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WORLD REFERENCE CENTER FOR ARBOVIRUSES

ANNUAL REPORT

ROBERT E. SHOPE

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New viruses were identified and new geography and pathogenicity were recognized for eady known viruses. Kagoshima virus from Japan caused congenital abnormalities in livestock was the same as Kasba virus, previously known from India. Batai virus was found for the st time in Africa and for the first time was recognized as a cause of human disease. A new navirus was isolated from spleen of a fatal human case in Venezuela. In addition, studies e done to revise the taxonomy of alphaviruses. U.S. Army patrol dogs in Korea were shown to infected frequently with Japanese encephalitis virus and acted as sentinels. By molecular hniques it was shown that the dengue-2 viruses in Venezuela and Brazil are very similar to se prevalent in Jamaica in 1981 and probably originated in Viet Nam. Vaccinia recombinant uses with structural JE gene inserts secreted particulate antigens and were highly unogenic. E and prM genes were needed for secretion of the particles. Immunization of 526 unteers with inactivated JE vaccine led to uniform responses of neutralizing antibody. The oratory distributed reagents in 1990 to 28 laboratories in 10 countries.

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TABLES AND FIGURES

FOREWORD

In conducting the research described in this report, the investigators 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 (DHEW Publication No. (NIH) 86-23, Revised 1985).

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46.

INTRODUCTION

The World Reference Center for Arboviruses is supported jointly by the U.S. Department of Defense, the National Institutes of Health, and the World Health Organization. The Center identifies and characterizes suspected arboviruses submitted from U.S. and overseas laboratories, diagnoses disease outbreaks, develops new techniques for rapid diagnosis and for characterization of arboviruses, prepares and distributes reference immune reagents and specific nucleic acid probes, prepares virus stocks for distribution through WHO regional reference centers and the American Type Culture Collection, prepares and distributes antigens on a limited basis, carries out limited serological surveys, and disseminates information through WHO and the American Committee on Arboviruses.

Emphasis has been placed on specific subprojects including molecular epidemiology using primer extension analysis of flavivirus RNA, adaptation of ELISA for field application to arboviruses of human disease importance, use of the extensive WHO reagent bank for characterization of monoclonal antibodies, and engineering of vaccinia vectored flavivirus cDNA for immunization of domestic animals and man, and for diagnostic antigens.

BODY OF REPORT

I. IDENTIFICATION AND CLASSIFICATION OF ARBOVIRUSES

A. Identification of Kagoshima virus from Japan as a strain of Kasba virus (B. Fonseca and R.E. Shope)

Kagoshima virus was isolated from Culicoides oxystoma collected in a cowshed in Kagoshima, Kyushu Island, Japan in November 1984. It was referred for identification by Dr. Y. Inaba, Nihon University, Fujisawa, Japan. It had previously been shown in Japan to be the same as Chuzan virus, an orbivirus causing epizootic hydranencephaly-cerebellar hypoplasia syndrome in calves.

CF tests with grouping ascitic fluids demonstrated a relationship of Kagoshima virus (strain KC-05Y84) to the Palyam group. Plaque reduction neutralization tests were done with eight viruses of the Palyam serogroup. The virus was not neutralized by mouse ascitic fluids to Bunyip Creek, CSIRO village, D'Aguillar, Palyam, Vellore, and Nyabira viruses, but was neutralized by Kasba virus (>1:320) and Marrakai virus (1:10). Cross-neutralization (Table 1) showed that Kagoshima virus was closely related or identical to Kasba virus.

Table 1

Cross-neutralization tests of Kagoshima virus and Kasba virus

Virus	Neutralization test titer of mouse ascitic fluid	
	Kagoshima	Kasba
Kagoshima	1:1280	1:640
Kasba	1:320	1:160

Kasba virus (IG 15534) has been known since 1957 when it was isolated from Culex vishnui mosquitoes in Vellore, South India. It has not been studied as a possible veterinary (or human) pathogen. This finding should lead to a search for disease in India.

B. Identification of Batai virus from febrile humans in Sudan (A. Main and N. Nevine)

Dr. A. Main and Ms. N. Nevine of U.S. Naval Medical Research Unit #3, Cairo, Egypt, during a visit to Yale, identified two strains of an agent isolated from febrile humans in Sudan. The agents were isolated from blood of two febrile patients (KV-66 and KV-141) during October 1988 in Kassala, Sudan. Blood was inoculated intracerebrally into suckling mice at NAMRU-3. Preliminary identification in Cairo indicated that the virus was in the Bunyamwera serogroup.

Plaque reduction neutralization tests at Yale confirmed the relationship to the Bunyamwera serogroup, and showed that the two agents were closely related or identical to Batai virus (Table 2).

All previous isolates of Batai virus were from mosquitoes. This is the first time the virus was encountered in Africa, and the first time the virus was associated with human illness.

Table 2

Plaque reduction neutralization tests comparing SudHKV-66 and SudHKV-141 with other members of the Bunyamwera serogroup

	Antibodies								
Virus	KV-66	KV-141	Batai	Birao	Belief	Ilesha	Bunyamwera	Germiston	Shok
SudHKV-66	640	1280	640	80	40	<10	<10	<10	<10
SudHKV-141	640	1280	1280	180	40	<10	<10	<10	<10
Batai	5120	5120	2560						
Birao	40	40		1280					
Ilesha	<160	<160				80			
Bunyamwera	20	40					>5120		
Germiston	<10	<10						160	
Shokwe	320	>320							160

C. Identification of a new arenavirus from Venezuela (R. Rico-Hesse, R. Tesh, and R. Shope)

During studies of dengue hemorrhagic fever, acute phase sera from patients in Venezuela were tested for virus isolation in order to obtain strains for limited sequence analysis of the RNA. No viruses were isolated from several of these patients, after routine culture of their sera on mosquito cells. However, Dr. Rosalba Salas, National Institute of Hygiene, Caracas, cultured human spleen from two fatal cases and observed transient cytopathic effect in vertebrate cells. These cultures were referred to YARU where an agent was characterized that killed baby mice approximately two weeks after i.c. inoculation. The mice exhibited disequilibrium and convulsions after being spun by the tail, a sign typical of LCM and other arenaviruses.

Infected Vero cells reacted in IFA with sera from several arenaviruses; a sucrose-acetone extracted mouse brain antigen reacted by CF with Amapari, and to a lesser extent with sera of other arenaviruses from Latin America. This preliminary identification indicated that the virus from spleen of the patient from Venezuela was an arenavirus, and that it probably differed serologically from the known arenaviruses of the New World.

D. Identification of SP An 107237 virus, a variety IF VEE virus, and suggested revision of the VEE classification (B. Fonseca and R. Shope)

SP An 107237 virus was referred by the Instituto Adolfo Lutz, Sao Paulo, Brazil for identification. The virus was isolated in 1987 from a sentinel mouse exposed in Iguape County, on the seacoast of Sao Paulo State.

The virus was shown to be an alphavirus by CF test. Additional CF tests showed that it was closely related to VEE virus (Table 3). Plaque reduction neutralization tests were carried out to define better its relationship to the viruses of the VEE complex. The results are shown in Table 4. SP An 107237 was most closely related to VEE variety IF, 78V-3531 previously isolated in Sao Paulo State, and it was also closely related to strain AG80-663, originating in Argentina.

In the process of identifying SP An 107237, it was found by neutralizaiton test that Everglades (subtype II) was more closely related to TC-83 than previously described. If these results are confirmed, then the VEE complex should be revised, placing Everglades as a variety of subtype I, rather than as subtype II. The dendrogram showing the relationships as determined in this study is displayed in Figure 2.

E. Identification of chikungunya and Cache Valley viruses contaminating a veterinary vaccine culture (R. Shope and S. Tirrell)

A commercial firm requested assistance of the Reference Center to identify a putative alphavirus contaminating a culture of feline leukemia virus. The vaccine virus is normally non-cytopathogenic, however the manufacturer noted during quality control procedures that the cultured cells had cytopathogenic effect. Electron microscopy revealed what appeared to be an alphavirus, and the culture killed baby mice after intracerebral inoculation.

Two viruses were identified in the cell culture preparation. These were agents closely related to, or identical with chikungunya virus and Cache Valley virus.

The cell culture preparation, 9 BK 18, was inoculated into Vero cells which showed cytopathic effects in 2 days. An antigen was prepared from the infected cells by washing them in saline, then lysing the cells with detergent. This antigen was used in the ELISA.

The 9 BK 18 cell culture preparation was also inoculated into 3-day old mice intracerebrally. The mice were sick in about 40 hours. The brains of the mice were used as immunogen to immunize adult mice intraperitoneally, and the infected brains were also used to prepare an antigen for CF tests, using the sucrose-acetone technique. The mice that were immunized received two inoculations intraperitoneally, at weekly interval, of infected suckling mouse brain in Freund's complete adjuvant. The mice were bled on day 14 after the first inoculation.

The Vero cell lysate antigen reacted in ELISA with grouping antibodies to alphaviruses and Bunyamwera serogroup viruses, as well as to the homologous mouse antibody. The antigen was negative with 21 other arbovirus grouping fluids. Results of a second ELISA are shown in Table 5.

Table 3

Complement-fixation relationships of SP An 107237 with VEE complex viruses

	Antibody							
Virus	SP An 107237	VEE TC-83	78V-3531	Everglades	Mucambo	Pixuna	Cabassou	AG80-663
SP An 107237	256/512*	512/128	128/128	256/128	8/128	128/512	512/128	256/32
VEE TC-83	32/64	512/256						
78V-3531	256/512		512/512					
Everglades	128/128			256/512				
Mucambo	16/4				16/4			
Pixuna	64/16					256/16		
Cabassou	128/512						512/128	
AG80-663	128/512							512/128

*Reciprocal of serum titer/reciprocal of antigen titer

Table 4

Neutralization test relationships of SP An 107237 with VEE complex viruses

Virus	Antibody							
	VEE TC-83	78V-3531	Everglades	Mucambo	Pixuna	Cabassou	AG80-663	SP An 107237
VEE TC-83	320	160	80	10	10	20	20	160
78V-3531	160	5120	80	10	20	80	320	5120
Everglades	1280	1280	10240	160	20	40	80	2560
Mucambo	40	80	40	320	20	80	80	160
Pixuna	10	10	40	10	10240	40	40	20
Cabassou	<10	10	<10	<10	<10	320	10	10
AG80-663	40	2560	160	80	160	160	10240	1280
SP An 107237	40	10240	160	10	40	160	320	10240

Figure 1.

Antigenic similarity of VEE subtypes and varieties

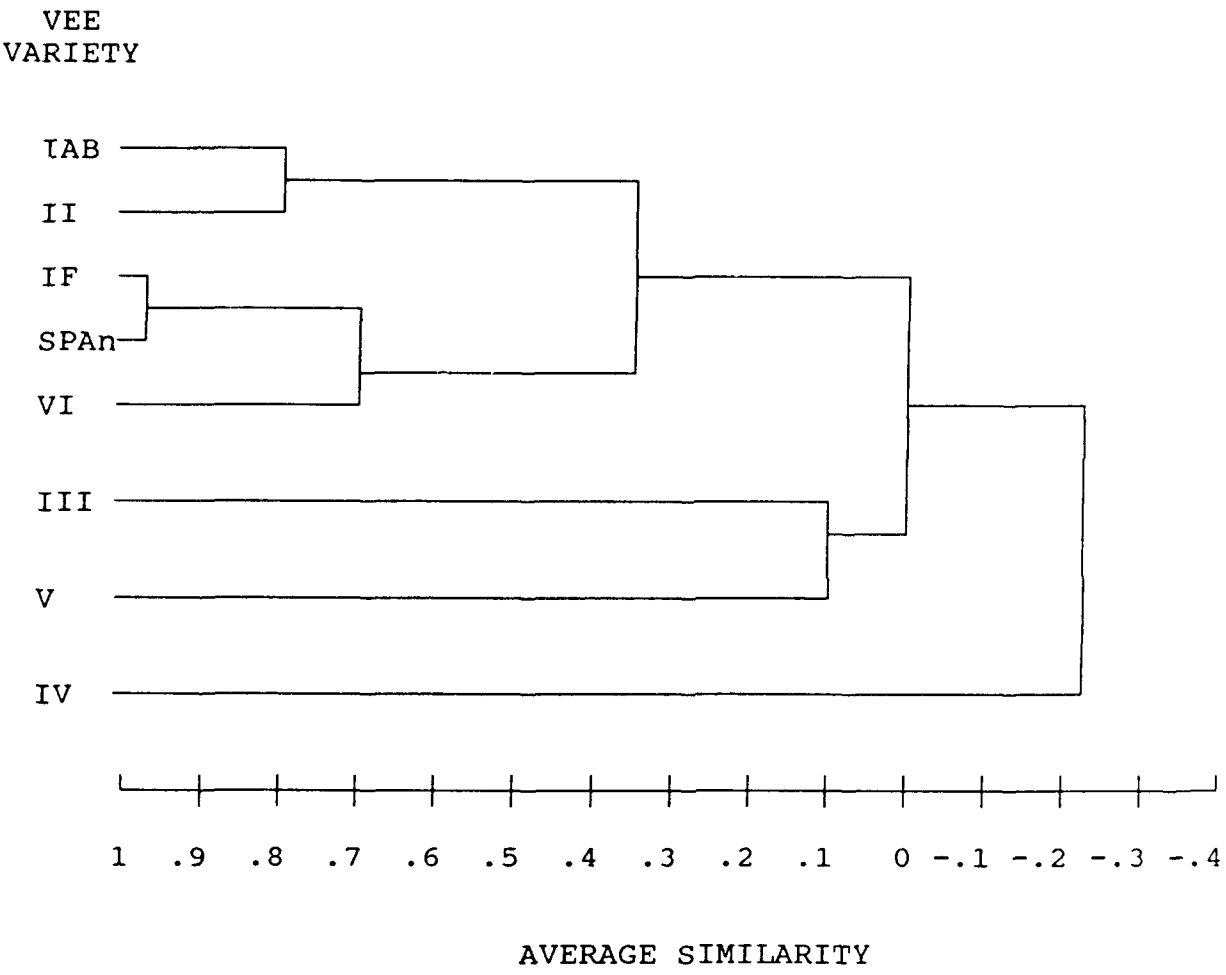

Table 5

ELISA reactions of 9BK18 agent with chikungunya virus

Antigen	Antibody chikungunya	9 BK 18
chikungunya	>6400	100
9 BK 18	6400	200

The 9 BK 18 antibody was also tested and found negative with ELISA antigens of Venezuelan encephalitis, eastern encephalitis, and western encephalitis viruses. Complement-fixation test results are shown in Table 6.

Table 6

Complement-fixation test reactions of 9BK18 agent with chikungunya and Cache Valley viruses

	Antibody		
Antigen	chikungunya	Cache Valley	9 BK 18
chikungunya	128/32*		256/16
Cache Valley		512/8	64/16
9 BK 18	16/4	>512/128	>256/128
Normal mouse brain	0/0	0/0	0/0

*titer of antibody/titer of antigen; 0=<8.

These results were unexpected. Chikungunya virus has been known to produce chronic infection of cell lines, but how it could have contaminated the line in question is not known. Cache Valley virus belongs to the family Bunyaviridae. It is ubiquitous in sheep and cattle in North America and could have contaminated the cell line through incompletely heat inactivated bovine serum. The Cache Valley virus has been incriminated in outbreaks of arthrogryposis and hydranencephaly in sheep in Nebraska and Texas (Chung et al., Vet. Microbiol. 21:297-307, 1990).

II. SEROLOGICAL SURVEY OF U.S. ARMY PATROL DOGS IN KOREA FOR FLAVIVIRUS ANTIBODY (S. Tirrell and R. Shope)

Sera of 104 dogs, mostly patrol dogs, were referred by MAJ Ross Graham, USAMRU-ROK. Sixty of the sera had previously been tested by plaque reduction neutralization test at the Armed Forces Research Institute for Medical Sciences at Bangkok, and 27 were found antibody positive to Japanese encephalitis virus. Tests at YARU were carried out to determine if other flaviviruses known in Asia were responsible for the high prevalence of flavivirus antibody.

Plaque reduction neutralization tests were done with JE, Negishi, Langat, Phnom Penh bat, Apoi, tembusu, Zika, and West Nile viruses. Eighteen sera tested with Negishi were negative, as were 51 sera tested with the closely related Langat virus. Some of these sera were positive to JE virus, thus the tick-borne encephalitis viruses do not appear to be responsible for the flavivirus positive reactions. Only one of 104 sera were postive for Zika virus, and that one was also positive for JE.

Twenty of 104 sera neutralized West Nile virus; 11 sera had West Nile titers equal to or greater than 1:40 (the highest dilution tested), and where tested, all but one were also positive for JE virus. Again, 20 of the 104 sera neutralized Tembusu virus; most also neutralized JE virus. One serum was positive for Tembusu and negative for all other viruses tested.

Eighteen of 57 sera tested were positive for Pnom Penh bat virus in the 1:10 screening dilution. Although there was not sufficient quantity of serum to determine the end-point titers, these reactors were independent of those reacting with JE virus and may represent specific antibody. Dogs positive for Pnom Penh bat virus were stationed in widely separated geograpic areas including Waegwan, Kunsan, Osan, Pusan, and Pupyung.

Patrol dogs in Korea are exposed extensively to bites of Culex mosquitoes. They appear to be excellent sentinel animals for detection of transmission of JE virus.

III. DEVELOPMENT OF NEW TECHNIQUES

A. Molecular epidemiology of dengue viruses (R. Rico-Hesse)

During the past two years there have been major epidemics of classical dengue in Venezuela, Brazil and Colombia, with a significant number of dengue hemorrhagic fever cases reported in the Venezuelan and Brazilian outbreaks. Most of this activity has been caused by dengue-2 virus. Representative strains of dengue-2 virus from Venezuela and Brazil were obtained from these outbreaks and were examined by limited primer-extension sequencing of their RNA. Dengue-2 strains currently active in Venezuela and Brazil are similar to each other and have a Southeast Asia genotype (Rico-Hesse, R., Virology 174:479-493, 1990). They are most closely related to strains of dengue-2 virus isolated in Jamaica in 1981, which we believe were also responsible for the Cuban epidemic of the same year. Epidemiologic evidence suggests that these strains were probably introduced into Cuba by returning Cuban military advisors who were working in Viet Nam

and Cambodia at that time. Although this Southeast Asia genotype has not been identified in the Americas since 1981, the appearance in 1990 of a very similar virus in Brazil and Venezuela suggests that it has persisited in this region. It may also explain in part the increased number of hemorrhagic fever cases observed during these two recent South American outbreaks.

B. Vaccinia virus recombinants expressing Japanese encephalitis E, NS1, and M proteins protect mice against lethal challenge (P. Mason, E. Konishi, B. Fonseca, R. Shope, S. Pincus, M. Fournier, T. Mason, and E. Paoletti)

Four recombinant vaccinia viruses were engineered expressing portions of the Japanese encephalitis virus, Nakayama strain, open reading frame extending from the prM to NS2B genes. A thymidine kinase mutant of the Copenhagen strain of vaccinia virus (vP410) was used to generate recombinant vP658. A recombinant vaccinia virus (vP425) containing the beta-galactosidase gene in the HA region under the control of the 11-kDa late vaccinia virus promoter was used to generate recombinants vP555, vP583, and vP650.

All four recombinant vaccinias contained the NS1 and NS2A genes, and each of these viruses specified the synthesis, glycosylation, and secretion of the nonstructural glycoprotein NS1. All four recombinants also contained the E gene, and each virus correctly directed the synthesis and glycosylation of the envelope glycoprotein E.

Four additional recombinant vaccinias contained portions of the JEV coding region extending from C through NS2B. These recombinants were generated in vaccinia vP410. Recombinant vP825 encoded the C, prM, E, NS1, and NS2A. Recombinant vP829 encoded the putative signal sequence of prM, prM and E. Recombinant vP857 encoded the predicted 30 aa signal sequence for NS1, NS1, and NS2A. Recombinant vP864 encoded the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal (TTTTTGT) in E was modified to TCTTTGT without altering the aa sequesnce. This change was made in an attempt to increase the level of expression of E since this sequence has been shown to increase transcription termination in in vitro transcription assays.

Two of these recombinants, vP555 encoding prM, E, and NS1, and vP829 encoding prM and E, induced the synthesis of extracellular particles in infected cells. These viruses elicited high levels of neutralizing and hemagglutination-inhibition antibodies in inoculated mice and they protected mice from a lethal JEV challenge Tables 7, 8, and 9. Recombinant viruses, vP583 and vP658, that encoded E but did not produce extracellular particles, induced lower levels of antibodies and protection. Moreover, vP829 which is superior in producing extracellular particles in infected cells relative to vP555 was also superior in protecting mice. We conclude from these results that the ability to induce the synthesis of extracellular forms of E is critical for vaccinia recombinant viruses to elicit high protective immunity in animals.

Table 7

Evaluation of ability of recombinant vaccinia virus vP555 and vP658 to protect mice from fatal JEV encephalitis

Immunizing virus*	Challenge dose (Log)**	Survival after one inoculation***	Survival after two inoculations****
vP410	-1	0/20	0/10
vP410	-2	0/20	1/10
vP410	-3	0/18	
vP555	-1	12/20	10/10
vP555	-2	15/20	10/10
vP555	-3	18/19	
vP658	-1	0/20	3/9
vP658	-2	4/22	3/10
vP658	-3	12/18	
-	-2	0/5	1/5
-	-3	1/10	3/5
-	-4	2/10	4/10
-	-5	3/10	6/10
-	-6	4/10	3/10
-	-7	3/5	7/10
-	-8		5/6

* Vaccinia recombinant used for immunization, or unimmunized lethal dose titration groups (-).

** Dilution of suckling mouse brain stock delivered in the challenge. Based on the simultaneous titration data shown in this table, the challenge dose of -1 log of virus was equivalent to 4.7x10/4 LD50 for the 6-week old animals challenged following one inoculation, and to 3.0x10/4 LD50 for the 10-week old animals challenged following two inoculations.

*** Live animals/total for each group; challenge delivered to 6-week old mice, 3 weeks following a single inoculation.

**** Live animals/total for each group; challenge delivered to 6-week old mice, 6 weeks following the first vaccinia inoculation and 3 weeks following a second inoculation with the same vaccinia recombinant.

Table 8

Neutralization and HI antibody titers in prechallenge sera of JEV and control recombinant vaccinia inoculation

Immunizing virus*	One inoculation		Two inoculations	
	Neut titer**	HI titer***	Neut titer**	HI titer***
vP410 group 1	<1:10	<1:10		
vP410 group 2	<1:10	<1:10	<1:10	<1:10
vP555 group 1	1:20	1:20		
vP555 group 2	1:20	1:20	1:320	1:80
vP825 group 1	1:10	1:10		
vP825 group 2	<1:10	1:10	1:320	1:40
vP829 group 1	1:80	1:40		
vP829 group 2	1:160	1:40	1:2560	1:160
vP857 group 1	<1:10	<1:10		
vP857 group 2	<1:10	<1:10	<1:10	<1:10
vP864 group 1	<1:10	<1:10		
vP864 group 2	<1:10	<1:10	<1:10	<1:10

* Vaccinia recombinant used for immunization; group 1 indicates mice challenged 3 weeks following a single vaccinia inoculation, and group 2 indicates mice challenged following two inoculations.
** Serum dilution yeilding 90% reduction in plaque number.
*** Serum dilution giving complete inhibition.

Table 9

Survival of vaccinia-immunized mice following a lethal JEV challenge

Immunizing virus*	Survival after one inoculation**	Survival after two inoculations***
vP410	0/10	0/10
vP555	7/10	10/10
vP825	8/10	9/10
vP829	10/10	9/10
vP857	0/10	5/10
vP864	1/10	6/10

* Vaccinia recombinant used for immunization.
** Live animals/total for each group, challenged with 4.9x10/5 LD50 of JEV 3 weeks following a single vaccinia inoculation.
*** Live animals/total for each group, challenged with 1.3x10/3 LD50 of JEV 6 weeks following the first inoculation, and 3 weeks following the second inoculation with the same vaccinia recombinant.

IV. EVALUATION OF JAPANESE ENCEPHALITIS PRE- AND POST-VACCINATION PLAQUE REDUCTION NEUTRALIZATION IN MILITARY SUBJECTS (S.J. Tirrell and R.E. Shope)

A trial of the Biken inactivated mouse brain vaccine for Japanese encephalitis (JE) was carried out in U.S. Army personnel in Hawaii. The project was a collaboration among Biken (the Research Foundation for Microbial Diseases, Osaka University, Suita, Japan), Connaught/Merieux Laboratories, the Centers for Disease Control, and the Yale Arbovirus Research Unit. The trial was designed to show that the vaccine had acceptable reactogenicity, and that it induced neutralizing antibody.

The vaccine from three consecutively manufactured lots was administered in a three dose series to 532 adult volunteer U.S. military personnel in Hawaii. The trial was under the supervision of Dr. Robert DeFraites of WRAIR.

The study was designed as an open label, randomized, prospective clinical trial with no placebo control. Participants were randomized to receive a three-dose series from one of three consecutively manufactured lots of JE vaccine. The series was administered in two regimens: Day 0, 7, 14 or Day 0, 7, 30. Serum for neutralizing antibody determination was obtained on Day 0, 60, and 180. Yellow fever antibody determinations were performed on sera obtained on Day 0.

Japanese encephalitis plaque reduction neutralization tests. Three test laboratories -- Yale, CDC, and Biken -- received sera under code. Plaque reduction neutralization antibody was detected in each of the three laboratories using the Nakayama-Yoken strain (Lot #JEV-N-9) as the challenge virus. Yale and CDC laboratories used Vero cell cultures in plastic multiple-well microplates, while Biken used chick embryo fibroblast cultures in glass Petri dishes. All laboratories used fresh human serum supplied by CDC. The neutralization end-point was 50% reduction for Biken, 80% for Yale, and 90% for CDC. The initial dilution at Yale was 1:10, at CDC and Biken it was 1:5. The results supplied by Yale (Table 10) as coded data were collated and analyzed by Dr. DeFraites.

All recipients developed detectable neutralizing antibody after the three dose series. Geometric mean antibody titer (GMT), by laboratory, vaccine lot, and dosing regimen are shown in Tables 11a, b, and c. For comparison of results among laboratories, GMT of the standard specimen 057A control for each laboratory is also presented. The differences in GMT among the laboratories were reflected in the differences in the estimate for this specimen; CDC=320, Yale=2560, and Biken=7448. Overall results from all three laboratories were similar.

One individual who had no detectable antibody in specimens tested at Yale had antibody when tested at Biken and CDC.

Both dosing schedules produced substantial titers of neutralizing antibody at 60 and 180 days. Antibody titers among recipients of the 0, 7, 30 day regimen were higher than those who received three doses in 14 days ($p=0.0001$). Vaccine from the three lots tested in this study were immunogenic. In the determinations performed at CDC and Yale, there was a statistically significant ($p=<0.05$) difference in mean log antibody titer

Table 10. Plaque reduction neutralization titers of JE vaccinees

number	A	B	C	number	A	B	C	number	A	B	C
001	2560	<10	640	057	-	<10	-	112	5120	1280	<10
003	640	<10	80	058	2560	<10	5120	113	5120	1280	<10
004	<10	2560	1280	059	<10	640	5120	114	640	<10	1280
005	320	<10	2560	060	40	320	640	115	5120	<10	640
006	80	320	<10	061	10240	<10	320	116	2560	<10	1280
007	5120	640	<10	062	20480	5120	<10	117	2560	640	<10
008	320	<10	2560	063	1280	<10	10240	118	<10	2560	640
009	320	160	<10	064	2560	80	1280	119	640	10	320
010	640	2560	40	065	<10	320	5120	120	160	<10	1280
011	<10	160	640	066	2560	10240	<10	121	640	<10	5120
012	5120	<10	40960	067	<10	1280	2560	122	320	<10	<10
013	2560	10240	80	068	<10	160	1280	123	<10	160	1280
014	<10	640	640	069	<10	-	-	124	320	2560	<10
015	320	<10	1280	070	<10	640	-	126	<10	5120	1280
016	160	2560	1280	071	<10	2560	20480	127	1280	320	<10
017	<10	5120	2560	072	<10	20480	10240	128	160	<10	1280
018	<10	-	-	073	10	1280	5120	129	1280	20480	<10
019	640	160	<10	074	<10	10240	2560	130	<10	5120	320
020	<10	-	-	075	10240	<10	2560	132	320	40	640
021	320	2560	<10	076	<10	10240	5120	133	640	256	<10
022	5120	160	10240	077	<10	2560	1280	134	<10	2560	10240
023	<10	640	5120	078	640	5120	<10	135	<10	2560	10240
024	40	640	5120	079	1280	5120	<10	136	<10	1280	5120
025	320	160	5120	080	-	<10	-	137	-	-	<10
027	160	5120	1280	081	80	5120	<10	138	<10	10240	2560
028	2560	<10	320	082	5120	80	640	139	<10	640	2560
029	640	320	<10	083	640	20	1280	140	<10	5120	20480
030	1280	320	<10	084	1280	10240	<10	141	2560	<10	10240
031	<10	-	-	085	<10	1280	5120	142	640	10240	<10
032	320	1280	<10	086	10240	<10	320	143	1280	<1	10240
033	<10	1280	320	087	<10	5120	2560	144	640	1020	<10
034	2560	<10	640	088	640	160	<10	145	2560	<10	160
035	<10	5120	320	089	640	<10	5120	146	10240	5120	<10
036	2560	5120	80	090	20480	1280	<10	149	2560	<10	10240
037	640	160	10	091	<10	2560	320	151	2560	640	<10
038	640	2560	<10	092	2560	1280	<10	152	<10	20480	81920
039	640	<10	320	093	10240	20	81920	153	2560	<10	1280
040	<10	160	1280	094	-	-	<10	156	5120	1280	<10
041	<10	2560	320	095	<10	20480	1280	157	10240	5120	<10
042	-	-	<10	096	1280	<10	20480	158	-	-	<10
043	5120	320	<10	097	10240	<10	2560	159	10240	-	<10
044	5120	<10	81920	098	640	<10	5120	160	5120	1280	<10
045	-	-	10	099	<10	320	640	161	<10	640	2560
046	2560	40	10240	100	640	1280	<10	162	<10	160	320
047	160	40	2560	101	10240	<10	1280	163	1280	10240	<10
048	<10	20480	5120	102	640	160	2560	165	640	<10	10240
049	2560	<10	160	103	2560	5120	<10	166	640	10240	<10
050	<10	20480	>80	104	20480	2560	<10	167	<10	640	5120
051	160	>80	<10	105	<10	2560	320	168	2560	<10	5120
052	2560	<10	5120	106	2560	5120	<10	169	5120	<10	640
053	5120	1280	<10	107	2560	<10	5120	171	640	<10	2560
054	1280	320	20	109	<10		1280	172	10240	<10	20480
055	2560	1280	<10	110	2560	<10	320	173	10240	<10	1280
056	<10	640	160	111	640	<10	160	175	-	<10	1280

Table 10 (continued)

number	A	B	C	number	A	B	C	number	A	B	C
176	10240	1280	<10	232	5120	10240	<10	287	320	5120	<10
177	80	1280	640	233	640	<10	2560	288	<10	640	1280
178	<10	1280	5120	234	160	<10	1280	289	640	5120	<10
179	<10	-	5120	235	640	2560	<10	290	<10	640	2560
180	2560	40	5120	236	320	1280	<10	291	2560	10240	20
181	2560	<10	320	237	<10	40960	5120	292	5120	<10	640
183	5120	10240	<10	238	320	2560	<10	293	5120	2560	160
184	<10	5120	640	239	320	<10	5120	294	<10	20480	5120
185	640	<10	20480	240	320	<10	2560	295	2560	640	<10
186	<10	5120	40960	241	10240	2560	<10	296	<10	2560	10240
187	640	2560	<10	242	320	<10	2560	297	320	2560	80
188	10240	<10	5120	243	1280	320	<10	298	160	1280	<10
189	5120	<10	320	244	5120	1280	<10	299	1280	2560	<10
190	40	10240	2560	245	160	<10	640	300	<10	5120	2560
191	10240	<10	5120	246	320	10240	<10	301	2560	10	320
192	1280	320	160	247	160	1280	<10	302	<10	160	160
193	2560	20480	<10	248	160	1280	<10	303	5120	<10	640
194	10240	640	<10	249	5120	<10	1280	304	1280	<10	160
195	5120	320	<10	250	<10	160	640	305	2560	640	<10
196	<10	5120	10240	251	320	40	2560	306	160	<10	80
197	1280	10240	<10	252	640	320	<10	308	1280	1280	<10
198	1280	10240	<10	253	10240	320	<10	309	320	80	<10
199	<10	640	2560	254	160	<10	640	310	320	<10	320
200	<10	10240	640	255	10240	640	<10	311	160	<10	320
201	5120	10240	<10	256	1280	5120	<10	312	640	-	<10
202	1280	5120	<10	257	<10	2560	10240	313	1280	<10	2560
203	160	640	<10	258	640	2560	<10	314	<10	1280	2560
204	10240	640	<10	259	<10	1280	10240	315	2560	640	<10
205	2560	1280	<10	260	1280	<10	2560	316	<10	320	1280
206	320	<10	5120	261	1280	10240	<10	317	<10	640	-
207	2560	320	<10	262	<10	640	2560	318	640	<10	640
208	<10	40960	10240	263	<10	2560	1280	319	80	<10	640
209	10	2560	640	264	<10	1280	2560	320	1280	20480	<10
210	320	<10	160	265	20480	<10	2560	321	5120	<10	640
211	80	1280	640	266	640	160	<10	322	320	<10	2560
212	<10	160	1280	267	<10	320	160	323	5120	<10	640
213	2560	5120	<10	268	1280	320	<10	324	640	5120	<10
214	<10	640	1280	269	<10	160	1280	325	<10	-	640
215	320	<10	2560	270	20	320	2560	326	<10	2560	640
216	2560	10240	<10	271	<10	2560	1280	327	1280	320	<10
217	<10	320	1280	272	10240	<10	20480	329	2560	640	<10
218	<10	10240	1280	273	2560	<10	40960	330	10240	2560	<10
219	<10	640	2560	274	160	5120	<10	331	160	<10	320
221	<10	-	-	275	40960	<10	320	332	<10	640	160
222	<10	2560	1280	276	<10	320	640	333	80	<10	160
223	1280	<10	10240	277	<10	640	20480	334	<10	160	2560
224	10240	<10	1280	278	1280	<10	2560	335	<10	1280	320
225	10240	5120	<10	280	<10	640	320	336	<10	10240	320
226	2560	<10	5120	281	5120	1280	<10	337	<10	2560	10240
227	<10	1280	320	282	<10	10240	640	338	320	<10	160
228	<10	20480	2560	283	640	5120	<10	339	2560	<10	320
229	5120	<10	320	284	640	<10	5120	340	2560	<10	1280
230	640	5120	<10	285	<10	320	160	341	640	1280	<10
231	640	<10	160	286	<10	80	320	342	640	<10	1280

Table 10 (continued)

number	A	B	C	number	A	B	C	number	A	B	C
343	<10	160	640	398	320	<10	5120	454	320	40	<10
344	160	<10	1280	399	160	<10	1280	455	160	320	<10
345	<10	80	160	400	<10	2560	320	456	320	<10	1280
346	<10	1280	10240	401	160	1280	<10	457	320	-	10240
347	5120	<10	320	402	320	<10	160	458	640	<10	320
348	<10	320	320	403	<10	-	320	459	640	-	<10
349	<10	320	160	404	20480	<10	10240	460	<10	640	2560
350	640	<10	320	405	2560	320	<10	461	-	<10	-
351	2560	10240	20	406	40	320	<10	462	1280	<10	160
352	320	80	<10	407	640	320	<10	463	1280	<10	640
353	320	<10	640	408	<10	320	80	464	320	<10	1280
354	160	320	160	409	320	320	<10	465	<10	2560	10240
355	640	5120	<10	410	320	<10	160	466	40	<10	320
356	1280	<10	5120	411	<10	640	-	467	640	<10	160
357	-	-	<10	412	<10	2560	320	468	80	320	<10
358	5120	1280	<10	413	<10	160	1280	469	10240	<10	-
359	640	<10	1280	414	2560	160	<10	470	<10	320	320
360	1280	640	<10	416	1280	320	<10	471	-	5120	<10
361	2560	<10	80	417	1280	<10	320	472	1280	<10	2560
362	80	<10	640	418	160	320	<10	473	20480	<10	1280
363	<10	320	1280	419	320	2560	<10	475	2560	<10	1280
364	320	160	<10	420	640	<10	160	476	1280	-	<10
365	<10	1280	1280	421	640	2560	<10	477	5120	<10	1280
366	5120	<10	-	422	<10	5120	640	478	10240	2560	<10
367	320	<10	1280	423	320	640	<10	479	320	2560	<10
368	80	10240	5120	424	160	1280	<10	480	<10	80	1280
369	640	<10	640	425	1280	<10	640	481	<10	-	5120
370	640	<10	1280	426	1280	10240	<10	482	640	640	<10
371	640	<10	160	427	1280	<10	320	483	<10	-	-
372	2560	<10	640	428	<10	1280	320	484	<10	1280	2560
373	320	<10	2560	429	10240	<10	1280	485	<10	320	2560
374	<10	320	640	430	2560	320	<10	486	10240	<10	2560
375	<10	2560	10240	431	1280	<10	640	487	320	<10	-
376	<10	5120	640	432	<10	1280	2560	488	160	<10	640
377	<10	320	320	433	<10	320	5120	489	<10	320	1280
378	<10	320	320	434	<10	1280	10240	490	2560	10240	<10
379	2560	<10	320	435	5120	2560	<10	491	<10	5120	320
380	160	<10	640	436	-	-	<10	492	<10	320	2560
381	320	1280	<10	437	5120	10240	<10	493	2560	<10	320
382	<10	320	160	438	10240	<10	5120	494	<10	320	640
383	640	2560	<10	439	-	640	<10	495	-	-	<10
384	10240	<10	640	440	2560	10	640	496	1280	<10	640
385	640	160	<10	441	<10	1280	-	497	5120	<10	640
386	320	<10	2560	442	10240	20480	<10	498	-	<10	10240
387	5120	2560	<10	443	640	<10	<10	499	1280	320	<10
388	10240	640	<10	444	320	640	<10	500	1280	10240	<10
389	5120	<10	640	446	<10	80	320	501	<10	80	40
391	320	<10	640	447	2560	640	<10	502	-	-	<10
392	2560	2560	<10	448	320	160	<10	503	2560	<10	10240
393	<10	1280	-	449	160	<10	1280	504	<10	320	2560
394	10240	<10	5120	450	2560	<10	5120	505	20480	<10	40960
395	1280	<10	5120	451	640	<10	160	506	2560	320	<10
396	320	2560	<10	452	<10	20480	163840	507	2560	<10	320
397	1280	40	80	453	<10	2560	10240	508	80	320	<10

Table 10 (continued)

number	A	B	C	number	A	B	C	number	A	B	C
509	320	<10	160	529	<10	-	-	550	1280	<10	640
510	320	<10	1280	530	10240	<10	640	551	<10	2560	5120
511	640	5120	<10	531	320	5120	10	553	-	<10	320
512	1280	<10	20480	532	-	<10	-	554	160	1280	<10
513	<10	2560	640	533	<10	320	1280	555	5120	10240	<10
514	640	<10	10240	535	640	10	5120	556	320	<10	320
515	2560	1280	<10	537	<10	640	2560	557	20	320	320
516	1280	320	<10	538	10240	<10	2560	558	640	1280	<10
517	<10	640	5120	540	10240	<10	1280	561	-	<10	320
519	160	<10	1280	541	-	640	40	562	1280	<10	160
520	2560	20480	<10	542	<10	2560	320	563	80	<10	320
522	20	640	80	543	<10	320	2560	564	5120	640	<10
523	320	<10	40	544	<10	-	320	565	640	1280	<10
524	-	5120	-	545	160	640	<10	566	<10	5120	20480
525	160	<10	1280	546	160	<10	640	568	160	<10	640
526	320	<10	640	547	<10	-	2560	569	640	<10	1280
527	<10	320	160	549	<10	10240	640	570	320	<10	1280

Table 11a: Yale Results**

Neutralizing antibody response to three injections of Japanese encephalitis vaccine, by dosing regimen and vaccine lot: Yale Arbovirus Research Unit. Mean log titer was higher at day 60 than day 180 (paired t-test, $p<0.0001$). Mean log titer was higher for recipients of the 0, 7, 30 day regimen at day 60 and day 180 (F-ratio, $p<0.0001$). At day 180, mean log titer was higher in recipients of lot 030 than lot 029 (F ratio, $p<0.03$). There were no other differences in antibody responses among the lot groups. Standard serum 057A had a GMT of 2560 on a sample of 33 runs. Titer of this standard did not vary from its mean by more than one two-fold dilution on any run.

Lot	0, 7, 14 Day Schedule						0, 7, 30 Day Schedule					
	Day 60			Day 180			Day 60			Day 180		
	n	minimum AB level	GMT*	*n*	minimum AB level	GMT*	*n*	minimum AB level	GMT*	*n*	minimum AB level	GMT*
029	*84*	<10†	1479	*80*	<10†	372	*67*	320	3890	*66*	80	631
030	*74*	160	2041	*67*	80	537	*74*	320	4169	*74*	80	871
031	*88*	80	1513	*82*	40	437	*70*	640	5128	*68*	160	1000
TOTAL	*246*	<10	1660	*229*	<10	437	*211*	320	4365	*208*	80	832

*Geometric mean titer for lot group.

†One participant had no detectable antibody after immunization (lower limit of antibody detection at Yale 1:10).

**Analyses and Table supplied by Dr. R. DeFraites

Table 11b: BIKEN Results **

Neutralizing antibody response to three injections of Japanese encephalitis vaccine, by dosing regimen and vaccine lot: Kanoji Institute, (BIKEN). Mean log titer was higher at day 60 than day 180 (paired t-test, $p<0.0001$). Mean log titer was higher for recipients of the 0, 7, 30 day regimen at day 60 and day 180 (F-ratio, $p<0.0001$). There were no significant differences in antibody response among the vaccine lot groups. Standard specimen 057A had a GMT of 7448 on 59 runs (mean log titer ±s.e. = 3.87 ± 0.014, max.=4.17, min.= 3.68).

Lot	0, 7, 14 Day Schedule						0, 7, 30 Day Schedule					
	Day 60			Day 180			Day 60			Day 180		
	n	minimum AB level	GMT*	*n*	minimum AB level	GMT*	*n*	minimum AB level	GMT*	*n*	minimum AB level	GMT*
029	*82*	182	1778	*78*	40	708	*75*	363	3388	*74*	129	1148
030	*76*	240	2344	*69*	120	891	*80*	617	4074	*80*	120	1148
031	*88*	257	1862	*81*	91	776	*79*	603	4677	*77*	191	1445
TOTAL	*246*	182	1950	*228*	40	794	*234*	363	4074	*231*	120	1230

*Geometric mean titer for lot group.

**Analyses and Table supplied by Dr. R. DeFraites

Table 11c: CDC Results **

Neutralizing antibody response to three injections of Japanese encephalitis vaccine, by dosing regimen and vaccine lot: Results of analysis by DVD, Centers for Disease Control. Mean log titer was higher at day 60 than day 180 (paired t-test, $p<0.0001$). Mean log titer was higher for recipients of the 0, 7, 30 day regimen at day 60 and day 180 (F-ratio, $p<0.0001$). There were no statistically significant differences in antibody response among the vaccine lot groups. Standard specimen 057A had a GMT of 320 on 11 runs. Titer of this standard did not vary from its mean by more than one two-fold dilution on any run.

Lot	0, 7, 14 Day Schedule						0, 7, 30 Day Schedule					
	Day 60			Day 180			Day 60			Day 180		
	n	minimum AB level	GMT*	*n*	minimum AB level	GMT*	*n*	minimum AB level	GMT*	*n*	minimum AB level	GMT*
029	*84*	10	98	*77*	<5†	58	*73*	20	617	*72*	5	126
030	*74*	10	135	*66*	5	81	*80*	20	661	*80*	10	170
031	*81*	10	89	*77*	10	59	*78*	20	794	*76*	5	204
TOTAL	*239*	10	104	*220*	<5	65	*231*	20	692	*228*	5	164

*Geometric mean titer for lot group. †One individual had no detectable antibody at Day 180

**Analyses and Table supplied by Dr. R. DeFraites

between lot groups 029 and 030 at day 180. The magnitude of this difference was on the order of a single two-fold dilution of antibody or less.

Yellow fever ELISA. Preexisting yellow fever antibody was determined at Yale. All serum specimens drawn at study day 0 were tested for presence of yellow fever antibody with an ELISA. This test used a French neurotropic virus sucrose-acetone extracted mouse brain antigen (Barry, M. et al. Am. J. Trop. Med. Hyg. 44:79-82, 1991). All negative sera were confirmed by PRNT. Sixty-five soldiers (12.1%) had no detectable yellow fever antibody by ELISA and by PRNT on day 0.

In the analysis of antbody status, Dr. DeFraites reported that yellow fever positive participants developed higher JE antibody titer; the difference between the yellow fever positive and negative groups was not statistically significant except at day 60 and then only for JE PRNT done at the Biken laboratory (Table 12).

These data were submitted to the U.S. Food and Drug Administration to support the license application for Japanese encephalitis virus vaccine.

V. DISTRIBUTION OF REAGENTS (R. Shope, S. Tirrell, R. Tesh)

Reagents were distributed to 28 laboratories in 10 different countries. The reagents and their recipients are listed in Table 13.

VI. CONCLUSIONS

New viruses were identified and new geography and pathogenicity were recognized for already known viruses. Kagoshima virus from Japan caused congenital abnormalities in livestock and was the same as Kasba virus, previously known from India. Batai virus, a bunyavirus, was found for the first time in Africa and for the first time was recognized as a cause of human disease. A new arenavirus was isolated from spleen of a fatal human case in Venezuela. This new virus is an important human pathogen. In addition, studies were done to revise the taxonomy of alphaviruses, and to find arboviruses contaminating veterinary vaccine cultures. U.S. Army patrol dogs in Korea were shown to be infected frequently with Japanese encephalitis virus and were good sentinels for this infection. They were also infected with Pnom Penh or a closely related flavivirus. By molecular techniques it was shown that the dengue-2 viruses in Venezuela and Brazil are very similar to those prevalent in Jamaica in 1981. These viruses probably originated in Viet Nam or another Southeast Asia country. Vaccinia recombinant viruses with structural JE gene inserts secrete particulate antigens and are highly immunogenic. E and prM genes are needed for secretion of the particles. Japanese encephalitis vaccine was evaluated. Five hundred twenty-six human subjects were immunized with three inoculations of inactivated vaccine. All responded with plaque reduction neutralization antibody. The laboratory distributed in 1990 reagents to 28 laboratories in 10 countries.

Table 12. Effect of Pre-existing Yellow Fever Immunity on Immunogenicity of JEV.* Yellow fever immunity is defined as detectable YF antibody by ELISA on day 0, or written record of having received YF vaccine in the past, or both. Displayed are geometric mean titers of JE neutralizing antibody, by dosing regimen.

YARU

	Day 60				Day 180			
Yellow Fever Immunity	*n*	Day 0, 7, 14 GMT	*n*	Day 0, 7, 30 GMT	*n*	Day 0, 7, 14 GMT	*n*	Day 0, 7, 30 GMT
POS	*220*	1585	*201*	4266	*205*	437	*198*	794
NEG	*26*	2512	*10*	6310	*24*	407	*10*	1585

BIKEN

	Day 60*				Day 180			
Yellow Fever Immunity	*n*	Day 0, 7, 14 GMT	*n*	Day 0, 7, 30 GMT	*n*	Day 0, 7, 14 GMT	*n*	Day 0, 7, 30 GMT
POS	*220*	1862	*222*	3890	*204*	776	*219*	1202
NEG	*26*	2754	*12*	6457	*24*	832	*12*	2089

* F-ratio $p<0.001$ for difference in mean log titer between YF POS and YF NEG at day 60. Kruskal-Wallis test for differences in means (YF POS vs. YF NEG) within each dosing regimen group at Day 60 $p<0.05$

CDC

	Day 60				Day 180			
Yellow Fever Immunity	*n*	Day 0, 7, 14 GMT	*n*	Day 0, 7, 30 GMT	*n*	Day 0, 7, 14 GMT	*n*	Day 0, 7, 30 GMT
POS	*215*	102	*220*	676	*198*	66	*217*	158
NEG	*24*	126	*11*	1000	*22*	52	*11*	263

*Analyses and Table supplied by Dr. R. DeFraites

Table 13

Shipments from World Reference Center for Arboviruses - 1990

Requestor/Recipient	Item shipped	Date
Dr. Stephen Eley Chemical Defense Establishment Porton Down, England	ANTIGENS: 2 eastern encephalitis 2 western encephalitis 2 Venezuelan encephalitis	12/13/90
Dr. Jose Ribeiro Department of Entomology University of Arizona Tuscon, AZ	INSECTS: 200 Pupae of Lutzomyia longipalpis 20 Male Rhodnius prolixus	12/12/90
Dr. Whei-kuo Wu University of Notre Dame Department of Biology Notre Dame, IN	MOUSE ASCITIC FLUID: 2 Dengue-1	11/24/90
Dr. Zhang Hai-Lin Yunnan Provincial Epidemic Research Institute Dali City, Yunnan, China	MOUSE ASCITIC FLUIDS: 2 Japanese encephalitis 2 each, dengue 1, 2, 3, 4 Mab 1 Negishi	11/15/90
Dr. Kenneth Eckels WRAIR Washington, DC	VIRUS: 1 Dengue-1, Mochizuki	11/12/90
Dr. Dennis Trent Division of Vector-Borne Diseases Centers for Disease Control Fort Collins, CO	VIRUSES: 1 each of 5 strains of Japanese encephalitis	10/16/90
Dr. Vincent Deubel Institut Pasteur Paris, France	VIRUSES: 1 Spondweni 1 Ilesha 1 Japanese encephalitis	10/15/90
Dr. Charles Calisher Vector-Borne Virus Diseases Division Centers for Disease Control Fort Collins, CO	MOUSE ASCITIC FLUID 1 D'Aguilar	9/13/90

Table 13 (continued)

Requestor/Recipient	Item shipped		Date
Dr. Thomas Ksiazek Disease Assessment Division USAMRIID, Ft. Detrick Frederick, MD 21701-5011	VENEZUELAN EQUINE ENCEPH.STRAINS 1 1AB Trinidad Donkey 1 1AB TC 83 1 1C P676 1 1D 3880 1 1E Mena-II 1 1F 78 V 3531 1 2 Everglades,Fe3-7c 1 3 Mucambo,BeAn8 1 4 Pixuna, BeAr35645 1 5 Cabassou, CaAr508 1 6 AG80-663		08/22/90
Dr.P.K. Murray CSIRO,Australian Animal Health Laboratory Melbourne,Australia	VIRUSES: 2 VSV-Indiana 2(Cocal)TR40233 2 VSV-Indiana 3(Alagoas), strain CoAr171048 2 Chandipura,I653614 2 Porton S 1643 2 Isfahan, 91026-167 2 Piry,BeAn24232 2 EHD-New Jersey 2 EHD-Alberta 2 EHD-IbAr 22619 2 EHD-IbAr 33853 MOUSE ASCITIC FLUIDS: 9 VSV-Indiana 2(Cocal)TR40233 10 VSV-Indiana 3(Alagoas), strain CoAr171048 10 Chandipura, I653514 10 Porton S 1643 10 Isfahan, 91026-167 10 Piry,BeAn24232 10 EHD-New Jersey 8 EHD-Alberta 10 EHD-IbAr22619 10 EHD-IbAr33853		08/19/90
)r. Neal Nathanson)ept. of Microbiology)niv.Pennsylvania ;chool of Medicine)hiladelphia,PA 19104-6076	1 Cocal virus,TR40233 1 Chandipura virus,I653514 1 VSV-Indiana, lab strain		08/11/90
)r.Andrew J.Main [edical Zoology Div. '.S. NAMRU-3 'airo, Egypt	1 SudH KV-66 1 SudH KV-141 1 Chikungunya 1 West Nile,EgH-101 1 SF,Naples,Sabin Orig.	MIAF " " " "	08/09/90

Table 13 (continued)

	1 SF,Sicilian,Sabin Orig.		
	1 Gabek For.,SudAn754-61	"	
	1 Arumowot,SA Ar13532	"	
	1 Karimabad,I 58	"	
	1 St.Floris,DakAnBR512b	"	
	1 Sindbis,EgAr339	"	
	1 Quaranfil,EgAr1095	"	
	1 Dugbe,IbAr1792	"	
	1 Crimean-Congo,IbAn10200	"	
	1 Ilesha,original	"	
	1 Arbia,Iss.Phl.18	"	
	1 Orungo,UgMP359	"	
	1 Toscana,Iss.Phl.3	"	
	1 O'nong-nyong,UgMP 30	"	
	1 Chandipura,I65-3514	"	
	1 Tehran,I-47	"	
	1 Issyk-Kul,LEIV 315K	"	
	1 Bunyamwera,original	"	
	1 Tahyna,souche D	"	
	1 Wanowrie,IG-700	"	
	1 Beliefe,UGMP 6830	"	
	1 Batai,MM-2222	"	
	1 Germiston,SA Ar1050,	Rabbit serum	
	1 Birao,DakArB219b,	Rabbit serum	
	1 Rift Valley Fever,	Monoclonals	
Dr. Joseph Mangiafico Dis.Assessment Division USAMRIID,Ft.Detrick Frederick, MD 21701-5011	1 Cache valley	MIAF	08/06/90
	1 Xingu	"	
	1 Maguari	"	
	1 Ft.Sherman MSP-18	"	
	1 Playas, 75V3066	"	
Dr. Pamela J.Langer Dept. of Molecular Biology College of Agriculture University of Wyoming Laramie, Wyoming 82071-3944	150 Frozen **Lutzomyia longi-palpis** (females)		07/23/90
Dr. Thomas Ksiazek Dis.Assessment Division USAMRIID,Ft.Detrick Frederick,MD 21701-5011	1 Uganda S, MIAF		07/13/90
Dr. James LeDuc Dis.Assessment Division USAMRIID,Ft.Detrick Frederick, MD 21701-5011	1 Apeu, BeAn848	MIAF	07/06/90
	1 Caraparu, BeAn3994	"	
	1 Marituba, BeAn15	"	
	1 Murutucu, BeAn974	"	
	1 Oriboca, BeAn17	"	
	1 Itaqui BeAn12797	"	
	1 Nepuyo TR12797	"	
	1 Ossa	"	
	1 Restan, TR51144	"	
Dr. Charles H.Hoke,Jr. Dept.of Virus Diseases Div.of Comm.Dis.& Immuno. WRAIR,Washington,DC 20307	100 amps.Japanese encepha-litis MIAF		06/21/90

Table 13 (continued)

Dr. Anthony James Dept. of Mol.Biol.Biochem. University of California Irvine,CA 92715	1 culture of C6/36 cells	06/20/90
Dr. Tam David-West Dept. of Virology, University of Ibadan College of Medicine Univ.College Hospital Ibadan, Nigeria	1 Mokola virus,IbAn27377 2 Mokola MIAF, IbAn27377 1 Kotonkan virus,IbAr23388 2 Kotonkan MIAF, IbAr23380 1 Lagos bat virus 2 Lagos bat MIAF 1 culture of C6/36 clone of **Aedes albopictus** cells	06/15/90
Dr. Nigel K. Blackburn Natl.Inst. for Virology Private Bag X4 Sandringham, 2131,So Africa	chikungunya virus,1455/75 " " I63-263 " " PO731460 " " I634029 " " Ph H15483 " " JKT 23574 " " Ph H45056	06/05/90
Dr. Ying-Chang-Wu Taiwan Provincial Institute of Infectious Diseases Taipei, Taiwan	1 culture of Vero cells 1 culture of BHK-21 cells	06/01/90
Dr. Phillip Kogan Dept. of Entomology Cornell University Ithaca, New York	20 **Rhodnius prolixus**	04/16/90
Dr. Andy Comer SCWDS,College of Veterinary Medicine University of Georgia Athens, Georgia 30602	1 VSV-New Jersey MIAF	04/11/90
Dr.Duane J.Gubler Div.of Vector-Borne Viral Dis. CDC, Box 2087 Ft.Collins, Colorado 80522	1 Dengue-1 virus,strain INS 353117 1 Dengue-1 virus, strain INS 353178 1 Dengue-1 virus, strain GML-100063	04/10/90
Dr.Karen Blake PHLS Centre for Applied Microbiology & Research,Div.of Biologics,Porton Down, Salisbury,Wiltshire U.K.	1 mouse poliovirus,GD-7 strain	03/22/90
Mr.John Putnam Dept. of Entomology 130 Symons Hall University of Maryland College Park, MD 20742	2 Dengue-2,Vero cell antigen 2 Normal Vero cell antigen 1 Dengue-2 MIAF	03/20/90

Table 13 (continued)

Dr.Jorge Boshell Virology Group Instituto Nacinal de Salud Bogota, Colombia, S.A.	3 vials	fluorescein-labeled anti-mouse IgG conjugate	01/24/90
Dr.Thomas P.Monath Virology Division USAMRIID,Ft.Detrick Frederick,MD 21701-5011	50ml	Sicilian sandfly fever	MIAF 01/19/90

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