	65
87048422	3	1	11	6/25/91	24	65
87048422	4	1	13	6/27/91	24	65
87048422	5	1	15	6/29/91	24	65
87048422	6	1		11/02/91	24	65
87048423	1	1	7	6/23/91	24	41
87048423	2	1	8	6/24/91	24	41
87048423	3	1	9	6/25/91	24	41
87048423	4	2	10	6/26/91	54	41
87048423	5	1	12	6/28/91	56	41
87048423	6	1	14	6/30/91	57	41
87048423	7	1	17	7/03/91	58	41
87048423	8	1	34	7/20/91	40	41
87048424	1	1	13	7/16/91	54	41
87048424	2	1	14	7/17/91	55	41
87048424	3	1	15	7/18/91	58	41
87048424	4	1	16	7/19/91	58	41
87048424	5	1	18	7/21/91	58	41
87048424	6	1	20	7/23/91	40	41
87048424	7	1	23	7/26/91	40	41

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048425	1	1	14	7/30/91	54	41
87048425	2	1	15	7/31/91	56	41
87048425	3	1	16	8/01/91	59	41
87048425	4	1	17	8/02/91	40	41
87048425	5	1	19	8/04/91	40	41
87048425	6	1	23	8/08/91	40	41
87048426	1	1	10	8/05/91	24	41
87048426	2	1	11	8/06/91	24	41
87048426	3	1	12	8/07/91	24	41
87048426	4	1	13	8/08/91	24	41
87048426	5	1	15	8/10/91	24	41
87048426	6	1	17	8/12/91	24	41
87048426	7	1	20	8/15/91	24	41
87048426	8	1	51	9/16/91	24	41
87048427	1	1	7	8/05/91	24	57
87048427	2	1	8	8/06/91	24	57
87048427	3	1	9	8/07/91	24	57
87048427	4	1	10	8/08/91	24	57
87048427	5	1	12	8/10/91	24	57
87048427	6	1	14	8/12/91	24	57
87048427	7	1	17	8/15/91	24	57
87048428	1	1	4	8/08/91	24	57
87048428	2	1	5	8/09/91	24	57
87048428	3	1	6	8/10/91	24	57
87048428	4	1	7	8/11/91	24	57
87048428	5	1	9	8/13/91	24	57
87048428	6	1	11	8/15/91	24	57
87048428	7	1	14	8/18/91	24	57
87048428	8	1	31	9/04/91	24	57
87048429	1	1	5	8/13/91	24	59
87048429	2	51	5	8/13/91		59
87048429	3	71	5	8/13/91		59
87048429	4	33	5	8/13/91		59

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048430	1	1	6	8/14/91	24	63
87048430	2	1	7	8/15/91	24	63
87048430	3	1	8	8/16/91	24	63
87048430	4	1	9	8/17/91	24	63
87048431	1	1	5	8/19/91	24	65
87048431	2	1	6	8/20/91	24	65
87048431	3	1	7	8/21/91	24	65
87048431	4	1	8	8/22/91	24	65
87048431	5	1	9	8/23/91	24	65
87048431	6	1	10	8/24/91	24	65
87048431	7	1	11	8/25/91	24	65
87048431	8	1	13	8/27/91	24	65
87048431	9	1	15	8/29/91	24	65
87048431	10	1	18	9/01/91	24	65
87048431	11	1	33	9/16/91	24	65
87048432	1	1	14	8/20/91	24	61
87048432	2	1	17	8/23/91	24	61
87048432	3	1	18	8/24/91	24	61
87048432	4	1	19	8/25/91	24	61
87048432	5	1	21	8/27/91	24	61
87048432	6	1	23	8/29/91	24	61
87048432	7	1	25	8/31/91	24	61
87048432	8	1	28	9/03/91	24	61
87048433	1	1	7	8/23/91	24	41
87048433	2	1	12	8/28/91	24	41
87048433	3	1	13	8/29/91	24	41
87048433	4	1	14	8/30/91	24	41
87048433	5	1	15	8/31/91	24	41
87048433	6	1	16	9/01/91	24	41
87048433	7	1	18	9/03/91	24	41
87048433	8	1	20	5/09/91	24	41
87048433	9	1	49	4/11/91	40	41
87048434	1	1	7	9/03/91	24	57
87048434	2	1	8	9/04/91	24	57
87048434	3	51	8	9/04/91		57
87048434	4	71	8	9/04/91		57
87048434	5	33	8	9/04/91		57

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048435	1	1	4	9/24/91	24	57
87048435	2	1	5	9/25/91	24	57
87048435	3	1	6	9/26/91	24	57
87048435	4	1	6	9/27/91	24	57
87048435	5	1	8	9/28/91	24	57
87048435	6	51	9	9/29/91		57
87048435	7	77	9	9/29/91		57
87048435	8	33	9	9/29/91		57
87048436	1	1	14	9/26/91	24	73
87048436	2	1	15	9/27/91	24	73
87048437	1	1	3	9/29/91	24	
87048438	1	1	4	10/08/91	24	6
87048438	2	1	5	10/09/91	24	6
87048438	3	1	6	10/10/91	24	6
87048438	4	1	7	10/11/91	24	6
87048438	5	1	9	10/13/91	24	6
87048438	6	1	11	10/15/91	24	6
87048438	7	1	14	10/18/91	24	6
87048438	8	1	28	11/01/91	24	6
87048439	1	1	3	10/09/91	24	41
87048439	2	1	4	10/10/91	24	41
87048439	3	1	5	10/11/91	24	41
87048439	4	1	6	10/12/91	24	41
87048439	5	1	8	10/14/91	57	41
87048439	6	1	10	10/16/91	58	41
87048439	7	1	13	10/19/91	40	41
87048439	8	1	29	11/04/91	40	41
87048440	1	1	4	10/14/91	24	41
87048440	2	51	5	10/15/91		41
87048440	3	71	5	10/15/91		41
87048440	4	33	5	10/15/91		41

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048441	1	1	12	10/22/91	24	61
87048441	2	1	12	10/23/91	24	61
87048441	3	1	14	10/24/91	24	61
87048441	4	1	15	10/25/91	24	61
87048441	5	1	17	10/27/91	24	61
87048441	6	1	19	10/29/91	24	61
87048441	7	1	22	11/01/91	24	61
87048442	1	1	4	10/23/91	24	61
87048442	2	1	5	10/24/91	24	61
87048442	3	1	6	10/25/91	24	61
87048442	4	1	7	10/26/91	24	61
87048442	5	1	9	10/18/91	24	61
87048442	6	1	11	10/30/91	24	61
87048442	7	1	14	11/02/91	24	61
87048443	1	1	7	10/24/91	24	70
87048443	2	1	8	10/25/91	24	70
87048443	3	1	9	10/26/91	24	70
87048443	4	1	10	10/17/91	24	70
87048443	5	1	12	10/29/91	24	70
87048443	6	1	14	10/31/91	40	70
87048443	7	1	17	11/03/91	40	70
87048444	1	1	14	10/25/91	24	41
87048444	2	1	15	10/26/91	24	41
87048444	3	1	16	10/27/91	24	41
87048444	4	1	17	10/28/91	24	41
87048444	5	1	19	10/30/91	24	41
87048444	6	1	21	11/01/91	56	41
87048444	7	1	24	11/04/91	57	41
87048444	8					
87048445	1	1	6	10/28/91	24	53
87048445	2	51	6	10/28/91		53
87048445	3	71	6	10/28/91		53
87048445	4	33	6	10/28/91		
87048446	1	1	4	10/29/91	24	57
87048446	2	51	4	10/29/91		57

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048446	3	71	4	10/29/91		57
87048446	4	33	4	10/29/91		57
87048447	1	1	7	10/29/91	24	57
87048447	2	1	8	10/30/91	24	57
87048447	3	1	9	10/31/91	24	57
87048447	4	1	10	11/01/91	24	57
87048447	5	1	12	11/03/91	24	57
87048447	6	1	14	11/05/91	24	57
87048447	7	1	17	11/08/91	24	57
87048447	8	1	19	11/10/91	24	57
87048447	9	1	34	11/25/91	24	57
87048448	1	1	7	10/30/91	24	57
87048448	2	1	8	10/31/91	24	57
87048448	3	1	9	11/01/91	24	57
87048448	4	1	10	11/02/91	24	57
87048448	5	1	12	11/04/91	24	57
87048448	6	1	14	11/06/91	24	57
87048448	7	1	17	11/09/91		
87048448	8	1				
87048448	9	1				
87048448	10	1				
87048449	1	1	7	10/30/91	54	41
87048449	2	1	8	10/31/91	56	41
87048449	3	1	9	11/01/91	58	41
87048449	4	1	10	11/02/91	40	41
87048449	5	1	12	11/04/91	40	41
87048449	6	1	14	11/06/91	40	41
87048449	7	1	17	11/09/91	40	41
87048450	1	1	10	10/30/91	24	
87048450	2	1	11	10/31/91	24	
87048450	3	1	12	11/01/91	24	
87048450	4	1	13	11/02/91	24	
87048450	5	1	15	11/04/91	24	
87048451	1	1	7	11/07/91	24	
87048451	2	1	8	11/03/91	24	

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048451	3	1	9	11/04/91	24	
87048452	1	1	12	11/03/91	56	41
87048452	2	1	13	11/04/91	59	41
87048452	3	1	14	11/05/91	40	41
87048452	4	1	15	11/06/91	40	41
87048452	5	1	12	11/08/91	40	41
87048452	6	51	17	11/08/91		41
87048452	7	71	17	11/08/91		41
87048452	8	33	17	11/08/91		41
87048453	1	1	5	11/10/91	24	72
87048453	2	1	6	11/11/91	24	72
87048453	3	1	7	11/12/91	24	72
87048453	4	1	8	11/13/91	24	72
87048453	5	51	9	11/14/91		72
87048453	6	71	9	11/14/91		72
87048453	7	33	9	11/14/91		72
87048454	1	1	14	11/14/91	24	65
87048454	2	1	15	11/15/91	24	65
87048454	3	1	16	11/16/91	24	65
87048454	4	1	17	11/17/91	24	65
87048454	5	1	18	11/18/91	24	65
87048454	6	1	20	11/20/91	24	65
87048454	7	1	22	11/22/91	24	65
87048454	8	1	25	11/25/91	24	65
87048454	9	1	39	12/09/91	24	65
87048455	1	1	15	11/30/91	40	2
87048455	2	1	15	12/01/91	40	2
87048455	3	1	17	12/02/91	40	2
87048455	4	1	18	12/03/91	40	2
87048455	5	1	20	12/05/91	40	2
87048456	1	1	14	12/03/91	40	41
87048456	2	1	15	12/04/91	40	41
87048456	3	1	16	12/05/91	40	41
87048456	4	1	17	12/06/91	40	41
87048456	5	1	19	12/08/91	40	41

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048456	6	1	21	12/10/91	40	41
87048457	1	1	7	12/04/91	24	57
87048457	2	1	8	12/05/91	24	57
87048457	3	1	9	12/06/91	24	57
87048457	4	1	10	12/07/91	24	57
87048457	5	1	12	12/09/91	24	57
87048457	6	1	14	12/11/91	24	57
87048457	7	1	17	12/14/91	24	57
87048457	8	1	32	12/30/91	24	57
87048458	1	1	7	12/15/91	24	63
87048458	2	51	8	12/06/91		63
87048458	3	71	8	12/06/91		63
87048458	4	33	8	12/16/91		63
87048459	1	1	3	12/05/91	24	
87048459	2	1	4	12/06/91	24	
87048459	3	1	5	12/07/91	24	
87048459	4	1	6	12/08/91	24	
87048459	5	1	8	12/10/91	24	
87048459	6	1	10	12/12/91	24	
87048459	7	1			24	
87048460	1	1	7	12/05/91	24	
87048460	2	1	8	12/06/91	24	
87048460	3	1	9	12/07/91	24	
87048460	4	1	10	12/08/91	24	
87048460	5	1	11	12/09/91	24	
87048460	6	1	12	12/10/91	24	
87048460	7	1	14	12/12/91	24	
87048460	8	1	16	12/14/91	24	
87048461	1	1	6	12/09/91	24	41
87048461	2	1	7	12/10/91	24	41
87048461	3	1	8	12/11/91	54	41
87048461	4	1	9	12/12/91	56	41
87048461	5	1	11	12/14/91	57	41
87048461	6	1	13	12/16/91	59	41
87048461	7	1	16	12/19/91	40	41

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048462	1		7	12/09/91	24	57
87048462	2		8	12/10/91	24	57
87048462	3		9	12/11/91	24	57
87048462	4		10	12/12/91		57
87048462	5		10	12/12/91		57
87048462	6		10	12/12/91		57
87048463	1	1	12	12/12/91	24	57
87048463	2	1	13	12/13/91	24	57
87048463	3	1	14	12/14/91	24	57
87048463	4	1	15	12/15/91	24	57
87048463	5	1	17	12/17/91	24	57
87048463	6	1	19	12/19/91	24	57
87048463	7	1	22	12/22/91	24	57
87048463	8	1	37	1/06/92	24	57
87048464	1	1	5	12/16/91	24	
87048464	2	1	6	12/17/91	24	
87048464	3	1	7	12/18/91	24	
87048464	4	1	8	12/19/91	24	
87048464	5	1	10	12/21/91	24	
87048464	6	1	12	12/23/91	24	
87048464	7	1	15	12/26/91	24	
87048465	1	1	10	12/17/91	24	41
87048465	2	1	11	12/18/91	24	41
87048465	3	1	12	12/19/91	24	41
87048465	4	1	13	12/20/91	24	41
87048465	5	1	15	12/22/91	57	41
87048465	6	1	17	12/24/91	58	41
87048465	7	1	20	12/27/91	40	41
87048466	1	1	8	12/19/91	24	41
87048466	2	1	9	12/20/91	24	41
87048466	3	1	10	12/21/91	24	41
87048466	4	1	11	12/22/91	24	41
87048466	5	1	13	12/24/91	56	41
87048466	6	1	15	12/26/91	57	41
87048466	7	1	18	12/29/91	59	41

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048466	8	1	26	1/06/92	40	41
87048467	1	1	15	12/19/91	24	
87048467	2	1	16	12/20/91	24	
87048467	3	1	17	12/21/91	24	
87048467	4	1	18	12/22/91	24	
87048468	1	1	7	12/19/91	24	
87048468	2	1	8	12/20/91	24	
87048468	3	1	9	12/21/91	24	
87048468	4	1	10	12/22/91	24	
87048468	5	1	12	12/24/91	24	
87048468	6	1	14	12/26/91	24	
87048468	7	1	17	12/29/91	24	
87048469	1	1	14	1/06/92	24	57
87048469	2	1	15	1/07/92	24	57
87048469	3	1	16	1/08/92	24	57
87048469	4	1	17	1/09/92	24	57
87048469	5	1	19	1/11/92	24	57
87048469	6	1	21	1/13/92	24	57
87048469	7	1	24	1/16/92	24	57
87048469	8	1	38	1/30/92	24	57
87048470	1	1	8	1/06/92	24	41
87048470	2	1	9	1/02/92	24	41
87048470	3	1	10	1/08/92	24	41
87048470	4	1	11	1/09/92	24	41
87048470	5	1	13	1/11/92	24	41
87048470	6	1	15	1/13/92	56	41
87048470	7	1	18	1/16/92	40	41
87048471	1	1	7	1/16/92	24	2
87048471	2	1	8	1/17/92	24	2
87048471	3	1	9	1/18/92	24	2
87048471	4	1	10	1/19/92	24	2
87048471	5	1	12	1/21/92	56	2
87048471	6	1	14	1/23/92	59	2
87048471	7	1	17	1/26/92	40	2

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048472	1	1	14	1/17/92	24	2
87048472	2	1	15	1/18/92	54	2
87048472	3	1	16	1/19/92	55	2
87048472	4	1	17	1/20/92	57	2
87048472	5	1	19	1/22/92	40	2
87048472	6	1	21	1/24/92	40	2
87048472	7	1	24	1/27/92	40	2
87048473	1	1	10	1/20/92	24	2
87048473	2	1	11	2/21/92	24	2
87048473	3	1	12	2/22/92	24	2
87048473	4	1	13	1/23/92	24	2
87048473	5	1	15	1/25/92	58	2
87048473	6	1	16	1/17/92	40	2
87048474	1	1	7	1/20/92	24	
87048474	2	1	8	1/21/92	24	
87048474	3	1	9	1/11/92	24	
87048474	4	1	10	1/23/92	24	
87048474	5	1	12	1/25/92	24	
87048474	6	1	14	1/27/92	24	
87048475	1	1	6	1/21/92	24	6
87048475	2	1	7	1/22/92	24	6
87048475	3	1	8	1/23/92	24	6
87048475	4	1	9	1/24/92	24	6
87048475	5	1	11	1/26/92	24	6
87048475	6	1	30	2/14/92	24	6
87048476	1	1	5	1/22/92	24	2
87048476	2	1	6	1/23/92	24	2
87048476	3	1	7	1/24/92	55	2
87048476	4	51	8	1/26/92		
87048476	5	71	8	1/26/92		
87048476	6	33	8	1/26/92		
87048477	1	1	7	1/22/92	24	2
87048477	2	1	8	1/23/92	24	2
87048477	3	1	9	1/24/92	24	2
87048477	4	1	10	1/25/92	24	2

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048477	5	1	12	1/27/92	24	2
87048477	6	1	14	1/29/92	56	2
87048477	7	1	17	2/01/92	59	2
87048478	1	1	7	1/22/92	24	
87048478	2	1	8	1/23/92	24	
87048478	3	1	9	1/24/92	24	
87048478	4	1	10	1/25/92	24	
87048478	5	1	12	1/27/92	24	
87048478	6	1	14	1/29/92	24	
87048478	7	1	17	2/01/92	24	
87048478	8	1	26	2/10/92	24	
87048479	1	1	14	1/24/92	24	
87048479	2	1	15	1/25/92	24	
87048479	3	1	16	1/26/92	24	
87048480	1	1	10	1/25/92	40	2
87048480	2	1	11	1/26/92	40	2
87048480	3	1	12	1/27/92	40	2
87048480	4	1	13	1/28/92	40	2
87048480	5	1	15	1/30/92	40	2
87048481	1	1	7	1/28/92	24	2
87048481	2	1	8	1/29/92	24	2
87048481	3	1	9	1/30/92	24	2
87048481	4	1	10	1/31/92	55	2
87048481	5	1	12	2/02/92	58	2
87048481	6	1	15	2/05/92	40	2
87048482	1	1	14	1/29/92	24	2
87048482	2	1	15	1/30/92	24	2
87048482	3	51	16	1/31/92		2
87048482	4	71	16	1/31/92		2
87048482	5	33	16	1/31/92		2
87048483	1	1	14	1/29/92	24	
87048483	2	1	15	1/30/92	24	
87048483	3	1	16	1/31/92	24	

Accession #	Serial #	Spec. Type	Day dis.	Spec. date	Ab titer	Diagnosis
87048483	4	1	17	2/01/92	24	
87048483	5	1	19	2/03/92	24	
87048483	6	1	21	2/05/92	24	
87048483	7	1	24	2/08/92	24	
87048484	1	1	14	2/16/92	57	2
87048484	2	51	14	2/16/92		
87048484	3	71	14	2/16/92		
87048484	4	33	14	2/16/92		
87048485	1	1	5	2/18/92	24	
87048485	2	1	6	2/19/92	24	
87048485	3	1	7	2/20/92	24	
87048485	4	1	8	2/21/92	24	
87048485	5	1	10	2/23/92	24	
87048485	6	1	12	2/25/92	24	
87048486	1	1	7	2/18/92	24	76
87048486	2	1	8	2/19/92	24	76
87048486	3	1	9	2/20/92	24	76
87048486	4	1	10	2/21/92	24	76
87048486	5	1	12	2/23/92	24	76
87048486	6	1	14	2/25/92	24	76
87048487	1	1	7	2/19/92	24	2
87048487	2	1	8	2/20/92	24	2
87048487	3	1	9	2/21/92	24	2
87048487	4	51	9	2/21/92		2
87048487	5	71	9	2/21/92		2
87048487	6	33	9	2/21/92		2

ATTACHMENT 3

EVALUATION OF POLYMERASE CHAIN REACTION FOR DIAGNOSIS OF LASSA FEVER

[1]Sam G. Trappier, [1]Ann L. Conaty, [1]Bertha B. Farrar, [2]Dave D. Auperin, [1]Joseph B. McCormick, [1]Susan P. Fisher-Hoch

[1]Special Pathogens Branch
Division of Viral and Rickettsial Diseases
National Center of Infectious Diseases
Centers For Disease Control
Atlanta, Georgia 30333

[2]Pfizer Central Research
Department of Molecular Genetics and Protein Chemistry
Easton Point Rd.
Groton , CT 06340

Address for reprints: Sam G. Trappier
Division of Viral and Rickettsial Diseases MS G14
Centers for Disease Control
1600 Clifton Rd. NE
Atlanta, Georgia 30333

ABSTRACT

We evaluated a polymerase chain reaction (PCR) procedure for diagnosis of Lassa fever. Serum samples stored over a 14 year period from patients in Sierra Leone, West Africa were examined prospectively and retrospectively. Primers were derived from a region of the S segment of Lassa virus coding for the glycoprotein. Eighty virus-isolation-negative control sera were negative by PCR and hybridization. Thirty-three of 98 patient specimens examined retrospectively were positive for Lassa virus by PCR and virus isolation ($p=0.0006$ [Fisher's exact test]); 42 of 98 were positive when PCR and hybridization was performed ($p<0.0001$ FET). Fifteen were positive by PCR and hybridization but isolation-negative, and 9 were positive by isolation but PCR/hybridization-negative. Thirty-two were negative by all methods. In a prospective study of 195 patient sera, 51 were positive by PCR and virus isolation, and 24 were PCR-positive but virus-isolation-negative ($p<0.0001$ FET). After hybridization, 15 of 26 PCR-negative, virus isolation-positive sera were shown to be positive for Lassa fever, as were 24 of 94 PCR-negative, virus isolation-negative sera. All specimens from patients in whom Lassa fever was excluded by serologic tests were negative. PCR was positive longer in the disease course and was more sensitive than virus isolation. Twenty-two specimens from patients who had serologically confirmed Lassa fever were negative by PCR and hybridization (specificity 78%), as were RNA preparations from tissue culture of some Lassa virus isolates from other countries in West Africa. Lassa virus strain variation may be common even in a restricted geographic area such as eastern Sierra Leone. Though some cases may be missed, PCR

is a reliable, safe, and sensitive tool for the diagnosis of Lassa fever when used in conjunction with classic diagnostic techniques.

Written revised 5/89

ATTACHMENT 4

Prevalence of Lassa Fever in the Republic of Guinea: A cross-sectional survey

Austin Demby MPH , Marylou Solbrig MD.

ABSTRACT

In a 1980 cross-sectional serologic survey of Lassa Fever in the Republic of Guinea, the prevalence of human antibodies to Lassa virus ranged from 0% to 28.8%. Populations for study were selected on the basis of geography, age, sex, and occupation. Savannah and forest region populations had the highest seroprevalence, comparable to neighboring regions in Sierra Leone at the time. Such seroprevalence imply Lassa Fever in Guinea was a public health problem of comparable magnitude to that of other West African countries in 1980.

Introduction

The first descriptions of Lassa Fever were of sporadic outbreaks of hemorrhagic febrile illness with high (40%) mortality between 1969 and 1970 in the Jos plateau of Northern Nigeria. (1,2) The virus isolated from the 1969 cases was a previously undescribed virus belonging to the arenavirus group. (3) Reports of epidemics in rural hospitals in Sierra Leone in 1970 to 1971 (4) and in Liberia in 1972 (5) soon followed.

Rodent to human contacts (6) and nosocomial transmission (2) have both been implicated in Lassa Fever outbreaks. The reservoir of Lassa in Nigeria (7) and Sierra Leone (8) has been identified as a rat Mastomys natalensis. A study of the natural activity of Lassa in Guinea was begun in 1980.

From cross sectional population and hospital serosurveys conducted in 1980, several epidemiologic and ecologic issues were addressed : variability in prevalence of infection according to age, sex, occupation and variability in prevalence of infection in different geographic areas.

Subjects and Methods

Epidemiology Study

We undertook a prevalence survey of antibodies to Lassa virus in samples from two groups : village populations and hospital staff. Village populations to be bled were selected by numbering the

table; then numbering all the local revolutionary powers (PRL) of these 14 regions, and selecting 22 PRLs using a random number table. Within each of the 22 selected PRLs, 10 houses were selected , again by random sampling. If the selected family refused to participate, the next house on the random nunber table was utilized.

Seven hospitals were randomly chosen from all the republic's hospitals. All hospital staff members volunteered for the study and donated venous blood.

Antibody Testing

Serum samples were tested using the indirect immunofluorescent - antibody (IFA) test in our laboratory in Sierra Leone. (9) Cells infected with virus isolated in 1977 from a Sierra Leone patient, inactivated , then immobilized on glass slides, were the source of antigen. We chose a serum IgG antibody titer of >= 1:16 to represent past infection with Lassa virus.

Results

Antibody prevalence in PRLs

We surveyed populations in 22 different PRLs. Coastal, savannah forest and mountain regions were all represented. Antibody prevalences varied from 0 to 3.8 % in coastal PRLs , 0 to 28.8 % in savannah PRLs, 0 to 14.4 % in the forest PRLs and 0.0% in the mountain PRL. See Table 1.

Age-specific prevalence of antibodies in coastal, savannah and forest regions is shown in Table 2. The Prevalence of antibodies increased with age, with higher percentages in the over 20 than under 20 year age group. (chi sq. = 6.9).

Separating the sexes, in Tables 3 and 4, we found the highest prevalence of antibodies in older females (> 20 years) in the forest region. (Table 4) Segregating the participants by occupation, we found the highest antibody prevalence in housewives (but not significant at alpha 0.05 level) Table 5.

Antibody prevalence in hospital personnel

Hospital personnel were treated as a separate group to address the role of nosocomial infection in the spread of Lassa. (Table 6). Antibody prevalence of hospital staff closely matched those of the population within the same region, implying hospital workers were not at increased risk for acquiring Lassa at the time of our study.

Table 1. Distribution of Lassa Fever antibodies by regions in the Republic of Guinea.

Natural Region	Region	PRL No.	Sample Size	No. positive for Lassa antibodies	% positive
Costal	Conakry	23	97	0	0
		24	64	0	0
	Dubreka	3	186	4	3.8
	Forecariah	2	93	3	3.6
			350	7	2
Savannah	Faranah	20	77	21	28.8
		21	59	1	1.7
	Kankan	14	94	1	1.1
		15	100	0	0.0
		16	90	1	1.1
	Kouroussa	17	76	3	8.3
		18	78	0	0.0
		19	103	0	0.0
	Dabala	22	115	5	4.3
			788	32	4.1
Forest	Beyla	5	119	5	4.2
		6	104	5	4.8
		7	100	11	11.0
		8	92	1	1.1
	Kissidougou	13	104	15	14.4
	Macenta	12	100	0	0.0
	Nzerekore	4	109	4	3.7
		11	100	14	14
			828	55	6.6
Mountain	Telemala	1	74	0	0.0
All regions Combined			2040	94	4.6

Table 2. Age distribution of participants with Lassa antibodies in the different regions (both sexes included).

		AGE			
	Test Result	<= 20 years	> 20 years	Total	
Forest	Positive	9 (5.4%)	20 (14.6%)	29	
	Negative	158	117	275	
	Total	167	137	304	9.5% pos.
Savannah	Postive	16 (3.3%)	16 (5.2%)	32	
	Negative	462	294	756	
	Total	478	310	788	4.1% pos.
Coastal	Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	--
	Negative	82	78	160	
	Total	82	78	160	0.0% pos.

Table 3. Age distrubution of participants with Lassa antibodies in the different regions stratifying on sex (males).

		AGE			
	Test Result	<= 20 years	> 20 years	Total	
Forest	Positive	3 (4.9%)	8 (16.3%)	11	
	Negative	58	41	99	
	Total	61	49	110	10% pos.
Savannah	Postive	7 (3.4%)	6 (5.1%)	13	
	Negative	201	112	313	
	Total	208	118	326	4% pos.
Coastal	Positive	0 (0.0%)	0 (0.0%)	0	
	Negative	29	20	49	
	Total	29	20	49	0% pos.

Table 4. Age distrubution of participants with antibodies in the different regions controlling for sex (females).

		AGE					
	Test Result	<= 20 years		> 20 years		Total	
Forest	Positive	6	(5.8%)	12	(13.8%)	18	
	Negative	98		75		173	
	Total	104		87		191	9.4% pos.
Savannah	Postive	8	(3.0%)	9	(4.7%)	17	
	Negative	256		182		438	
	Total	264		191		455	3.7% pos.
Coastal	Positive	0	(0.0%)	0	(0.0%)	0	
	Negative	48		51		91	
	Total	48		51		91	0.0% pos.

Table 5. Distribution of antibody titers by occupation stratified on regions.

	Forest			Savannah			Coastal		
	Sample Size	No. Positive	% positive	Sample Size	No. Positive	% Positive	Sample Size	No. Positive	% Positive
Farmers	31	2	6.5	86	4	4.7	1	0	0
Sheperds	2	0	0	3	1	***33.3	0	0	0
Fishing	0	0	0	1	0	0	0	0	0
Mechanics	69	4	5.8	0	0	0	0	0	0
Pupils	65	4	6.2	161	7	4.3	21	0	0
Housewives	79	13	16.5	194	11	5.5	55	0	0
Miners	0	0	0	0	0	0	0	0	0
Children	77	3	3.9	270	5	1.9	50	0	0
Total	324	26	8.0	754	31	4.1	127	0	0

*** Probably an inflated percentage due to small sample size.

Table 6. Disbtribution of Lassa antibodies among 3 hospitals

Region	Hospital NO.	Sample Size	No. Positive	% Positive
Savannah	No. 34	29	1	3.4
Coastal	No. 35	97	3	3.1
Coastal	No. 36	97	1	1.0

time of our study.

Discussion

We demonstrated antibodies to Lassa virus in a number of PRLs throughout the forest, savannah and coastal regions of Guinea. There was a range of antibody prevalence with none found in the mountain areas, 0 to 3.8% in the coastal areas, intermediate levels to 14.4 % in the forest area, and levels to 28.8 % in 1 savannah PRL. Seroprevalence to Lassa was widely distributed through forest and savannah regions. No clusters with regard to demographic nor economic activities were apparent. Dry savanah ecology is similar to the region in Nigeria where the disease was first described, (1,2) and the tropical secondary forest areas are simillar to neighboring border regions of Sierra Leone where antibody levels up to 52 % have been found.(10) Such observation lends support to the idea that Lassa virus infection occurs in similar ecologic zones all over west Africa.

The low prevalence in coastal regions also matches the Sierra Leone experience. In Sierra Leone, Lassa does not originate in coastal areas. All have been imported cases, who left an interior hyperendemic area within the incubation period (personal observations). We suspect the same may be true in Guinea.

Both rodent-to-human and human-to-human transmissions are known from our studies in Sierra Leone (10). Housewives may have been

at higher risk because of increased contact with rodents through domestic chores or because of their role as primary caretakers for ill family members.

Of interest is the fact that Lassa antibody prevalence of hospital workers in 1980 closely matched those of the general population within the same region. Recent studies in Sierra Leone have shown that masks, gloves and barrier nursing practices have eliminated nosocomial spread of infection and greatly reduced occupational risk (11). Low antibody prevalence in Guinean hospital staff may have resulted either from staff exercising great care in handling patients and observing strict bodily fluid precautions, or resulted from the fact that staff prior to 1980 may have had limited occupational exposure if few Lassa cases may have made it to the hospital.

Four cases of Lassa Fever in Guinea were between 1965 and 1968 at a mission station at Telekoro. 3 adults from this station who had a prolonged, debilitating, febrile illness were diagnosed in retrospect when tested for neutralizing antibodies for Lassa using a Vero cell plaque reduction test. (12) The fourth case was a child who could not provide a history of illness. Important differences between clinical illness at Telekoro and that reported at Jos was the absence of pharyngitis, dysphagia and hemorrhagic manifestations. (12)

We wonder if Lassa is the same clinical illness in Guinea as in other West African regions, and query the relative contributions of rodent-human and human-human transmission in the spread of

infection. Investigation of rodent species in Guinea to determine natural reservoir(s), then attempting virus isolation from the same and from patients, along with a second population prevalence study to asses interval activity, would all clarify the natural history and activity of lassa in Guinea, upon which preventive public health programs could be based.

References

1. Frame JD, Baldwin jr JM, Gocke DJ, Troup JM. Lassa Fever, a new virus disease of man from West Africa I. Clinical description and pathologic findings.
Am. J. Trop. Med. Hyg. 1970; 19: 670-6

2. Troup JM, White WA, Fom ALMO, Carey DE. An outbreak of Lassa Fever on the Jos plateau, Nigeria, in Jan-Feb. 1970 : a preliminary report.
Am. J. Trop Med. Hyg. 1970; 19: 695-6

3. Buckley SM, Casals J, Downs WG. Isolation and antigenic characterization of Lassa virus .
Nature 1970; 227 :174

4. Monath TP, Maher M, Casals J, Kissling RE, Cacciapouti A. Lassa Fever in the Eastern province of Sierra Leone, 1970-1972. II. Clinical observations and virological studies on selected hospital cases.
Am. J. Trop. Med. Hyg. 1974; 23:1140-9

5. Mertens PE, Patton R, Baum JJ, Monath TP.
Clinical presentation of Lassa Fever cases during the hospital epidemic at Zorzor, Liberia, March-April 1972.
Am. J. Trop. Med. Hyg. 1973; 22:780-4

6. Keenlyside RA, McCormick JB, Webb PA, Smith E, Elliott L, Johnson KM. Case-control studies of Mastomys natalensis and humans in Lassa virus infected households in Sierra Leone. Am. J. Trop. Med. Hyg. 1983; 32:829-37.

7. Wulff H, Fabayi A, Monath TP. Recent isolations of Lassa virus fron Nigerian rodents.
Bull WHO 1975 ; 52: 609-13

8. Monath TP, Newhouse VF, Kemp GE, Setzer HW, Cassiapouti A. Lassa virus isolation from Mastomys natalensis rodents during an epedemic in Sierra Leone.
Science 1974; 185: 263-5

9. Johnson KM, Elliott LH, Heymann DL.
Preparation of polyvalent viral immunofluorescent intracellular antigens and use in human serosurveys.
J. Clin Mirobiol 1981; 14: 527-9.

10. McCormick JB, Webb PA, Krebs JW, Johnson KM, Smith ES. A prospective study of the epidemiology and ecology of Lassa Fever. J. Inf. Dis. 1987; 155: 437-444.

11. Helmick CG, Webb PA, Scribner CL, Krebs JW, McCormick JB. No evidence of increased risk of Lassa Fever infection in hospital staff. Lancet 1986 ; 2 :1202-5

12. Henderson BE, Gary GW, Kissling RE, Frame JD, Carey DE. Lassa Fever : Virological and serological studies. Trans. R. Soc. Trop. Med. Hyg. 1972; 66: 409-416.