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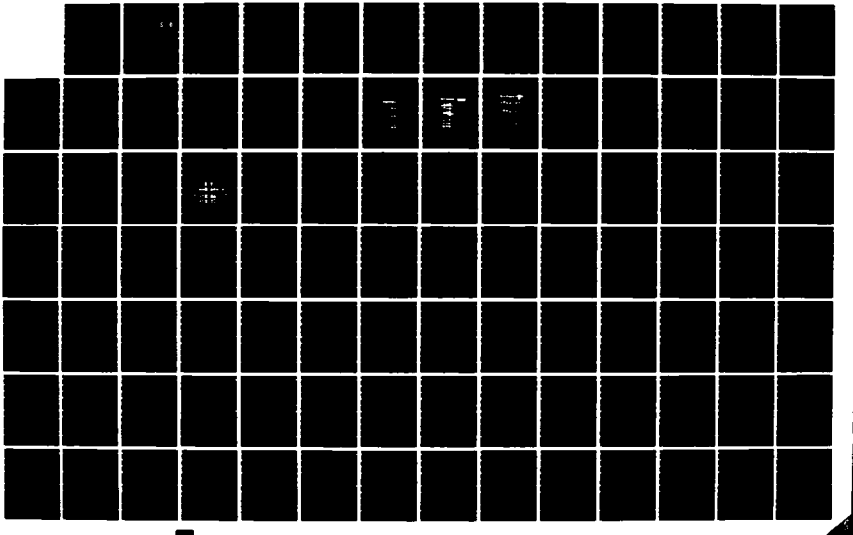

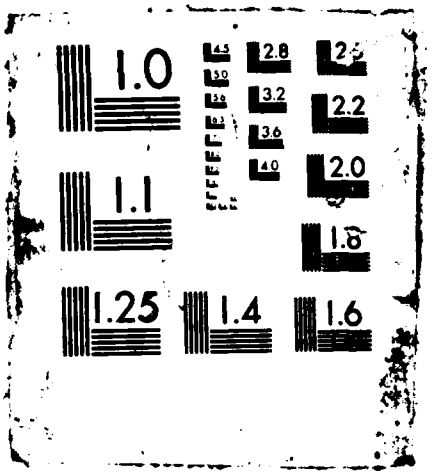
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WORLD REFERENCE CENTER FOR ARBOVIRUSES

ANNUAL PROGRESS REPORT

Robert E. Shope, M.D.

January 1984

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SUMMARY

Virus classification. Taxonomic study of the Changuinola and Palyam serogroups of the orbiviruses indicated 12 and 10 serotypes respectively. Considerable genetic variance was noted among Changuinola strains when PAGE analysis of RNA was undertaken. In contrast, there was considerable homogeneity among Palyam strains. Corriparta serotypes shared antigenicity in complement fixation tests although some differences were discernible. The Great Island complex of the Kemerovo serogroup was defined and several new serotypes were evident.

The Mapputta and Tete serogroups in the family Bunyaviridae were revised. A virus from a mynah bird from India was recognized as a new serotype of the Tete group.

Virus identification. A new flavivirus from Ornithodoros ticks from France was identified. It is in the West Nile-Tyuleniy complex. Two new vesiculoviruses were recognized from phlebotomine flies of Brazil. One of these, Maraba, shows significant relationship to VSV-Indiana virus. A new Uukuniemi group virus was identified from a bird from the USA. Okelbo virus, isolated from mosquitoes in Sweden in the region of an outbreak of fever and arthritis, was identified as Sindbis virus. Two strains of Eyach virus, related to Colorado tick fever virus, were identified from ticks collected in France. Several ungrouped viruses were studied including a strains from Argas ticks from Australia and Tanzania, and 3 isolates from Aedes lineatopennis from Thailand.

Large numbers of strains isolated in mosquito tissue culture in Japan, Indonesia, Israel, Thailand, and China are being studied. Some of these were identified as Japanese encephalitis, and some as orbiviruses. Others are still unidentified.

Diagnosis of disease. Eastern encephalitis was diagnosed in a child from Rhode Island by virus isolation and IFA of brain biopsy material. Attempts to link AIDS serologically with mouse parvovirus, arboviruses, or African hemorrhagic fever agents were so far unsuccessful. Dengue patients were studied for IgM response. The serological response was consistent with that of original antigenic sin. In secondary infections, the IgM correctly identified the infecting virus.

Serologic survey. The Congo-Crimean hemorrhagic fever ELISA test was developed and applied for serosurvey. Surveys were carried out with sera from Sudan, Indonesia, and Cuba. In a study of yellow fever vaccine responses in Brazilians, it was found that persons vaccinated 40 years previously, still had neutralizing antibody. These individuals had an anamnestic response to revaccination.

Development of techniques. Monoclonal antibodies to Rift Valley fever and to Crimean-Congo hemorrhagic fever were produced and characterized. The ELISA was used successfully to detect IgM antibody in cases of Rift Valley fever encephalitis. CSF and sera were positive. A sandwich ELISA was developed and applied to determine antibody in

sheep and cattle. The ELISA for yellow fever functioned well with filterpaper blood collections. The ELISA was also applied to rapid diagnosis of dengue and to detection of antigen in avian sera containing EEE virus.

Collection of low passage virus reference strains. Twenty-three unpassaged or low passage arboviruses were added to the reference collection in 1983. These were aliquoted and lyophilized to be sent to interested scientists and to be stored for future experimental approaches. The new accessions include strains of SLE, WEE, California encephalitis, Jamestown Canyon, Sicilian sandfly fever, VSV-New Jersey, and EEE viruses.

Distribution of reagents. The reference center distributed 1,008 ampoules of reference sera, antigens, and viruses during 1983; mosquito cells and colonized insects were also distributed. Of the viruses distributed, there were represented 222 different serotypes.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

I. VIRUS CLASSIFICATION

ORBIVIRUSES

Although the 1982 Annual Report contained preliminary data on the Changuinola and Palyam serogroups, the complete reports on these serogroups are presented here.

Characterization of the Changuinola Serogroup Viruses (Reoviridae: Orbivirus) (A.P.A. Travassos da Rosa, R.B. Tesh, F.P. Pinheiro, J.F.S. Travassos da Rosa, P.H. Peralta, and D.L. Knudson). The antigenic, biological, and chemical properties of 24 selected Changuinola serogroup viruses were examined. The viruses tested were chloroform resistant, and they were lethal to newborn hamsters after intracerebral inoculation. The prototype Changuinola virus strain (BT-436) replicated in mosquito and sandfly cell cultures. In complement-fixation tests, the viruses were broadly cross-reacting and indistinguishable; but by neutralization test at least 12 distinct serotypes were identified and by polyacrylamide gel electrophoresis of dsRNA, 22 distinct profiles were found. These data suggest that the Changuinola serogroup may be comprised of a large number of genetically different viruses.

Table 1 lists the Changuinola serogroup virus strains that were used in this study. The strains are listed by their country of origin and year of isolation. The lack of species identification of the Lutzomyia from which many of these viruses have been isolated is due to inherent difficulties in their identification.

Since the complement-fixation (CF) test has been the basis for serologic grouping of orbiviruses, this procedure was done to establish that each of the isolates was antigenically related. Results of the cross CF tests with 12 Changuinola group virus antigens and immune sera are given in Table 2. In general, these agents were broadly cross-reactive with most of the viruses being indistinguishable.

Table 3 summarizes the results of pathogenicity studies with 10 selected Changuinola group viruses. The passage history, titer and average survival time of each virus are shown. Although these agents were practically indistinguishable by CF tests, biological differences were detected among them. The average survival time of baby hamsters inoculated with these 10 viruses ranged from 3.7 to greater than 12 days indicating marked variation in their animal pathogenicity. The least pathogenic virus was VP-188G; only 2 of 12 hamsters inoculated with this agent died. There was no significant correlation between hamster pathogenicity, virus titer, or passage history.

Table 3 also demonstrates the effect of chloroform treatment on the same 10 Changuinola group viruses. Titers of the control and chloroform-treated specimens were almost identical, indicating that the viruses were resistant to lipid solvents. In contrast, the titer of vesicular stomatitis virus (family Rhabdoviridae) dropped more than 10^4 PFU after chloroform treatment.

The comparative growth of the prototype strain of Changuinola virus (BT-436) in sandfly (LL-5) and mosquito (C6/36) cell cultures is summarized in Table 4. Virus replication occurred in both cell lines with comparable rates of growth. Virus cytopathic effect (CPE) was observed in the infected LL-5 cells

between the seventh and ninth day when many of the cells began to detach from the glass surface and to lyse. However, new cells appeared within 10 to 14 days and a new monolayer was formed. The infected cells were subsequently subcultured at weekly intervals for 6 weeks. Samples of the resulting cell suspensions were frozen and later titrated. The weekly samples continued to yield 10^4 to 10^5 PFU/ml of Changuinola virus, indicating that a persistent infection may have been established in the sandfly cells. In contrast, the C6/36 cells did not show CPE, and they were not tested for persistent infection.

Table 5 shows the mouse neutralization test results with 12 Changuinola group viruses and antisera. Since each of the viruses could be differentiated by this technique and were antigenically distinct, the Changuinola serogroup is comprised of at least twelve distinct serotypes.

When the dsRNA of Changuinola serogroup viruses was analyzed by polyacrylamide gel electrophoresis (PAGE), distinctive profiles of the 10 dsRNA segments were observed for most of the isolates (Figs. 1, 2, and 3). The apparent molecular weights of the dsRNA segments were calculated using the Dearing strain of reovirus type 3 as a molecular weight standard in linear regression analyses. These data are presented in Table 6 to allow comparisons to be made of dsRNA profiles analyzed under different conditions.

Twenty-two unique dsRNA profiles were found for the 24 isolates that were examined. Strains, BT-436 (Changuinola) and BT-766, were indistinguishable by PAGE. Likewise, the mobilities of the dsRNA segments of BT-2164 and BT-2365 were identical. Seven of the 10 segments of BT-104 were identical to BT-436 and BT-766, but minor electrophoretic variations were seen consistently in segments 2, 3, and 5 (Fig. 1 and Table 6). The dsRNA profiles for six of the viruses also exhibited additional minor molar species (Figs. 1, 2, and 3). These data are in press in Intervirology.

Table 1

Changuinola serogroup viruses included in this study

Virus*	Strain number	Source	Geographic locality**	Date of isolation
Changuinola	BT-436	Lutzomyia sp. (sandfly)	Bocas del Toro, Panama	1960
-	BT-104	Lutzomyia sp.	Bocas del Toro, Panama	1960
-	BT-766	Lutzomyia sp.	Bocas del Toro, Panama	1960
-	BT-2164	Lutzomyia sp.	Bocas del Toro, Panama	1961
-	BT-2365	Lutzomyia sp.	Bocas del Toro, Panama	1961
-	BT-2380	Lutzomyia sp.	Bocas del Toro, Panama	1961
-	VP-19A	Lutzomyia sp.	Panama, Panama	1969
-	VP-46F	Lutzomyia sp.	Panama, Panama	1969
-	VP-188G	Lutzomyia trapidoi	Panama, Panama	1970
-	VP-202A	Lutzomyia sp.	Panama, Panama	1970
-	CoAr 2837	Lutzomyia sp.	Valle, Colombia	1964
Irituia	BeAn 28873	Oryzomys sp. (rice rat)	Para, Brazil	1961
Gurupi	BeAr 35646	Lutzomyia sp.	Para, Brazil	1962
Ourem	BeAr 41067	Lutzomyia sp.	Para, Brazil	1962
Caninde	BeAr 54342	Lutzomyia sp.	Para, Brazil	1963
Jamanxi	BeAr 243090	Lutzomyia sp.	Para, Brazil	1973
Altamira	BeAr 264277	Lutzomyia sp.	Para, Brazil	1974
Purus	BeAr 361064	Psorophora albipes (mosquito)	Acre, Brazil	1977
Jari	BeAn 385199	Choloepus didactylus (sloth)	Para, Brazil	1980
Saraca	BeAr 385278	Lutzomyia sp.	Para, Brazil	1980
Monte Dourado	BeAn 385401	Dasypus novemcinctus (armadillo)	Para, Brazil	1980
Almeirim	BeAr 389709	Lutzomyia umbratilis	Para, Brazil	1980
-	BeAr 385274	Lutzomyia sp.	Para, Brazil	1980
-	BeAr 385279	Lutzomyia sp.	Para, Brazil	1980

* Hyphen denotes unnamed strain.
** State province department, country

Table 2

Complement fixation tests with selected Changuinola group viruses

Antigen	Antiserum											
	CGL	IRI	GUR	OUR	CAN	JAM	ALT	PURUS	JARI	SAR	MD	AMR
Changuinola	32/32	0	8/32	≥256/32	≥256/32	64/32	8/8	128/≥128	128/128	128/128	128/32	≥256/≥128
Irituia	16/8	32*/32	64/32	≥256/32	256/32	≥256/32	32/32	128/32	128/128	256/128	256/82	256/32
Gurupi	16/32	32/≥128	64/≥128	≥256/128	≥256/≥128	128/128	16/32	64/128	128/≥128	256/≥128	256/128	128/≥128
Ourem	16/128	8/8	64/128	≥256/≥128	≥256/128	128/128	32/32	≥128/128	256/≥128	256/≥128	≥256/128	256/≥128
Caninde	8/32	32/≥128	64/≥128	≥256/128	512/128	128/128	32/32	32/128	64/≥128	128/128	256/128	128/128
Jamanxi	<16/8	16/32	32/32	256/128	≥256/128	128/128	32/128	32/32	64/≥128	≥256/≥128	≥256/≥128	256/≥128
Altamira	<16/8	8/32	16/32	256/128	≥256/32	64/≥128	32/32	32/32	64/≥128	≥256/≥128	≥256/≥128	256/≥128
Purus	16/32	0	8/8	256/32	128/8	32/32	0	256/32	128/128	64/32	64/32	≥256/32
Jari	8/32	0	8/32	≥256/≥128	256/32	64/32	8/32	64/≥128	256/≥128	128/≥128	128/128	256/≥128
Saraca	<16/8	8/8	32/8	256/32	256/32	32/32	8/8	16/32	64/32	256/128	256/128	64/32
Monte Dourado	<16/8	8/32	32/32	≥256/≥128	≥256/≥128	128/≥128	32/128	64/128	64/≥128	≥256/≥128	≥256/≥128	256/≥128
Almeirim	8/32	8/8	16/≥128	≥256/≥128	256/≥128	64/128	8/32	64/≥128	128/≥128	128/≥128	128/≥128	≥256/≥128

* Reciprocal of highest antiserum dilution/reciprocal of highest antigen dilution.
0=<8/8

Table 3

Biological studies with selected Changuinola group viruses

Virus or strain	Passage history*	Virus titer, control**	Virus titer after chloroform treatment	Avg. survival time+
Changuinola	SM_{13}, $Vero_3$	5.3	5.6	7.3
BT-766	SM_{10}, $Vero_2$	6.4	6.2	9.8
BT-2380	SM_8, $Vero_3$	5.9	6.0	7.8
VP-19A	$Vero_3$	6.2	6.6	10.4
VP-46F	$Vero_3$	6.6	6.2	6.6
VP-188G	$Vero_2$	5.9	6.0	(12.0)++
VP-202A	$Vero_3$	5.4	5.7	8.7
Irituia	SM?, $Vero_1$	6.1	5.7	3.9
Gurupi	SM_{11}, $Vero_2$	6.4	6.5	3.7
Caninde	SM_6, $Vero_1$	6.3	5.8	3.8
Vesicular stomatitis-Indiana	$Vero_5$	7.3	3.2	-

* SM = suckling mouse, Vero = Vero cell cultures
** Titer given as $\log_{10}$ plaque forming units/ml. Inoculum for newborn hamsters was 0.02 ml of virus stock.
\+ Average survival time (days) of inoculated newborn hamsters
++ Two of 12 inoculated newborn hamsters died on day 12 post-inoculation; the remainder survived.

Table 4

Growth of Changuinola virus (BT-436) in *Lutzomyia longipalpis* (LL-5) and *Aedes albopictus* (C6/36) cells

Day post-inoculation	Virus titer* LL-5	C6/36
1	1.0	0.7
2	3.5	3.9
3	5.5	5.2
4	6.0	6.2
5	6.2	6.4
6	5.9	6.6
7	6.4	6.7

*Titer expressed as $\log_{10}$ of PFU/ml of frozen cell harvest.

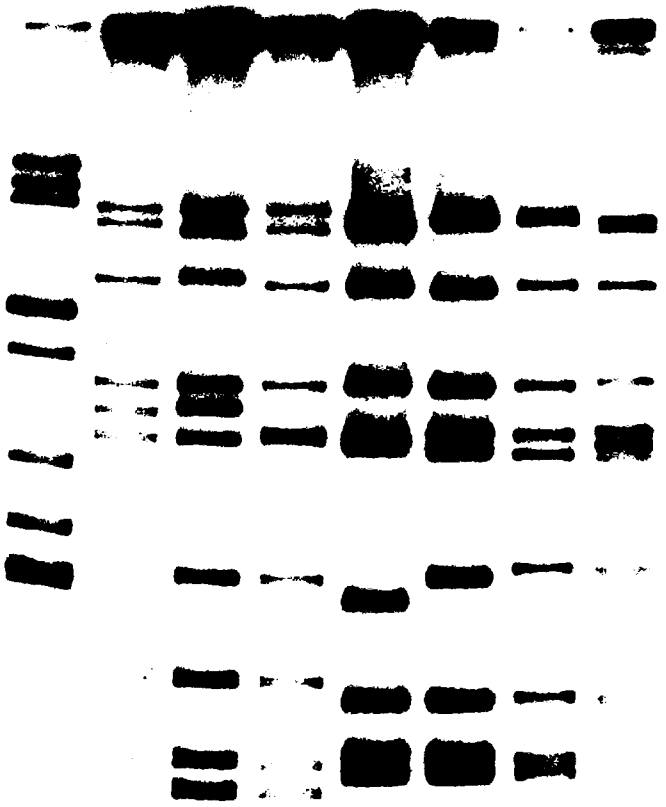
Table 5

Mouse neutralization tests with selected Changuinola group viruses

Virus	Antiserum											
	CGL	IRI	GUR	OUR	CAN	JAM	ALT	PURUS	JARI	SAR	MD	AMR
Changuinola	2.1	0	≤1.1	0	0	≤1.1	0	0	0	0	≤1.1	0
Irituia	0	2.4*	0	0	1.2	0	0	0	0	0	0	0
Gurupi	0	0	2.6	0	0	0	0	1.2	0	0	0	0
Ourem	0	0	0	3.7	0	0	0	1.2	1.2	0	0	0
Caninde	0	0	0	0	3.0	0	0	0	0	1.9	0	0
Jamanxi	0	0	0	0	0	3.1	0	0	0	0	0	0
Altamira	0	1.1	1.2	0	0	0	3.6	1.2	0	0	0	0
Purus	0	0	0	0	0	0	0	2.5	0	0	0	0
Jari	0	0	0	0	0	0	0	0	1.7	0	0	0
Saraca	1.2	0	0	0	1.7	0	0	1.1	0	3.0	0	≤1.1
Monte Dourado	0	0	0	0	1.4	0	0	1.2	0	0	3.0	0
Almeirim	0	0	0	0	0	0	0	0	0	0	0	2.4

*Log_{10} neutralization index. 0 = ≤1.0

Figure legends Figures 1, 2, and 3. Autoradiogram depicting the resolution of the segmented dsRNA genome of selected members of the Changuinola serogroup by electrophoresis of 3' end-labeled dsRNA through tris-glycine buffered 10% polyacrylamide gel. The viruses are from left to right for Fig. 1: Reovirus, BT-104, Changuinola (BT-436, BT-2308, VP-19A, VP-46F, VP-188G, and VP-202A); for Fig. 2: Reovirus, Changuinola, CoAr 2837, Irituia, Gurupi, Ourem, Caninde, Jamanxi, BeAr 385274, BeAr 385279; and for Fig. 3: Reovirus, Changuinola, Irituia, Altamira, Purus, Jari, Saraca, Monte Dourado, Almeirim.



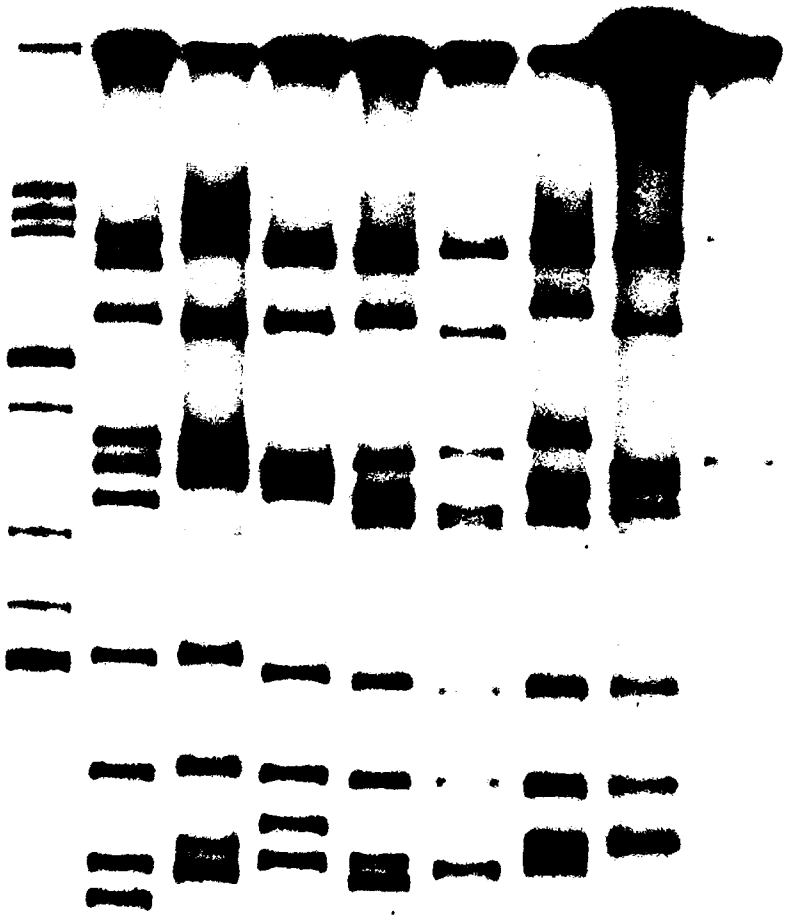


Table 6
Changuinola Serogroup Viruses:
Apparent Molecular Weight of the dsRNA Segments

Virus Strain	DsRNA Segment and Total Genome Molecular Weights (Molecular Weight X 10^{-6} daltons) (Standard deviation)										
	1	2	3	4	5	6	7	8	9	10	Sum
Changuinola											
BT-436	2.28	2.12	1.80	1.25	1.15	1.04	0.65	0.47	0.36	0.32	11.44
	0.04	0.03	0.02	0.02	0.03	0.03	0.02	0.01	0.01	0.01	0.16
BT-104	2.29	2.17	1.78	1.24	1.12	1.03	0.64	0.47	0.37	0.33	11.42
	0.06	0.06	0.04	0.03	0.02	0.02	0.01	0.02	0.02	0.02	0.25
BT-766	2.29	2.12	1.79	1.23	1.11	1.02	0.64	0.46	0.36	0.32	11.34
	0.03	0.02	0.02	0.05	0.06	0.05	0.02	0.01	0.00	0.00	0.19
BT-2164	2.29	2.17	1.73	1.19	0.99	0.99	0.62	0.45	0.35	0.32	11.11
	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.02
BT-2365	2.30	2.18	1.73	1.19	0.99	0.99	0.62	0.45	0.34	0.32	11.14
	0.04	0.03	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.03
BT-2380	2.41	2.15	1.75	1.25	1.05	1.05	0.65	0.46	0.34	0.32	11.46
	0.15	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.13
VP-19A	2.19	2.10	1.72	1.22	1.00	0.99	0.58	0.43	0.36	0.33	10.93
	0.03	0.04	0.03	0.05	0.04	0.04	0.02	0.01	0.01	0.01	0.21
VP-46F	2.20	2.20	1.70	1.21	1.03	0.96	0.64	0.43	0.36	0.33	11.06
	0.03	0.03	0.03	0.05	0.05	0.04	0.03	0.02	0.01	0.01	0.25
VP-188G	2.22	2.19	1.76	1.26	1.07	1.00	0.69	0.45	0.36	0.35	11.37
	0.03	0.06	0.03	0.04	0.04	0.03	0.03	0.02	0.02	0.01	0.29
VP-202A	2.20	2.11	1.75	1.29	1.09	1.04	0.98	0.69	0.45	0.37	11.96
	0.05	0.04	0.04	0.04	0.03	0.03	0.04	0.03	0.03	0.02	0.33
CoAr 2837	2.29	2.18	1.79	1.14	1.06	1.06	0.55	0.45	0.35	0.33	11.21
	0.03	0.02	0.01	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.08
Irituia											
BeAn 28873	2.41	2.22	1.73	1.21	1.11	1.11	0.65	0.47	0.38	0.35	11.65
	0.04	0.04	0.03	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.26
Gurupi											
BeAr 35646	2.39	2.31	1.74	1.21	1.09	0.99	0.99	0.63	0.45	0.34	12.17
	0.04	0.04	0.01	0.04	0.02	0.02	0.02	0.01	0.02	0.01	0.23
Ourem											
BeAr 41067	2.27	2.06	1.73	1.14	1.07	0.98	0.58	0.45	0.37	0.34	11.00
	0.05	0.05	0.04	0.05	0.04	0.04	0.02	0.02	0.02	0.01	0.31
Caninde											
BeAr 54342	2.39	2.06	1.73	1.21	1.10	1.07	0.63	0.47	0.37	0.34	11.39
	0.04	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.26
Jamanxi											

BeAr 243090	2.15	2.15	1.72	1.16	1.08	0.98	0.57	0.44	0.39	0.33	11.01
	0.00	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.09
Altamira											
BeAr 264277	2.24	2.18	1.79	1.18	1.14	1.09	0.63	0.47	0.41	0.37	11.51
	0.04	0.04	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.16
Purus											
BeAr 361064	2.21	2.12	1.80	1.18	1.07	1.01	0.62	0.46	0.37	0.35	11.22
	0.01	0.01	0.03	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01
Jari											
BeAn 385199	2.22	2.22	1.74	1.22	1.04	1.00	0.61	0.47	0.36	0.36	11.24
	0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.05
Saraca											
BeAr 385278	2.30	2.19	1.88	1.28	1.10	1.01	0.60	0.45	0.38	0.36	11.58
	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.12
Monte Dourado											
BeAn 385401	2.25	2.17	1.77	1.16	1.11	1.04	0.61	0.46	0.39	0.39	11.35
	0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.19
Almeirim											
BeAr 389709	2.26	2.14	1.72	1.19	1.18	0.99	0.58	0.47	0.38	0.35	11.26
	0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.14
-											
BeAr 385274	2.21	2.20	1.76	1.17	1.10	0.96	0.60	0.45	0.38	0.38	11.24
	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.05
-											
BeAr 385279	2.22	2.22	1.75	1.19	1.10	0.97	0.61	0.46	0.38	0.35	11.29
	0.01	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.08
Reovirus 3											
Dearing	2.60	2.42	2.29	1.57	1.57	1.35	0.93	0.75	0.65	0.63	14.76
	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02

*Reovirus 3 included as the molecular weight standard in the calculations.

Characterization of the Palyam Serogroup Viruses (Reoviridae: Orbivirus) (D.L. Knudson, R.B. Tesh, A.J. Main, T.D. St. George, and J.P. Digoutte). Thirty-one Palyam serogroup viruses were examined by complement-fixation and plaque reduction neutralization tests and by polyacrylamide gel electrophoresis of the dsRNA segmented genome. Although the viruses were indistinguishable by complement-fixation tests, 10 distinct virus serotypes were identified by plaque reduction neutralization methods. Palyam group viruses which were distinct by the neutralization test had unique dsRNA profiles, whereas those agents which were indistinct by the neutralization test had identical dsRNA profiles. Twenty isolations of three Palyam serotypes were made from bovines and Culicoides midges in Australia over a 9-year period. When the dsRNA genome of these isolates was examined electrophoretically, the dsRNA profiles of virus isolates within a given serotype were identical.

Tables 7 and 8 list the Palyam serogroup virus strains and isolates which were used in this study. The strains are listed by their country of origin and year of isolation.

Since the complement-fixation (CF) test has been the basis for serologic grouping (serogroup) of orbiviruses, this procedure was done initially to establish that the viruses were antigenically related. Results of cross CF tests with 11 Palyam serogroup viruses are given in table 9. The viruses were indistinguishable by this technique indicating that they share common antigens.

The results of PRN tests are summarized in table 10. Ten of the 11 Palyam serogroup viruses examined by the neutralization test appeared to be antigenically distinct using the criterion of a four-fold or greater difference between homologous and heterologous antisera titers.

When the segmented dsRNA genome of ten of the Palyam serogroup viruses was examined by polyacrylamide gel electrophoresis (PAGE), 9 distinctive profiles of their dsRNA segments were observed (Fig. 4). Abadina and Ar K 58 were indistinguishable by PAGE, and this finding was confirmed by co-electrophoresis of these two virus isolates (data not shown). The apparent molecular weights of their dsRNA segments were calculated by linear regression analyses using the Dearing strain of reovirus type 3 as the molecular weight standard (Table 11).

Twenty-three isolates of D'Aguilar, Bunyip Creek, and CSIRO Village virus serotypes were also examined by PAGE. The results indicated that those viruses belonging to the same serotype had identical dsRNA profiles (data not shown). For example, the 9 virus isolates identified as D'Aguilar by the neutralization test (Table 8) had identical dsRNA patterns. Likewise, the Bunyip Creek isolates were indistinguishable, and each of the CSIRO Village isolates was similar.

When D'Aguilar, Bunyip Creek, and CSIRO Village were co-electrophoresed in pairwise combinations (data not shown), the dsRNA profiles were identical for nine of the ten dsRNA segments. Only the second largest segment varied among the three distinct serotypes (Fig. 4). The apparent serologic classification of this serogroup is presented in table 12. These data are in press in Intervirology.

Table 7

Palyam Group Viruses

Virus	Strain	Source	Geographical origin	Date of isola
Palyam	G5287	Culex "vishnui"	Vellore, India	1956
Kasba	G15534	Culex "vishnui"	Vellore, India	1957
Vellore	68886	Culex pseudovishnui	Vellore, India	1966
D'Aguilar	B8112	Culicoides brevitarsis	Bunya, Q., Australia	1972
CSIRO Village	CSIRO 11	Culicoides spp.	Beatrice Hill, N.T., Australia	1974
Marrakai	CSIRO 82	Culicoides schultzei + peregrinus	Beatrice Hill, N.T., Australia	1975
Bunyip Creek	CSIRO 87	Culicoides schultzei	Beatrice Hill, N.T., Australia	1976
Abadina	Ib Ar 22388	Culicoides spp.	Ibadan, Nigeria	1967
Nyabira	792/73	calf (aborted fetus)	Nyabira, Zimbabwe	1973
Petevo	Ar TB 2032	Amblyomma variegatum	Bangui, Central African Republic	1978
-	Ar K 58	Amblyomma variegatum	Kindia, Rep. of Guinea	1978

Hyphen (-) denotes unnamed virus.

Table 8

Australian Palyam Group Virus Isolates

Virus	Isolate	Source	Geographical origin*
D'Aguilar	B8112	Culicoides brevitarsis	Bunya, Q.
	CSIRO 208	cow	Mt. Bundy, N.T.
	CSIRO 209	cow	Tortilla, N.T.
	CSIRO 211	cow	Douglas Daly, N.T.
	CSIRO 330	cow	Tortilla, N.T.
	CSIRO 353	cow	Tortilla, N.T.
	CSIRO 662	Cu. brevitarsis	Peachester, Q.
	CSIRO 681	Cu. brevitarsis	Peachester, Q.
	CSIRO 703	Cu. brevitarsis	Peachester, Q.
Bunyip Creek	CSIRO 87	Cu. schultzei	Beatrice Hill, N.T.
	CSIRO 112	cow	Kununurra, W.A.
	CSIRO 113	Cu. brevitarsis	Camden, N.S.W.
	CSIRO 166	cow	Beatrice Hill, N.T.
	CSIRO 155	cow	Peachester, Q.
	CSIRO 186	cow	Peachester, Q.
	CSIRO 422	cow	Tortilla, N.T.
	CSIRO 674	Cu. brevitarsis	Peachester, Q.
	CSIRO 722	Cu. brevitarsis	Peachester, Q.

Table 8 (continued)

Virus	Isolate	Source	Geographical origin	Date
CSIRO Village	CSIRO 11	Culicoides spp.	Beatrice Hill, N.T.	1974
	CSIRO 55	cow	Peachester, Q.	1976
	CSIRO 193	cow	Paterson, N.S.W.	1978
	CSIRO 205	Cu. brevitarsis	Kairi, Q.	1979
	CSIRO 305	cow	Kairi, Q.	1980

* Q. = Queensland; N.T. = Northern Territory; W.A. = Western Australia and N.S.W. = New South Wales.

Table 9

Complement Fixation Tests with Palyam Serogroup Viruses

	ANTISERUM										
ANTIGEN	PALYAM	CSIRO VILLAGE	KASBA	ABADINA	Ar K 58	MARRA-KAI	VELLORE	BUNYIP CREEK	D'AGUILAR	PETEVO	NYABIRA
PALYAM	128/32*	64/32	256/64	256/32	64/16	128/64	256/128	64/32	256/64	256/32	128/64
CSIRO VILLAGE	16/16	128/8	128/4	64/8	8/4	32/4	128/64	64/32	256/16	128/8	128/8
KASBA	128/64	128/64	512/128	256/64	64/32	128/64	256/≥128	128/64	512/64	256/64	128/≥128
ABADINA	128/32	64/32	256/64	256/64	64/64	128/64	256/≥128	128/64	256/64	128/64	128/>128
AR K 58	32/16	128/16	256/16	256/16	64/8	128/16	256/64	64/32	256/16	128/16	128/32
MARRAKAI	128/64	128/64	256/64	256/128	64/32	256/64	256/128	128/64	512/64	128/64	128/>128
VELLORE	128/64	128/64	256/64	256/64	64/32	128/64	512/≥128	64/64	256/64	256/64	128/>128
BUNYIP CREEK	128/32	64/64	256/64	128/64	32/32	128/64	128/≥128	128/128	256/64	128/64	128/≥128
D'AGUILAR	128/64	64/64	256/64	256/64	32/32	128/64	128/≥128	128/64	512/≥128	128/64	128/≥64
PETEVO	128/64	128/64	256/128	256/128	64/64	128/128	256/≥128	64/128	256/≥128	256/128	128/>128

*Reciprocal of highest antiserum dilution/highest antigen dilution.

Table 10

Plaque Reduction Neutralization Tests with Palyam Serogroup Viruses

VIRUS	ANTISERUM										
	PALYAM	CSIRO VILLAGE	KASBA	ABADINA	Ar K 58	MARRA-KAI	VELLORE	BUNYIP CREEK	D'AGUILAR	PETEVO	NYABIRA
PALYAM	320*	320	0	0	0	0	0	0	0	0	0
CSIRO VILLAGE	40	5,120	0	0	0	0	0	0	0	0	0
KASBA	0	0	>20,480	2,560	320	40	0	0	0	0	0
ABADINA	0	0	1,280	5,120	1,280	0	0	0	0	0	0
Ar K 58	0	0	2,560	2,560	1,280	0	0	0	0	0	0
MARRAKAI	0	0	10	20	0	>20,480	0	0	0	0	0
VELLORE	0	0	0	0	0	0	10,240	20	0	0	0
BUNYIP CREEK	0	0	0	0	0	0	640	80	0	0	0
D'AGUILAR	0	0	0	0	0	0	20	0	320	0	1,280
PETEVO	0	0	0	0	0	0	10	0	0	10,240	0

*Reciprocal of highest antiserum dilution producing ≥90% plaque inhibition.

Figure legend Figure 4. Autoradiogram depicting the resolution of the segmented dsRNA genome of members of the Palyam serogroup by electrophoresis of 3' end-labeled dsRNA through tris-glycine buffered 10% polyacrylamide gel. The viruses are from left to right Reovirus, Palyam, CSIRO Village, Kasba, Abadina, Ar K 58, Marrakai, Vellore, Bunyip Creek, D'Aguilar, Petevo, and Reovirus.

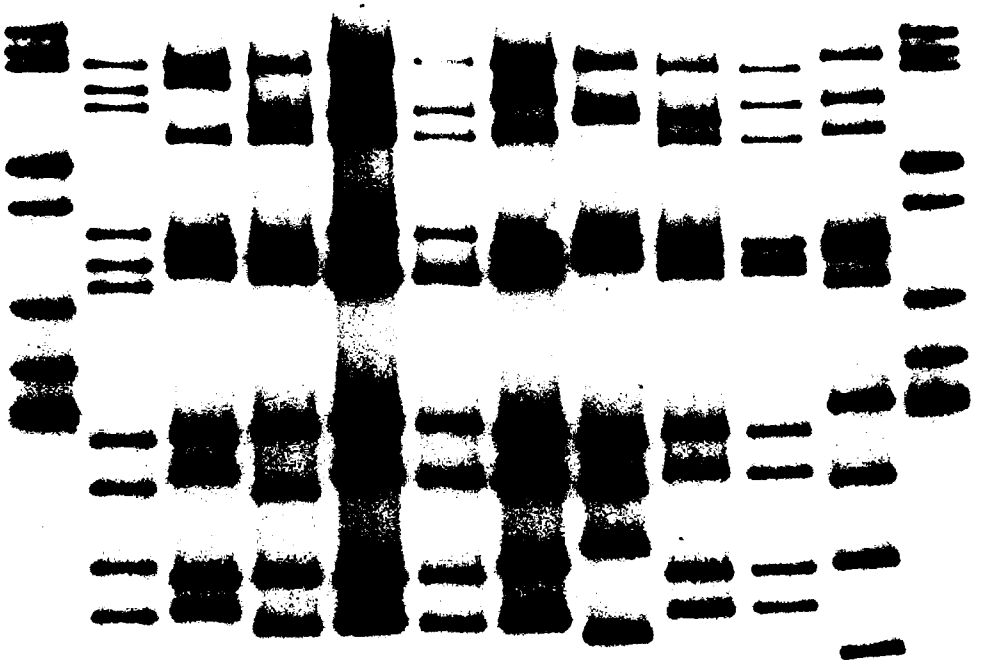


Table 11
Palyam Serogroup Viruses:
Apparent Molecular Weight of the DsRNA Segments

Complex: Virus Strain Variant	DsRNA Segment and Total Genome Molecular Weights (Molecular Weight X 10^{-6} daltons) (Standard deviation) 1	2	3	4	5	6	7	8	9	10	SUM
Palyam complex:											
Palyam											
G5287	2.31	2.09	1.95	1.23	1.09	1.01	0.58	0.49	0.37	0.32	11.45
	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.00	0.01	0.01	0.10
CSIRO Village											
CSIRO 11	2.34	2.18	1.77	1.20	1.12	1.08	0.60	0.52	0.36	0.32	11.48
	0.03	0.03	0.03	0.03	0.02	0.04	0.02	0.02	0.01	0.01	0.16
Kasba complex:											
Kasba											
G15534	2.31	1.91	1.77	1.13	1.09	1.04	0.60	0.49	0.36	0.30	11.00
	0.04	0.05	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.16
Abadina											
Ib Ar 22388	2.40	1.94	1.78	1.22	1.03	1.03	0.61	0.51	0.36	0.30	11.20
	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04
Marrakai											
CSIRO 82	2.38	2.08	1.81	1.17	1.05	1.05	0.60	0.50	0.37	0.31	11.32
	0.03	0.04	0.04	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.19
Vellore complex:											
Vellore											
68886	2.37	1.95	1.93	1.20	1.10	1.10	0.57	0.49	0.40	0.29	11.39
	0.03	0.04	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.11
Bunyip Creek											
CSIRO 87	2.30	1.90	1.76	1.18	1.09	1.06	0.59	0.51	0.36	0.31	11.08
	0.02	0.03	0.03	0.00	0.02	0.01	0.01	0.01	0.01	0.01	0.08
D'Aguilar complex:											
D'Aguilar											
B8112	2.29	2.00	1.75	1.17	1.09	1.06	0.59	0.51	0.36	0.31	11.15
	0.05	0.04	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.17
Petevo complex:											
Petevo											
ArTB-2032	2.43	2.07	1.82	1.20	1.14	1.03	0.65	0.50	0.37	0.27	11.47
	0.05	0.05	0.05	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.21
Reoviridae:*											
Reovirus 3											
Dearing	2.59	2.42	2.30	1.57	1.57	1.36	0.93	0.75	0.65	0.62	14.75
	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.09

*Reovirus 3 Dearing strain included as the molecular weight standard in the calculations.

Table 12
Palyam Serogroup Viruses:
Apparent Serologic Classification

Complex	Serotype	Strain
Palyam	Palyam	G5287
	CSIRO Village	CSIRO 11
Kasba	Kasba	G15534
	Abadina	Ib Ar 22388
	Marrakai	CSIRO 82
Vellore	Vellore	68886
	Bunyip Creek	CSIRO 87
D'Aguilar	D'Aguilar	B8112
	Nyabira	792/73
Petevo	Petevo	ArTB-2032

Classification of the Corriparta serogroup by complement fixation test (A.J. Main). The 4 described members of the Corriparta serogroup were compared by CF test as were 3 additional isolates from Australia supplied by Dr. T.D. St. George of the CSIRO, Brisbane. The results are presented in Table 13. Corriparta, Acado, Jacareacanga, and Bambari cross-reacted significantly, although showing 2- to 4-fold higher titers with the homologous antigen. CSIRO 134 could also marginally be distinguished from the 4 described members. The antibody preparations for CSIRO 76 and 109 were low-titered, not permitting interpretation.

Classification of the Great Island complex of the Kemerovo serogroup by neutralization test (A.J. Main). Viruses of the Great Island complex were compared by plaque reduction neutralization test. It was determined that Cape Wrath, Okhotskiy, Tindholmur, Mykines, Yaquina Head, Great Island, Bauline, Poovoot, and RML-85 are distinct serotypes. Kenai, Nugget, FinV808, FinV873, and FinV962 viruses have not yet been tested, but may also possibly be distinct serotypes. The results are shown in Table 14.

BUNYAVIRUSES

Classification of the Mapputta serogroup by complement fixation test and identification of AusAr96037 (A.J. Main). AusAr96037 virus was isolated from a pool of Anopheles annulipes from New South Wales and submitted by Dr. M.J. McCloonan. It was shown by CF tests to be a member of the Mapputta serogroup, similar or identical to Trubanaman virus. The CF reactions of AusAr96037 and of the Mapputta serogroup are demonstrated in Table 15.

Classification of the Tete serogroup by complement fixation, HI and neutralization tests, and identification of I 612045 (A.J. Main and G. Modi). I 612045 virus, from a mynah bird in India was referred by the National Institute of Virology, Pune, India. The virus appears to be a new Tete group virus by CF and HI tests; neutralization tests are not yet complete (Table 16). Each of the viruses in the group appears to be a distinct serotype but the definitive neutralization testing for at least 2 of the viruses awaits preparation of more potent sera or development of a more sensitive test.

Table 13

Complement-fixation tests comparing members of the Corriparta serogroup

Ascitic Fluid

Antigen	CORR	ACADO	JAC	BAM	134	76	109	Group CORR
Corriparta	64/16*	32/8	32/8	<8/<4	32/16	<8/<4	<8/<4	32/16
Acado	32/32	128/64	64/32	16/32	32/32	<8/<4	8/8	128/>64
Jacareacanga	16/8	16/8	128/32	16/16	8/8	<8/<4	<8/<4	32/32
Bambari	32/16	32/16	32/16	64/32	16/8	<8/<4	<8/<4	32/32
CSIRO 134	32/8	32/8	16/8	8/4	64/16	<8/<4	<8/<4	32/8
CSIRO 76	64/16	32/8	32/4	8/4	128/16	<8/<4	8/4	32/16
CSIRO 109	128/16	32/8	32/8	8/16	32/16	<8/<4	16/8	64/16

*reciprocal of serum titer/reciprocal of antigen titer.

Table 14

Kemerovo group. Plaque reduction neutralization tests comparing prototype strains of the Great Island complex, Kemerovo serogroup

	VIRUSES								
ASCITIC FLUIDS	CW	OKH	MYK	YH (15)	YH (62)	GI	BAU*	PVT*	RML-85
Cape Wrath	40-80	<10	<10	<10	<10	<10	<10	<10	<10
Okhotskiy	<10	160-320	<10	<10	<10	<10	<10	<10	<10
Tindholmur	<10	<10	<10	<10	<10	<10	<10	<10	<10
Mykines	<10	<10	40	<10	<10	<10	<10	<10	<10
Yaquina head (RML-15)	<10	<10	10	-	40-80	-	<10	<10	-
Yaquina head (RML-62)	<10	-	<10	<10	80	<10	-	<10	<10
Great Island	<10	<10	<10	<10	<10	80-160	<10	<10	<10
Bauline	<10	<10	<10	<10	<10	<10	40	<10	<10
Kenai	<10	<10	<10	<10	<10	<10	<10	<10	<10
Poovoot	<10	<10	<10	<10	<10	<10	<10	40	<10
Nugget	<10	<10	<10	<10	<10	<10	<10	<10	<10
FinV808	<10	<10	<10	<10	<10	<10	<10	<10	<10
FinV873	<10	<10	<10	<10	<10	<10	<10	<10	<10
FinV962	<10	<10	<10	<10	<10	<10	<10	<10	<10
RML-85	<10	<10	<10	<10	<10	<10	<10	<10	40

*reciprocal of highest ascitic fluid dilution inhibiting >70% of plaques; rest >80%.

Table 15

Results of complement-fixation tests comparing AusAr 96037 with other members of the Mapputta serogroup.

Ascitic Fluids

Antigen	- AusAr 96037	Trubanaman MRM 3630	Maprik MK 7532	Mapputta MRM 186	Polyvalent #3*
AusAr 96037	256/>512**	8/256	128/>128	8/256	512/>512
Trubanaman	256/64	16/64	256/64	16/64	512/128
Maprik	16/16	<8/<4	1024/128	32/64	-
Mapputta	32/>128	<8/<4	256/>128	128/>128	512/>128

*mice immunized with Koongol, Wongal, Ketapang, Mapputta, Trubanaman, and Maprik.

** reciprocal of serum titer/reciprocal of antigen titer.

Table 16. Serological tests comparing I 612045 with other Tete group viruses

Ascitic Fluids

-	Tete	Batama	Bahig	Matruh	Tsuruse
I 612045	SAAn4511	DakAnB1292	EgB 90	EgAn 1047	Mag 271580
Complement-fixation					
I	512/>256*	128/>256	128/>256	64/>256	32/8	<8/<4
TET	128/>128	1024/>128	512/>128	64/>128	32/32	16/8
BAT	128/>128	1024/>128	>1024/>128	64/>128	128/64	8/>128
BAH	256/16	<8/<4	128/128	256/>128	256/>128	16/8
MAT	<8/<4	<8/<4	16/16	256/>32	256/>32	<8/<4
TSU	32/>128	<8/<4	128/>128	64/>128	16/64	64/>128
Hemagglutination-inhibition					
I	>10240/8*	80/8	160/8	20/8	80/8	10/8
TET	10/2	40/2	320/2	<10/2	<10/2	<10/2
BAH	160/8	10/8	160/8	40/8	20/8	40/8
TSU	>10240/1	20/1	160/1	40/1	20/1	320/1
Plaque-reduction Neutralization					
I	1280*	<10	40	20	<10	<10
TET	<10	<10	<10	<10	<10	<10
BAT	<10	<10	640	<10	<10	<10
BAH	>320	<10	20	80	<10	<10
MAT	<10	<10	20	20	20	<10
TSU	160	<10	40	20	<10	160

*reciprocal of serum titer/reciprocal of antigen titer

*reciprocal of serum titer/HA units

*reciprocal of highest dilution reducing plaques by 90%.

II. IDENTIFICATION OF VIRUSES

Characterization of recent Indonesian and Israeli virus isolates made in mosquito cells (R.B. Tesh, J. Converse, J. Peleg, and D.L. Knudson). During the past 2 years, we have received approximately 165 virus isolates which were recovered from mosquitoes collected in Indonesia, Thailand, Japan and Israel. All of these agents were originally recovered in mosquito cell cultures (C6/36 clone of Aedes albopictus or AP-61 line of Aedes pseudoscutellaris) instead of the usual vertebrate isolation systems (i.e. newborn mice or mammalian or avian cells). This group of viruses produces viral cytopathic effect (CPE) in mosquito cells, but most of the isolates do not kill newborn mice or cause CPE in mammalian cells. Because of the frequency with which these viruses have been recovered, we accorded priority to their study and identification.

The largest group of these agents (153) was submitted by Dr. J. Converse, U.S. Naval Medical Research Unit NO.2, Jakarta, Indonesia. The 153 viruses were isolated from a variety of mosquito species collected on several different islands in the Indonesian archipelago (Sumatra, Kalimantan, Java, Bali and Flores). Table 17 summarizes the mosquito species processed and the number of viruses isolated.

Initially, spot slides were prepared using C6/36 cells infected with each of the isolates. The infected cells were then examined by indirect fluorescent antibody technique (IFAT), using a variety of arbovirus grouping sera. By this technique 9 of the Indonesian isolates were shown to be flaviviruses, later identified as Japanese encephalitis (Tables 18 and 19), and 5 were identified as members of the epizootic hemorrhagic disease of deer (EHD) serogroup. The remaining 138 agents did not react with any of the grouping sera tested (Group A, Group B, Australia group and Polyvalent #8). The latter viruses were then inoculated into cultures of C6/36 cells and each sample was examined by polyacrylamide gel electrophoresis (PAGE). About 40% of the Indonesian isolates were shown to be double stranded RNA viruses. Most had 10 RNA segments but a few had 9 and 12. The double stranded RNA viruses did not react by IFAT with any of the known arbovirus grouping sera, and their gel patterns appeared different from any of the recognized arboviruses. Some of these viruses gave identical RNA patterns in PAGE, suggesting that they were the same. Others were distinct by PAGE but cross-reacted by IFAT, indicating that they were distinct but members of the same serogroup. Two of the 4 virus isolates from Israel reacted in IFAT with several viruses from Indonesia, indicating antigenic similarity among viruses from widely distant regions.

Because of the avirulence of these viruses for mice amd mammalian cells we have had to work with them in mosquito cell cultures. Large quantities of representative viruses were grown in C6/36 cells which were subsequently harvested and purified to extract the virus. The resulting antigens have been used to prepare hyperimmune mouse ascitic fluids. Eventually these immune ascitic fluids will be used to type and to identify the virus isolates.

Table 17

Virus isolations made from Indonesian mosquitoes 1981-83

Genus	Species	Geographic Region: Bali	Java	West & South Kalimantan	East Kalimantan	Flores	Sumatra	Total
Aedes	albopictus	9*	-	-	2	-	143	154
	caecus	-	241	-	-	-	-	241
	lineatopennis	455	25	18	-	36	-	534
	paecilius	14	57	-	-	-	-	71
	scutellaris	-	-	-	-	15	-	15
	vexans	610	2,508 (1)	2	4	1,728	-	4,882 (1)
	(unidentified spp.)	-	-	2	75	-	-	77
	TOTAL	1,088	2,831 (1)	22	81	1,774	143	5,974 (1)
Armigeres	moultoni	1	-	-	-	-	-	1
	subalbatus	9	32	-	-	-	-	41
	TOTAL	10	32	-	-	-	-	42
Mansonia	annulifera	-	1,550	-	12	-	-	1,562
	bonneae/dives	-	-	24	57	-	20	101
	indiana	-	4	-	-	-	-	4
	uniformis	64	386 (1)	241	194 (1)	-	654 (3)	1,539 (5)
	TOTAL	64	1,940 (1)	265	263 (1)	-	674 (3)	3,206 (5)

*No. mosquitoes processed (No. virus isolates).

Table 17 (continued)

Virus isolations made from Indonesian mosquitoes 1981-83

Genus	Species	Geographic Region					
		Bali	Java	West & South Kalimantan	East Kalimantan	Flores	Sumatra	Total
Anopheles								
	aconitus	-	434 (1)	20	-	86	-	540 (1)
	annularis	2,945(1)*	618	-	-	168	-	3,731 (1)
	barbirostris	616	994	424	2	10	-	2,046
	indefinitis	-	17	-	-	-	-	17
	karwari	-	4	-	-	-	-	4
	kochi	850	68	-	-	8	132	1,058
	maculatus	-	-	-	-	1	6	7
	nigerrimus	-	127	1,366	68	-	109	1,670
	peditaeniatus	-	-	878	-	-	-	878
	pujutensis	-	-	1	-	-	-	1
	schueffneri	-	417	-	-	-	-	417
	subalbatus	-	198	-	-	-	-	198
	subpictus	4,348	2,093 (2)	-	-	864 (1)	-	7,305 (3)
	sundaicus	-	-	-	-	32	-	32
	tessellatus	687	197	86	-	13	-	983
	umbrosus	-	-	156	-	-	-	156
	vagus	34,455(15)	7,476 (7)	8	-	891 (1)	-	42,830 (23)
	(unidentified spp.)	-	-	3,630	-	-	8	3,638
	TOTAL	43,901(16)	12,643 (10)	6,569	70	2,073 (2)	255	65,511 (28)

*No. mosquitoes processed (No. virus isolates).

Table 17 (continued)

Virus isolations made from Indonesian mosquitoes 1981-83

Genus / Species	Geographic Region: Bali	Java	West & South Kalimantan	East Kalimantan	Flores	Sumatra	Total
Culex							
bitaeniorhynchus	1,335(1)*	1,054	17	24	4	6	2,440 (1)
fuscocephala	24,643(3)	6,663 (6)	6	-	71	3	31,386 (9)
gelidus	866	589 (1)	5,336	50	174 (1)	991	8,006 (2)
pseudosinensis	-	86	-	-	-	-	86
quinquefasciatus	135	349 (1)	95	115	15	73	782 (1)
sinensis	-	-	1,479	114	-	1,009	2,602
sitiens	-	13	36	-	-	56	105
tritaeniorhynchus	24,058(15)	14,046 (9)	6,941 (5)	2,168 (11)	2,787 (10)	1,113 (2)	51,113 (52)
vishnui	8,499(11)	17,355 (20)	653	1,319 (13)	861	621 (1)	29,308 (45)
whitae	-	-	-	-	-	4	4
whitmorei	9	-	-	-	-	-	9
(unidentified spp.)	-	100**	5,243 (7)	170	-	-	5,513 (7)
TOTAL	59,545(30)	40,255 (37)	19,806 (12)	3,960 (24)	3,912 (11)	3,876 (3)	131,354 (117)
Coquilletidia							
crassipes	-	-	1,430	41 (1)	-	-	1,478 (1)

*Number of mosquitoes processed (No. virus isolates obtained).
**One additional isolate made from a mixed pool of 50 Culex and Anopheles spp. from Java.

Table 18

Flaviviruses recovered from mosquito pools from Indonesia submitted to YARU by Dr. Jim Converse of NAMRU-2 Jakarta Detachment

JKT Arbo #	mosquito species	location	date collected
5441	Anopheles vagus	Bali	7 July 80
6468	Cx. tritaeniorhynchus	Flores	5-6 Jan 81
7003	Cx. vishnui	Java	15 Jun 81
7180	Cx. tritaeniorhynchus	Java	13 Jul 81
7887	Cx. tritaeniorhynchus	Java	26-27 Jul 81
8442	Cx. tritaeniorhynchus	Bali	10 Dec 80
9092	Cx. vishnui	Bali	25 Mar 81
6844	Cx.fuscocephala	Java	30 May 81
9744	Cx. vishnui	Java	29 Sep 81

Table 19

Microneutralization testing of flaviviruses recovered from pooled mosquitoes from Indonesia.

Mouse immune ascitic fluids	Reference Viruses					JKT Arbo #		
	JE	MVE	SEP	TMU	ZIKA	5441	7180	9092
Japanese encephalitis (JE)	320	40			<5	160	40	80
Murray Valley encephalitis (MVE)	5	10			<5	<5	<5	<5
Sepik(SEP)	<5	<5	40		<5	<5	<5	<5
Tembusu (TMU)	<5	<5		320	<5	<5	<5	5
Zika (ZIKA)	5	5			20	5	10	5
JKT Arbo # 5441	320	20			<5	160	ND	20

ND = not done

As more workers begin to use mosquito cells for primary isolation of arboviruses, undoubtedly additional viruses of this type will be recovered. It seems probable that many of these agents are mosquito viruses, possibly many of them represent new serogroups or even new families of viruses. We hope to determine in the near future whether these viruses are of any public health importance or whether they might hold some potential as mosquito control agents.

TOGAVIRIDAE

Association of Sindbis Virus with Ockelbo Disease in Sweden (R.B. Tesh and A.J. Main). Recently a new disease, characterized by fever, arthritis and rash, has been described in Sweden, Finland and adjacent regions of the Soviet Union. In these three countries, the disease is referred to as Ockelbo disease, Pogasta disease and Karelian fever, respectively. The disease occurs during the summer months mainly among picnickers, berry collectors and other persons entering wooded areas. Because of the similarity in the clinical symptoms between Ockelbo disease and the illnesses caused by several mosquito-borne alphaviruses (i.e. chikungunya, Mayaro, Ross River and o'nyong-nyong), the etiology of the new disease is thought to be an arthropod-borne virus. The demonstration of antibodies against Sindbis virus (Togaviridae: Alphavirus) in the convalescent sera of Swedish Ockelbo patients supports this view.

In the summer of 1982 Swedish workers isolated an unknown alphavirus from Culiseta mosquitoes collected in the Ockelbo endemic region. This agent (EDS-14) was submitted to us for study by Dr. A. Espmark, Dept. of Virology, National Bacteriological Laboratory, Stockholm, along with paired sera from 10 Ockelbo patients.

Hemagglutination-inhibition (HI), complement-fixation (CF) and plaque reduction neutralization (PRN) tests were done comparing these specimens with a variety of other alphaviruses and specific immune sera. The results are summarized in Tables 20, 21, and 22. By HI and CF tests, EDS-14 and Sindbis viruses are indistinguishable (Tables 20 and 21). By PRN test, there is only a slight difference between the two agents (Table 22). The paired sera of Ockelbo patients all showed a rise in titer in the convalescent specimens to both Sindbis and ESD-14 viruses (Table 23). In fact titers to both agents were similar. Results of these studies suggest that the Swedish mosquito isolate is a geographic variant of Sindbis virus. The serologic response of the 10 Ockelbo patients indicates recent infection with an alphavirus which is antigenically identical or closely related to Sindbis. For these reasons, we conclude that Ockelbo disease is caused by Sindbis virus or a closely related agent. Actually the symptoms described in Ockelbo patients are similar to those observed in the few reported human infections with Sindbis virus. The interesting epidemiologic question is why Sindbis virus, which is widely distributed throughout the Old World, only causes significant human infection and disease in Scandinavia?

Isolation of EEE from equine brains (A.J. Main and M. Fletcher). Viruses M-210-83 and M-236-83 were isolated from two of three equine

Table 20

Complement-fixation tests comparing Edsbyn 5/82 with other alphaviruses in the WEE-Sindbis complex

	Ascitic Fluid								
Antigen	Edsbyn 5/82	Sindbis	Whataroa	Kyzylagach	WEE	Y-62-33	Highlands J	Ft.Morgan	Aura
Edsbyn 5/82	256/16*	32/16	128/>32	0	0	0	0	0	0
Sindbis	256/>32	64/>32	64/>32	8/8	0	8/8	0	0	0
Whataroa	16/4	16/8	128/>32	0	0	0	0	0	0
Kyzylagach	0	16/4	16/32	16/16	0	0	0	0	0
WEE	32/>32	16/>16	32/>32	0	128/>32	128/>16	0	0	0
Y-62-33	64/>32	32/>16	128/>32	8/16	16/>16	128/>32	8/>16	0	0
Highlands J	0	0	128/>32	8/8	8/8	8/8	16/8	0	0
Ft.Morgan	0	0	16/>32	8/8	8/4	0	0	16/4	0
Aura	8/16	0	32/>16	8/8	8/4	8/>16	32/>16	0	32/>32

*Reciprocal of highest antibody (ascitic fluid) dilution/highest antigen dilution.

Table 21

Kinetic hemagglutination-inhibition tests comparing Edsbyn 5/82 with alphaviruses in the WEE-Sindbis complex

Antigen	Ascitic Fluid								
	Edsbyn 5/82	Sindbis	Whataroa	Kyzylagach	WEE	Y-62-33	Highlands J	Ft.Morgan	Aura
A. 10 minute incubation									
Edsbyn 5/82	80*	20							
Sindbis	40	20							
B. 1 hour incubation									
Edsbyn 5/82	>20480	160	5120	20	40	40	10	<10	<10
Sindbis	>20480	160	>20480	20	40	160	20	20	<10
Whataroa	2560	40	20480	10	20	40	10	10	<10
Kyzylagach	1280	80	1280	320	20	80	10	10	<10
WEE	640	640	1280	20	5120	1280	40	20	<10
Y-62-33	2560	80	>20480	40	640	1280	80	80	<10
Highlands J	160	80	1280	20	160	160	80	40	<10
Aura	40	<10	80	<10	<10	20	<10	<10	40
C. 20 hour incubation									
Edsbyn 5/82	>20480	320	5120	40	320	80	20	<10	<10
Sindbis	>20480	2560	>20480	40	160	320	80	20	<10
Whataroa	>20480	160	>20480	40	80	160	40	20	10
Kyzylagach	>20480	320	10240	640	80	160	20	<10	<10
WEE	>20480	80	>20480	40	>20480	>20480	320	80	10
Y-62-33	>20480	160	>20480	80	>20480	>20480	160	80	20
Highlands J	640	40	1280	<10	2560	1280	640	40	<10
Aura	40	<10	80	10	<10	20	<10	<10	80

*Reciprocal of serum titer.

Table 22

Results of plaque reduction neutralization tests with Edsbyn 5/82 and other selected alphaviruses

	Antiserum							
Virus	Edsbyn 5/82	Sindbis	Whataroa	Y-62-33	Kyzylagach	Chikungunya	Ross River	Mayaro
Edsbyn 5/82	320*	10	<10	<10	<10	<10	<10	<10
Sindbis	2,560	320	10	<10	<10	<10	<10	<10
Whataroa	20	<10	320	<10	<10	<10	<10	<10
Y-62-33	20	<10	NT	2,560	<10	<10	<10	<10
Chikungunya	<10	<10	NT	<10	NT	160	<10	40
Ross River	<10	<10	NT	<10	NT	<10	80	<10
Mayaro	<10	<10	NT	<10	NT	<10	<10	160

*Reciprocal of highest antiserum dilution producing 80% plaque inhibition.
NT=Not Tested.

brains submitted by the Department of Pathobiology at the University of Connecticut. The 2 viruses were identified as EEE by CF. These two horses exhibited typical signs of equine encephalitis. Virus was not isolated from the third horse and the cause of death could not be determined.

Paired blood samples taken less than 24 h apart, from a fatal case of equine encephalitis showed a four-fold increase in EEE antibody.

EEE and Highlands J viruses from New Jersey birds (A.J. Main and L. Lee). DV349, DV465, DV556, DV50, DV60, DV86, DV89, DV260, and WC415 were isolated from the blood of various species of birds collected in New Jersey by Dr. Wayne Crans. They were identified as strains of EEE by CF test.

DV447, DV448, and WC431, also from birds were identified by CF as Highlands J virus strains.

An apparently new flavivirus, Brest/Ar/T707 from ticks from France (A.J. Main). This virus was isolated by Dr. Claude Chastel of the Faculte de Medecine de Brest in France from Ornithodoros maritimus collected from a sea bird colony off the coast of Brittany. It is tentatively classified as a new Flavivirus, possible in the West Nile-Tyuleniy complex by complement-fixation and hemagglutination-inhibition (Table 24).

RHABDOVIRIDAE

Viruses from sandflies: two new vesiculovirus from Brazil (R.B.Tesh and A.J. Main). Two viruses, tentatively identified as members of the vesicular stomatitis virus (VSV) serogroup, were submitted for study by Amelia P.A. Travassos, Instituto Evandro Chagas, Belem, Brazil. These agents, BeAr 411391 and BeAr 411459, have been designated as Carajas and Maraba viruses, respectively. Both viruses were originally recovered from pools of phlebotomine sandflies (Lutzomyia spp.)collected at Serra Norte, Para State, Brazil, the site of large iron ore deposits in the Amazon Basin. Carajas virus was isolated from a pool of male sandflies.

Carajas and Maraba antigens and immune ascitic fluids were tested in complement-fixation and plaque reduction neutralization tests against 14 known members of the VSV serogroup. Results of these studies are summarized in Tables 25 and 26. They indicate that both Carajas and Maraba viruses represent new VSV serotypes, bringing the total number of known vesiculoviruses to 16. Maraba virus is especially interesting because of its close antigenic relationship with VSV-Indiana, Cocal and VSV-Alagoas viruses. The previously noted CF reactions of Keuraliba and LaJoya with other rhabdoviruses was not found in these tests.

An experiment was carried out to demonstrate the effect on the CF reaction of immunizing mice with multiple VSV serogroup viruses. One set of mice was immunized with types New Jersey, Indiana, and Cocal; another set with Carajas, New Jersey, Indiana, Cocal, Chandipura, and Isfahan; and still another set with Isfahan, Chandipura, and Piry. The results are shown in Table 27.

Table 23

Results of plaque reduction neutralization tests with an acute and convalescent sera of patients with Ockelbo disease

Patient number	Acute serum		Convalescent serum	
	Sindbis	Edsbyn 5/82	Sindbis	Edsbyn 5/82
1	<10*	<10	80*	80
2	<10	<10	20	20
3	<10	<10	40	80
4	<10	<10	40	20
5	<10	<10	20	20
6	<10	<10	40	20
7	<10	<10	20	40
8	<10	<10	40	80
9	10	<10	40	80
10	<10	<10	80	20
11	NT	NT	160	80
12	NT	NT	80	40
13	NT	NT	40	40

*Reciprocal of highest sera dilution producing ≥80% plaque reduction.

NT = Not Tested.

Table 24. Complement-fixation and hemagglutination-inhibition tests comparing Brest/Ar/T707 with other Group B viruses.

	BREST/AR/T707			
	ANTIGEN		ANTIBODY	
	CF	HI	CF	HI
	Ht/Ho	Ht/Ho	Ht/Ho	Ht/Ho
Murray Valley Encephal.	16/64	10/160	256/4096	640/8192
Tyuleniy (FinV-724)	16/128	40/1280	256/4096	5120/81920
Sepik	128/>1024	160/>10240	256/4096	320/81920
CSIRO 122	<8/64	<10/40	256/4096	160/81920
Saumarez Reef	128/256	80/160	128/4096	160/81920
Usutu	128/256	160/1280	128/4096	2560/81920
Tyuleniy (TAR)	64/256	160/>10240	128/4096	1280/81920
Banzi	<8/32	<10/20	128/4096	640/81920
Ilheus	64/512	10/520	128/4096	2560/81920
Weselsbron	<8/64	<10/40	128/4096	1280/81920
Apoi	<8/128	10/160	128/4096	160/81920
Edge Hill	<8/128	10/80	128/4096	160/81920
TBE. RSSE	64/128	20/1280	64/4096	80/81920
Tyuleniy (LEIV6c)	512/>1024	320/2560	64/4096	160/81920
Entebbe bat	<8/16	<10/40	64/4096	40960/81920
Israel turkey enceph.	64/256	320/640	64/4096	640/81920
Kadam	16/64	40/640	64/4096	1280/81920
Royal Farm	64/256	10/5120	64/4096	80/81920
Ntaya	<8/32	<10/160	64/4096	10240/81920
Stratford	<8/32	-	64/4096	-
Tembusu	<8/32	<10/160	64/4096	640/81920

Table 24 (continued) BREST/AR/T707

	ANTIGEN		ANTIBODY	
	CF	HI	CF	HI
	Ht/Ho	Ht/Ho	Ht/Ho	Ht/Ho
West Nile	64/512	10/>10240	64/4096	640/81920
Langat	16/128	10/640	64/4096	320/81920
Dakar bat	<8/64	<10/10	64/4096	80/81920
Sponweni	<8/64	<10/40	64/4096	40/81920
Saboya	32/512	160/10240	64/4096	10240/81920
Dengue 3	16/512	10/80	64/4096	80/81920
Bussaquara	<8/256	<10/80	64/4096	640/81920
Uganda S	32/64	<10/80	32/4096	640/81920
Dengue 4	32/64	<10/40	32/4096	320/81920
Kyasanur forest disease	64/256	10/160	32/4096	160/81920
Louping ill	64/512	40/640	32/4096	80/81920
Batu Cave	16/128	10/640	32/4096	1280/81920
Powassan	<8/64	<10/40	32/4096	80/81920
BeAn 3276000	32/512	10-	32/4096	-
TBE, Central European	32/1024	40/640	32/4096	160/81920
Kunjin	16/120	80/2560	16/4096	2560/81920
US bat salivary gland	128/1024	80/520	16/4096	320/81920
St. Louis encephalitis	128/1024	160/2560	16/4096	640/81920
Alfuy	16/256	20/160	16/4096	160/81920
Carey Island	16/256	40/-	16/4096	-
Jugra	32/512	20/640	16/4096	<10/81920
Negrishi	-	-	16/4096	40/81920
Cowbone Ridge	16/8	10/320	16/4096	320/81920

Table 24 (continued) BREST/AR/T707

	ANTIGEN		ANTIBODY	
	CF	HI	CF	HI
	Ht/Ho	Ht/Ho	Ht/Ho	Ht/Ho
Bukalasa bat	<8/64	10/40	8/4096	320/81920
Sokulu	-	-	8/4096	10/81920
Dengue 1	32/64	<10/80	< 8/4096	160/81920
Bouboui	64/256	160/320	< 8/4096	-
Kokobera	<8/32	<10/10	< 8/4096	160/81920
Japanese encephalitis	<8/32	<10/20	< 8/4096	321/81920
Dengue 2	8/64	<10/20	< 8/4096	< 10/81920
Phnom Penh	64/512	80/1280	< 8/4096	320/81920
Rocio	32/512	20/-	<8/4096	-
Modoc	8/128	10/80	<8/4096	40/81920
Yellow fever	8/128	10/320	<8/4096	640/81920
Montana Myotis Leucoenc.	68/>1024	<10/-	<8/4096	-
Tamana	<8/128	<10/80	<8/4096	<10/81920
Zika	16/512	20/160	<8/4096	320/81920
Jutiapa	16/512	10/320	<8/4096	160/81920
Yokose	-	-	<8/4096	20/81920
Aroa	-	-	<8/4096	<10/81920
Koutango	-	-	<8/4096	-
Karshi	-	-	<8/4096	-
Omsk hemorrhagic fever	16/-	20/-	-	-
Bagaza	128/-	40/-	-	-
polyvalent group B	32/-	4-/10-640	-	-

Table 25

Results of complement-fixation tests with 15 known or suspected vesiculoviruses

ANTIGEN	IMMUNE ASCITIC FLUID														
	VS-NJ	VS-I	COC	MBA	VS-A	CRJ	ISF	JUR	CHP	PIRY	JBD	PER*	LJ	P-S	KEU
New Jersey	256/>128**	16/64	8/32	16/32	0	32/>128	0	0	8/4	0	0	16/64	0	0	0
Indiana	0	1024/>128	256/>128	256/2128	16/>128	64/>128	0	0	8/4	0	0	8/16	0	0	0
Cocal	0	128/>128	1024/>128	512/2128	16/>128	64/>128	0	0	0	0	0	16/32	0	0	0
Maraba	0	256/>128	256/>128	2048/>128	32/>128	64/>128	0	0	0	0	0	16/32	0	0	0
Carajas	0	16/>128	16/>128	32/32	0	>1024/>128	0	0	0	0	0	16/16	0	0	0
Isfahan	0	16/16	0	32/32	16/32	16/32	256/>512	0	0	0	16/>32	16/>32	0	0	0
Jurona	0	0	0	0	0	0	16/16	128/128	0	0	0	16/32	0	0	0
Chandipura	0	0	0	0	0	0	0	0	64/64	0	0	8/4	0	0	0
Piry	0	0	0	0	0	0	0	0	0	256/>128	0	16/32	0	0	0
Jug Bogdanovac	0	16/64	0	0	0	0	0	0	0	0	512/ 128	8/8	0	0	0
Perinet	0	8/4	0	0	0	16/8	8/8	0	0	0	8/4	1024/>128	0	0	0
La Joya	0	0	0	0	0	0	0	0	0	0	0	8/16	32/>128	0	0
Porton-S	0	0	0	0	0	0	0	0	0	0	8/8	16/8	0	1024/32	0
Keuraliba	0	0	0	0	0	0	0	0	0	0	0	16/32	0	0	512/>128

* The Perinet ascitic fluid was anti-complementary to a dilution of 8/4.

** Reciprocal of antiserum titer/reciprocal of antigen titer.

Table 26

Results of plaque reduction neutralization tests with selected vesiculoviruses

Immune Serum

Virus	CJS	MBA	VS-NJ	VS-I	COC	JUR	PIRY	CHP	ISF	JBD	VS-A
Carajas	20,480*	10	10	10	0	0	0	0	0	0	0
Maraba	0	81,920	20	160	0	10	0	0	0	0	20
New Jersey	20	0	2,621,466*	10	0	0	0	0	0	0	0
Indiana	20	1,280	10	327,680	10	0	0	0	0	0	20
Cocal	20	320	0	20	5,120	0	0	0	80	0	20
Jurona	0	0	0	0	0	40,960	0	0	0	0	0
Piry	0	0	0	0	0	0	40,960	0	0	0	0
Chandipura	0	0	0	0	0	80	80	10,240	0	0	0
Isfahan	0	0	0	0	10	10	0	0	5,120	0	0
Jug Bogdanovac	0	0	0	20	0	0	0	0	0	10,240	0

*Reciprocal of highest antiserum dilution producing >95% plaque reduction. 0=<1:10

Table 27

Complement fixation tests of mice immunized with multiple VSV serogroup viruses

ANTIGENS	GROUP I	GROUP I &II	GROUP II
BeAr 411391	16/>128	>64/>128**	0
BeAr 411459	-	>64/>128	0
VSV-N.J.	256/>128**	>64/>128**	0
VSV-Ind.	256/>128**	>64/>128**	0
Cocal	256/>128**	>64/>128**	8/16
Isfahan	0	>64/>64**	>128/>128**
Perinet	0	32/32	16/32
Jug Bogdanovac	0	0	0
Jurona	0	16/64	-
Porton S	0	0	0
Chandipura	0	>64/32**	>256/>128**
Keuraliba	0	64/32**	0
LaJoya	0	0	0
Piry	0	> 64/>64	> 256/128**
Normal	0	0	0

**viruses used to immunize the mice

LaJoya and Keuraliba viruses did not react with these sera casting doubt on their membership in the VSV serogroup.

Electron microscopic examination of Vero cells infected with Carajas virus showed typical bullet-shaped virons within the cytoplasm. Maraba virus was not examined by EM.

Since BeAr 411391 as well as six other VSV group viruses (VSV-Indiana, Isfahan, Chandipura, Perinet, Maraba and Jug Bogdanovac) have been recovered from naturally infected phelbotomine sandflies, studies were done to determine if BeAr 411391 grew in Lutzomyia longipalpis. Female sandflies were inoculated intrathoracically and were fed suspensions of the virus. The insects were subsequently held at 25°C and sampled daily to determine if the virus replicated. Results of these experiments are shown in Tables 28 and 29. BeAr 411391 replicated in L. longipalpis after inoculation but disappeared after ingestion. BeAr 411391 virus was also recovered from the F1 progeny of experimentally infected female L. longipalpis, indicating transovarial transmission of the virus in sandflies.

Attempts to confirm a relationship of Charleville to other rhabdoviruses (A.J. Main). We had reported in the 1981 Annual Report that Charleville mouse ascitic fluid reacted by CF with Mossuril, Kamese, Bangoran, Barur, Cuiaba, Kern Canyon and Marco antigens. To try to confirm this relationship with the reciprocal Charleville antigen, it was tested with 35 grouping fluids and 255 specific sera including 47 rhabdoviruses. This antigen reacted with only those sera containing Charleville antibody. Complement was not fixed in the presence of Mossuril, Bangoran, Cuiaba, Kern Canyon, Marco, and Flanders specific ascitic fluids or group Hart Park, Bwamba-Nyando-Mossuril, Marco-Chaco-Timbo-Pacui, and Hart Park-Flanders-Kern Canyon-Klamath-Mt. Elgon bat grouping fluids. We could find no evidence (using Charleville antigen) of a "Charleville serogroup".

Flanders virus from Connecticut (A.J. Main). Ar-40-83, Ar-61-83, Ar-80-83, Ar-98-83, and Ar-99-83 were isolated from mosquitoes in Connecticut; all were identified as Flanders virus by CF test.

BUNYAVIRIDAE

Viruses from sandflies: a new Phlebovirus from Greece (R.B.Tesh and A.J. Main). This agent, designated PaAr 814, was isolated from sandflies collected in Corfu, Greece and was submitted for identification by Dr. Claude Hanoun, Institute Pasteur, Paris. In initial indirect fluorescent antibody tests, using spot slides of infected Vero cells, PaAr 814 viral antigen reacted with a number of phlebovirus antisera as well as with a phlebotomus fever grouping reagent. In complement fixation tests, PaAr 814 and Sicilian sandfly fever viruses were indistinguishable as shown in Table 30. However, by plaque reduction neutralization tests (Table 30), PaAr 814 and Sicilian viruses were antigenically distinct. Thus PaAr 814 represents a new member of the phlebotomus fever serogroup (genus Phlebovirus). Epidemics of sandfly fever have occurred in Greece during the past 50

Table 28

Growth of BeAr 411391 virus in Lutzomyia longipalpis following intrathoracic inoculation

Day post-inocultion	Range of virus titers in positive flies	Mean virus titer
0	1.4 - 1.9*	1.7*
1	3.0 - 3.6	3.4
2	2.8 - 4.0	3.7
3	3.3 - 4.4	3.9
4	4.3 - 5.3	4.9
5	4.6 - 6.0	5.1
6	4.4 - 6.2	5.3
7	4.0 - 5.2	4.6

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 29

Survival of BeAr 411391 virus in Lutzomyia longipalpis following ingestion by the insect

Day of post-feeding	Virus titers in sand flies sampled
0	3.0, 3.0, 3.2, 3.3, 3.4*
1	2.3, 2.6, 2.6, 2.7, 2.7
2	<0.7, 1.2, 1.2, 1.2, 1.4
3	<0.7, <0.7, <0.7, <0.7, 3.0
4	<0.7, <0.7, <0.7, <0.7, <0.7
5	<0.7, <0.7, <0.7, <0.7, <0.7
6	<0.7, <0.7, <0.7, <0.7, <0.7
7	<0.7, <0.7, <0.7, <0.7, <0.7

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 30

Complement-fixation and neutralization tests comparing PaAr814 and IssPhl 18 with other members of the Phlebotomus fever group.

Complement fixtion test

Ascitic Fluids

	PaAr814	Sicilian	Salehabad	IssPhl 18
PaAr814	512/32*	512/32	16/8	0
Sicilian	256/64	1024/64	0	0
Salehabad	0	0	256/>128	128/>64
IssPhl18	0	0	128/64	512/16

*reciprocal of serum titer/reciprocal of antigen titer
0 = <8/<4

Plaque reduction neutralization tests

Ascitic fluids

Virus	PaAr 814	Sicilian
PaAr 814	<1:160	1:10
Sicilian	1:10	<1:80

years, but it is unknown whether PaAr 814 virus causes disease in humans.

Identification of Murre virus (A.J. Main and T. Schwan). This strain was received from Dr. Conrad Yunker formerly of the Rocky Mountain Laboratory. Results of complement-fixation tests suggest that it is a new Uukuvirus (Table 31).

Viruses from French shrews (A.J. Main). Brest/An219 and Brest/An221 were recovered from shrews in France by Dr. Claude Chastel. The antigens submitted by Dr. Chastel were tested by complement-fixation against 45 grouping fluids; reactions were observed with the polyvalent Anopheles A, Anopheles B, Turlock ascitic fluid. The ascitic fluids prepared against these viruses in France indicate that it may be a member of the Turlock serogroup. Thus far, we have not been able to establish either strain in suckling mice at YARU.

Sakhalin group viruses from ticks from France (A.J. Main). Brest/Ar/T261 and Brest/Ar/T439, isolated from Ixodes uriae in France by Dr. Claude Chastel, are members of the Sakhalin serogroup, similar or identical to Avalon virus by complement-fixation test (Table 32). We have not been able to establish these strains in suckling mice at YARU; serological tests were performed with antigens and ascitic fluids prepared in France.

Cache Valley-related viruses from Connecticut and New Jersey (A.J. Main and L. Lorenz). Ar-560-79 virus was isolated from Aedes triseriatus in Connecticut and 14 strains were isolated from Aedes sollicitans from New Jersey. They have been identified as Cache Valley-like, but additional studies are underway comparing "Cache Valley-like" isolates from Connecticut, New Jersey, Ohio, Manitoba, and Ontario with the prototype strain from Utah.

REOVIRIDAE (Orbivirus)

RML-85 from a bird (A.J. Main and M. Fletcher). Work is progressing on the identification of RML-85, a strain of virus isolated from the blood of a murre (Uria sp.) in Alaska by Dr. Conrad Yunker of the Rocky Mountain Laboratory in Hamilton, Montana. RML-85 was previously shown to be a member of the Great Island complex of the Kemerovo serogroup by complement-fixation (YARU Annual Report 1982). Plaque-reduction neutralization tests comparing this virus with prototype strains of the Great Island complex are almost completed. Tests completed to date indicate that RML-85 is distinct from other members of the complex (Table 33).

Eyach virus from ticks collected in France (A.J. Main). Brest/Ar/577 and Brest/Ar/578 were isolated from Ixodes ricinus and Ixodes ventalloi taken from a rabbit in France by Dr. Chastel. They were identified by complement-fixation and neutralization tests as Eyach virus (Table 34).

POSSIBLE ARENAVIRIDAE

I 772366-17 from Indian bat ticks (A.J. Main and G. Modi). This virus was isolated from bat ticks (Ornithodoros pipirformis) in India by Dr. M.A. Sreenivasan. No reactions were observed in complement-fixation tests with a

Table 31

Complement-fixation tests comparing Murre virus with other members of the Uukuniemi serogroup.

	Murre Virus	
	Antigens Ht/Ho	Ascitic Fluid Ht/Ho
Uukuniemi	128/256	<32/2048*
EgAn 1825-61	32/>512	64/2048
Manawa	<8/>512	32/2048
Grand Arbaud	<8/>512	32/2048
Zaliv Terpeniya	8/512	<32/2048
Oceanside	<8/256	<32/2048
FT/254	8/512	<32/2048
Fin V-707	8/>512	<32/2048

*Murre ascitic fluid anticomplementary at 1:16

Table 32

Complement-fixation tests comparing Brest/Ar/T261 and Brest/Ar/T439 with Sakhalin group viruses.

	Brest/Ar/T261		Brest/Ar/T439	
	Antigen Ht/Ho	Antibody Ht/Ho	Antigen Ht/Ho	Antibody Ht/Ho
Avalon	512/>1024	64/128	1024/>1024	128/128
Clo Mor	-	-	<4/32	<4/128
Taggert	-	4/128	<4/>1024	<4/128
Tillamook	4/512	4/128	<4/512	<4/128
Sakhalin	-	4/128	<4/64	<4/128

Table 33. Complement-fixation and plaque-reduction neutralization tests comparing RML-85 with members of the Kemerovo serogroup.

	RML-85			
	ANTIGEN		ASCITIC FLUID	
	CF	PRNT	CF	PRNT
Kemerovo	64/256*	-	64/128	-
Tribec	64/64	-	64/128	-
Lipovnik	16/32	-	16/128	-
Cape Wrath	128/128	<10/40-80	64/128	<10/40
Okhotskiy	512/128	<10/160-320	32/128	<10/40
Tindholmur	512/128	-	32/128	<10/40
Mykines	32/64	<10/40	16/32	<10/40
Yaquine Head (15)	256/64	<10/40-80	64/128	-
Yaquina Head (62)	-	<10/80	-	<10/40
Great Island	128/128	<10/80/160	64/128	<10/40
Bauline	128/128	<10/40	64/128	<10/40
Kenai	128/32	-	64/128	-
Poovoot	256/128	<10/40	32/32	<10/40
Nugget	256/256	-	64/128	10/40
Chenuda	<16/256	-	<8/128	-
Mono Lake	<8/128	-	8/128	-
Huacho	16/128	-	16/128	-
Baku	64/>512	-	8/128	-
Sixgun City	16/128	-	<8/32	-
Wad Medani	<8/32	-	<8/128	-
Seletar	16/128	-	<8/32	-

*Heterologous serum titer/Homologous serum titer

Table 34
Complement-fixation tests comparing Brest/Ar/T577 and Brest/Ar/T578 with members of the Colorado Tick fever serogroup

	Brest/Ar/T578	Eyach-38	CTF
Brest/Ar/T577	256/16*	128/32	32/8
Brest/Ar/T578	64/64	64/64	32/32
Eyach-38	64/32	64/64	16/16
CTF	16/16	16/16	64/≥128

*Reciprocal of serum titer/reciprocal of antigen titer.

battery of antigens and ascitic fluids (Table 35). Virus particles observed by electron microscopy resembled arenaviruses, but the antigen failed to fix complement in the presence of LCM, Amapari, Junin, Machupo, Pichinde, Tacaribe, and Tamiami ascitic fluid or Tacaribe grouping fluid. This strain has been named Muroor virus after type locality.

CORONAVIRIDAE

M-1-83 from a bat captured in Connecticut (A.J. Main). This virus, presumably isolated from the brown fat of a silver-haired bat in Connecticut, has been tentatively identified as mouse hepatitis virus, but additional studies are necessary.

UNCLASSIFIED

CSIRO 704 virus from ticks collected in Australia (A.J. Main). This virus was isolated from Argas robertsi collected in Australia by Dr. Toby St. George. Serologic relationships have not been detected by complement-fixation tests (Table 35). It has been tentative classified as a new ungrouped virus and named Lake Clarendon.

Ethiopian isolates (M. Fletcher with A. Smith). Eleven isolates, 5 from the blood of vertebrates and 6 from arthropods, from Ethiopia were selected for identification. Preliminary studies with these isolates were done by Dr. Owen Wood when he was stationed at NAMRU 3 in Egypt and later at YARU. Further studies are now underway (Table 36).

Identification of viruses from Thai mosquitoes (A.J. Main, R. Tesh, L. Lorenz, and M. Fletcher). Three strains - BKM-1028-70, BKM 1122-70, and BKM-1173-70 -failed to react by complement-fixation tests with 33 grouping fluids. These strains were isolated from Aedes mosquitoes and referred to YARU by the AFRIMS laboratory in Bangkok.

RML 64423-8 from Tanzanian ticks (A.J. Main). This virus, from Argas brumpti collected in Tanzania and submitted to YARU for identification by Dr. Conrad Yunker, did not show any serological relationship by complement-fixation with 35 grouping fluids, 85 togaviruses, 42 bunyaviruses, 51 reoviruses, 47 rhabdoviruses, and 30 other viruses. It appears to be a new ungrouped virus.

O. coriaceus '76 from California ticks (A.J. Main). This isolate, from Ornithodoros coriaceus collected in California and submitted by Dr. Yunker, also failed to react with any other sera listed under RML 64423-8. This is tentatively designated as a new ungrouped virus.

III. DIAGNOSIS OF DISEASE

Diagnosis of eastern encephalitis by brain biopsy (A.J. Main, G.H. Tignor and M. Fletcher). Hu-3-83 virus was isolated from brain biopsy in suckling mice and identified by CF test as eastern encephalitis virus. The brain material from a 9-month old girl was submitted by Dr. Warren Andiman of Yale Department

Table 35

Antisera used in CF tests with RML 64423-8, Brest/Ar/T261 and T439, I772366-17, CSIRO704, O. coraceus '76, Brest ArT577 and T578, and M-1-83F

GROUPING FLUIDS:

Group A (NIH G209-701-567)
Group B 8/6/80
Group B 12/11/66
Group Vesicular Stomatitis (NIH G204-701-567)
Group Bunyamwera (NIH G205-701-567)
Group California (NIH G206-701-567)
Group Kemerovo 4/71
Group Tacaribe 5/23/68
Group Guama 5/10/66
Group Sakhalin 2/22/74
Group Hughes 4/23/74
Group Phlebotomus Fever 2/72
Group Phlebotomus Fever, New World 2/5/83
Group Phlebotomus Fever, Old World 2/5/83
Group Hart Park 3/19/71
Group Uukuniemi 3/5/76
Group Capim 6/17/66
Polyvalent CCHF-HAZ-GAN-DUG-BHANJ 7/5/74
" QUAR-BAND-KAI 4/9/69
" Rabies-LCM 1/7/69
" JA-QUAR-KAI-LANJ-BAND-QAL-SIL 6/10/68
" Bwamba-Nyando-Mossuril 4-5/71
" Rabies-LCM-Herpes-NDV-Vaccinia 3/28/74
" ALM-BEL-CHV-JAP-MR-WALL-WARR-WON 7/29/76
" Corriparta-Palyam, etc. 7/70
" Anopheles A- AnophelesB- Turlock-Lukuni-SpAr 395 6/17/66
" KOO-WON-BAK-KET-MAP-TRU-MAPRIK 5/70
" NYA-UUK-GA-THO 5/71
" Hughes-Sawgrass-MAT-Lone Star-Soldado 5/71
" Marco-Chaco-Timbo-Pacui 2/27/81
" Hart Park-Flanders-Kern Canyon-Klamath-Mt Elgon Bat 6/71
" BTU-EHD-Ib22619-CGL-IRI-CTF 10/70
" NAV-TNT-ARU-PCA 1/71
" UPO-DGK-WAN-DHO 9/71
" OKO-OLI-WIT-TAT-Dak1569 11/71

ANTIBODY TOGAVIRIDAE

Alphavirus

Eastern equine encephalomyelitis 5/28/74
Western equine encephalomyelitis (McMillan) 12/14/73
Highlands J (B230) 11/22/74
Fort Morgan (CM 4-146) 12/81
Y 62-33 11/21/67

Table 35, Alphavirus (continued)

Sindbis (EgAr 339) 11/76
Sindbis (Zim Bat Pool A/81) 12/1/81
Sindbis (Ockelbo ?) 5/11/83
Whataroa (M 78) 6/3/83
Kyzylagach (LEIV 65A) 6/3/83
Aura (BeAr 10315) 9/15/65
Middelburg (SAAr 749) 4/27/67
Ndumu (SAAr 2211) 1966
Semliki Forest (Smithburn) 4/23-27/73
Chikungunya (Ross) 4/20/66
O'nyong-nyong (Gulu) 8/15/66
Getah (MM 2021) 7/13/66
Bebaru (MM 2354) 3/11/66
Ross River (T 48) 11/18/69
Mayaro (BeH 256) 3/30/83
Una (BeAr 13136) 4/27/67
Venezuela equine encephalitis (Beck & Wyckoff) 3/11-24/75
Mucambo (BeAn 8) 3/22/74
Pixuna (BeAr 356465) 4/27/67

Flavivirus

Royal Farm (EgArt 285) 1/27/71
Royal Farm (EgArt 371) 1/12/72
Powassan (Byers) 10/26/66
Louping Ill 2/20/74
Kunjin (MRM 16) 5/66
Omsk (Bogo) 11/28-29/67 (mouse sera)
Tick-borne encephalitis, central European 11/3-6/64 (mouse sera)
Saumarez Reef (CSIRO4) 7/14/76
Tyuleniy (Fin V724) 12/23/75
Langat (TP21) 6/20/66
Kysanur Forest disease (W371) 7/11/66
Kadam (AMP 6640) 11/10/69
Tick-borne encephalitis, RSSE (Sophy) 1/31/66
Karshi (LEIV 2247US) 5/2/75
Brest/Ar/T707 8/25/82
West Nile (B956) 11/25/69
Wesselsbron 8/20/65
Usutu (SAAr 1776) 8/23/65
Tembusu (MM 1775) 9/23/66
Stratford (C 388) 7/27/66
Spondweni (SAAr94) 11/29/65
SLE (Parton) 1/29/74
Sepik (MK 7148) 10/9/70
Ntaya 5/23/66
Murray Valley (MVE/1/1951) 10/12/66
Bouboui(Dak Ar490) 7/9/73
Banzi (H336) 1/16/68
Alfuy (MRM 3929) 2/13/67 (mouse sera)
Kokobera (MRM 32) 1/16/68
Japanese encephalitis 7/17/68

Table 35, Flavivirus (continued)

Ilheus (BeH 7445) 1/31/58 (mouse sera)
Edge Hill (C 281) 12/14/67
Dengue 4 (H 241) 2/2/66
Dengue 3 (H 87) 11/6/67
Dengue 2 (New Guinea C) 3/6/73
Dengue 1 (Th S Man) 4/4/66
Yellow Fever (4090) 5/5/82
Yellow Fever (17D) 7/18/69
Saboya (Dak An D 4600) 12/22/69
Bussuquara (BeAn 4073 6/1/66
Ar 19786 12/16/80
Modoc (M544) 5/18/66
Jutiapa (JG128) 5/30/72
Israel Turkey encephalitis 6/20/66
Cowbone Ridge (W10986) 7/27/66
Apoi 4/13/67
Phnom-Penh (Cam A38D) 9/21/71
Entebbe bat (I1-30) 1/16/68
Dakar bat (IPD/A 249) 4/17/67
Jugra (P9-314) 12/23/81
U S Bat salivary gland 1/26/74
Montana Myotis leucoencephalitis 2/9/74

ANTIBODY-BUNYAVIRIDAE

Bunyavirus

Cache Valley (6V633) 12/22/82
Murutucu (BeAn 974) 5/18/66
California encephalitis (BFS 283) 10/20-25/78 (hamster sera)
Jamestown Canyon (61V2235) 10/17-19/78 (hamster sera)
Trivittatus 10/20-24/78 (hamster sera)
Keystone (B64-558705) 11/21/75 (hamster sera)
Snowshoe Hare 10/20-22/78 (hamster sera)
Koongol (MRM 31) 11/21/67
Wongal (MRM 168) 4/27/67
Pahayokee (Fe 3-52F) 5/12/67
Shark River (Fe 4-1R) 5/3/67
Tete (SAAr 4511) 11/20/67
Bahig (EgB90) 12/19/66
Matruh (EgAn 1047) 5/12/67
Batama (Dak An B1292) 2/15/82

Phlebovirus

Sicilian Sandfly Fever 6/18/82
Salehabad (I 18) 7/13/66
Arbia (ISS.Phl.18) 8/5/82
PA Ar814

Table 35 (continued)

Uukuvirus

Manawa (Argas T461) 1/27/76
Grand Arbaud (Argas 2) 1/20/76

Nairovirus

Congo (Dak 8194) 9/30/74
Sakhalin (LEIV 71c) 6/15/74
Avalon (Can Ar173) 6/15/74
Clo Mor (Scot Ar7) 6/25/74
Tillamook (RML 86) 10/8/73
Taggert (Aus MI-14850) 11/19/81
Hughes 5/3/74
Farallon 5/4/74
Zirqa (A 2070-1) 6/7/77
Soldado (TRVL 52214) 5/4/74
Punta Salinas (Cal Ar888) 5/3/74
Sapphire II 7/2/77
Qalyub (Eg Ar370) 5/15-16/80 (mouse sera)
Brest/Ar/T439 1/22/82 (mouse sera)
Brest/Ar/T261 11/21/80 (mouse sera)

genus unknown

Bakau (MM 2325) 8/3/66
Ketapang (MM 2549) 4/25/66
Mapputta (MRM 186) 3/21/66
Maprik (MK 7532) 10/16/70
Trubanaman (MRM 3630) 6/16/67
Aus 96037 3/14/83

ANTIBODY - REOVIRIDAE

Orbivirus

Alaska Bird Blood (RML085) 11/24/82
Bauline (Can Ar14) 6/6/72
Cape Wrath (Scot Ar20) 11/11/81
Fin V808 12/31/75
Fin V808 12/31/75
Fin V962 12/31/75
Great Island (Can Ar41) 5/9/72
Kenai (RML 71-1629) 11/2/81
Mykines (Den Ar12) 10/16/74
Nugget (Aus MI-14847) 3/7/73
Okhotskiy (LEIV 70c) 12/17/74
Poovoot (RML 57493-71) 10/12/81
Tindholmur (Den Ar2) 10/6/74
Kemerovo (R10) 6/24/68 (mouse sera)

Table 35, Orbivirus (continued)

Yaquina Head (RML 15) 5/7/72
Baku (LEIV 46A) 12/17/74
Chenuda (EgAr 1152 5/26/66
Huacho (Cal Ar883) 2/25-26/69 (mouse sera)
Mono Lake (Cal Ar861) 10/22/81
Sixgun City (RML 52451) 10/27/81
Brest/Ar/T222 3/14/80
UK FT 363 2/19/81
Kemerovo (EgAn 1169-61) 5/22/67
Lipovnik (Lip 91) 2/19/82
Tribec 7/11/66
Wad Medani (Eg Ar492) 8/23/66
Wad Medani (Jamaica Tick) 12/8/81
Seletar (Mal SM-214) 12/8/81
Palyam (IG 5287) 11/20/67
CSIRO Village (CSIRO 11) 4/8/82
Kasba (IG 15534) 4/8/82
Abadina (Ib Ar22388) 4/8/82
Marrakai (CSIRO 82) 4/8/82
Vellore (I 68886) 10/16/68
Bunyip Creek (CSIRO 87) 7/22/82
Dak Ar K58 4/22/82
D'Aguilar (Aus B8112) 6/13/69
Nyabira (Rd An792/73) 12/12/80
Petevo (Dak Ar B2032) 4/8/82
Acado (Eth Ar1846-64) 11/5/82
Bambari 1/4/83
Corriparta (Aus MRM 1) 11/5/82
Jacareacanga (Eb Ar295042) 11/5/82
CSIRO 109 11/5/82

Orbivirus cont.

CSIRO 76 10/29/82
CSIRO 134 11/5/82
EHD-New Jersey 11/20/67

Coltivirus

Colorado Tick Fever (Condon) 7/13/66
Eyack-38 5/30/82
Saulges (Brest/Ar/T577) 1/11/82
Saulges (Brest/Ar/T578) 1/31/82

Table 35 (continued) ANTIBODY - RHABDOVIRIDAE

Vesiculovirus

VSV-Indiana (VP 98F) 6/18/81
VSV-New Jersey 4/24/69
Cocal (Tr 40233) 7/18/66
Chandipura (I 653514) 12/5/65
Isfahan (I Ar91026-167) 6/18/81
Jug Bogdanovac (YU 4/76) 6/22/81
Jurona (Be Ar40578) 7/6/81
Keuraliba (Dak An D5314) 8/10/81
LaJoya (J 134) 11/1/79
Piry (Be An24232) 7/23/81
Porton S-1643 8/26/82
Be Ar 411391 6/83
VSV-Alagoas
Perinet (Dak Ar Mg 802) 2/28/81

Lyssavirus

Lagos Bat

Other rhabdoviruses

Charleville (Aus CH9824) 6/28/82
Bangoran (Dak Ar B2053)
Barur (I 6235)
Cuiaba (Be An227841) 2/28/79
Kamese (MP 6186)
Kern Canyon (M-206)
Marco (Be An40290)
Mossuril (Sa Ar1995)
Kwatta (A-57) 11/17/67
Hart Park (USA Ar 70) 8/15/66
Flanders (NY 61-7484) 8/25/69
Mosqueiro (Be Ar185559) 9/22-27/77
Sawgrass (64A-1247)
New Minto (0579)
Connecticut (Ar-1152-78) 8/5/79
Chaco (Be An42217) 11/21/67
Sena Madureira (Be An303197)
Aruac (TRVL 9223) 11/21/67
Bovine Ephemeral Fever (Ib An59689) 10/25/72
Gray Lodge (BFN 3187)
Inhangapi (Be Ar177325) 2/11/76
Joinjakaka (Aus MK 177325) 12/10/70
Mt. Elgon Bat (BP 846) 3/24/69
Navarro (Cali 874) 8/29/66
Poona 733646
Yata (Dak Ar B2181) 9/21/81
Kimberley 4/8/82

Table 35 (continued)

ANTIBODY - Miscellaneous and unclassified viruses

Mouse Hepatitis Virus (Ar-232-77) 10/27/77
Mouse Hepatitis Virus (Ar-159-77) 10/27/77
Bocas (BT 25) 2/7/74
Ectromelia (Ib An34325) 1/2/70
Newcastle Disease Virus 10/24/69
Newcastle Disease Virus (JKT H 2541) 2/16/82
Herpes (M-2513b) 5/14/71
Microtus (NY 64-7947) 11/14/74
C. gapperi (NY 64-7855) 11/22/74
Cotia (Sp An232) 2/16/71
O. coriaceus '76 5/1/81
Argas brumpti (RML 64423-8) 12/23/76
Argas brumpti (RML 64423-8) 2/23/82
Hilo (Conn An114) 7/69
Y. tularensis (Can 50-51) 8/16/81 (rabbit sera) not a virus
Yogue (Dak An5634)
BKM 1122-70
Lake Clarendon (CSIRO 704) 11/21/81
Lake Clarendon (CSIRO 704) 5/14/81
I 772366-17 7/19/82
Upolu (C 5581) 4/23/74
Aransas Bay 10/19/81
Quaranfil (Ar 1113) 11/20/67
Johnston Atoll 1/17-18/67 (mouse sera)
Issyk-Kul (LEIV 315k) 6/18/80
Keterah (P6-1361) 10/22/81
Chobar Gorge (I 701700-8) 5/3/74
Dhori (I 611313 clone 3) 11/2/81
Dhori (Po Ti461)
I 64434 7/69

Table 36

Identification of viruses isolated in Ethiopia.

strain	host	murine virus contaminants: serology*	murine virus contaminants: isolation attempts**
Ar 1618	Mansonia uniformis	MVM, GD VII	negative
Ar 3201	Culicoides spp.	MVM, GD VII	negative
Ar 1180	Anopheles spp.	not tested	GD VII
An 792	Arvicanthis sp	MVM, GD VII Sendai, MHV-SDA	GD VII
Ar 662	Rhipicephalus spp.	not tested	not tested
Ar 4848	mosquitoes	Sendai, MHV GD VII	not tested
An 3490	Arvicanthis sp.	MVM, GD VII	negative
Ar 3102	Amblyomma cohaerens	MVM, GD VII, Reo-3	negative
An 3530	Boubou (bird)	MVM, GD VII, PVM	negative
An 4255	rodent	MVM, GD VII, Reo-3	CPE in L cells
An 3024	rodent	MVM, GD VII, Sendai, MHV-SDA	not tested

*on ascitic fluids prepared in Ethiopia

** From crude mouse brain antigens

of Pediatrics. Immunofluorescence testing of the brain using mouse hyperimmune ascitic fluids to SLE, LAC, WEE, herpes simplex, and EEE viruses was positive only to EEE. The diagnosis was available within 4 hours of receipt of the specimen. The child also had EEE HI antibody in the initial hospitalization serum.

Acute and convalescent sera from three other encephalitis patients also submitted by Dr. Andiman were negative by CF and HI against a battery of antigens.

Interim report on arbovirus and African hemorrhagic fever virus serologic testing of human sera from Hopkins AIDS study (G.H. Tignor). Coded samples of cobalt-irradiated human sera collected from AIDS patients, suspect AIDS patients, and non-AIDS patients were sent to YARU for testing against arboviruses. Those human sera which we had in sufficient quantity have been tested for antibody to a variety of arboviruses which are known to cause human disease in various zoogeographic regions of the world. We have assayed these sera for antibodies reacting by immunofluorescence to tissue culture cells infected with both group A (alphaviruses) and group B (flaviviruses) togaviruses (spot slides). Virus-infected cells are dispensed onto printed slides, human serum is added followed by a fluorescein-isothiocyanate conjugated anti-human globulin. Routinely, cells infected with a variety of viruses from the same antigen group are mixed together and added to one printed slide. This technique effectively increases the number of viruses which can be tested at one time. This is a standard procedure which as been widely used during sero-surveys in recent years. The advantage of this technique is that, by choosing viruses carefully, one can detect not only homologous antibody, but heterologous antibody to a wide range of viruses. Thus, sera negative on a given polyvalent slide are negative to all known viruses belonging to the serologic group. Human sera have been tested for antibody to polyvalent group A viruses including WEE, VEE, chikungunya, and EEE; polyvalent group B viruses (tick-borne) including Powassan, Langat, Karshi, Kadam, and Tyuleniy; polyvalent group B viruses including St. Louis, Roccio, JBE, Sepik, dengue serotypes, and yellow fever. There were three positive reactions to group B mosquito-borne viruses. See Table 37 for details.

Human sera were also tested with minute virus of mice (MVM) and with spot slides for antibody to African hemorrhagic fever (CRE_2LM) viruses. These slides contained cells infected with the following viruses: Crimean-Congo hemorrhagic fever, Ebola (Zaire strain), Ebola (Sudan strain), Rift valley fever virus, Lassa fever virus, and Marburg virus. In addition human sera were tested for antibody to the agent causing Korean hemorrhagic fever. There were four positive reactions on the CRE_2LM slides. See Table 37 for details.

Pediatric inpatients with neurologic illnesses, Sumber Waras Hospital, Jakarta, Indonesia (J.G. Olson). A total of 108 patients with acute and convalescent sera was tested for serologic evidence of infection with viral agents which had potential for causing the neurologic illnesses observed. Flaviviruses were implicated (either very high titer or 4-fold rise in HI titer) as a probable cause of illness in 8 (7%) of 108 patients tested. The results of neutralization tests are shown in Table 38. Confirmation of dengue viral infections were made (4 fold rise in NT assay from acute to convalescent phase) in 1 of the 8. There was no evidence of Japanese encephalitis or Zika viral infections.

Table 37

RESULTS OF SEROLOGIC TESTING OF HUMAN SERA FROM HOPKINS AIDS STUDY

SERUM NUMBER	MONOVALENT OR POLYVALENT SLIDE					
	MVM	CRE2LM	POLY A	POLY B TICK	POLY B MOSQUITO	KHF
1051	0	0	0	0	0	0
2614	0	0	0	0	0	0
2182	0	+	0	0	0	0
3624	0	0	0	0	0	0
1305B	0	+	0	0	+	0
1262	0	0	0	0	0	0
1849	0	0	0	0	0	0
3247	0	0	0	0	0	0
1595	0	0	0	0	0	0
266	0	0	0	0	0	0
2380	0	0	0	0	0	0
1304	0	0	0	0	0	0
3576	0	+	0	0	+	0
1059	0	+	0	0	0	0
1943	0	0	0	0	0	0
1717	0	0	0	0	0	0
3739	0	0	0	0	0	0
1726	0	0	0	0	0	0
3267	0	0	0	0	0	0
1742D	0	0	0	0	+	0
2030	0	0	0	0	0	0
1346	0	0	0	0	0	0
2805	0	0	0	0	0	0
1301	0	0	0	0	0	0
2432	0	0	0	0	0	0
3402	0	0	0	0	0	0
2831	0	0	0	0	0	0
3703	0	0	0	0	0	0
1286	0	0	0	0	0	0
2648	0	0	0	0	0	0
1523	0	0	0	0	0	0
2650	0	0	0	0	0	0

*Sera were tested at a 1:4 dilution (+) means positive immunofluorescence while (0) means that no immunofluorescence was detected.

Table 38

Serologic test results of encephalitis patients with evidence of flaviviral infection

Serum		HI (JE) titer	NT titer DEN-1	DEN-2	DEN-3	DEN-4	JE	Zika
10314	A	≥640	-	160	80	20	<5	<5
	C	≥640	-	≥640	80	160	<5	<5
10469	A	<10	<10	<10	<10	<5	<5	<5
	C	160	<10	40	<10	10	<5	<5
10516	A	20	<5	20	< 5	<5	<5	<5
	C	160	5	≥320	10	20	<5	<5
10587	A	40	<20	<10	10	<5	<5	<5
	C	≥640	<5	100	100	<5	<5	<5
10590	A	320	<5	<5	<5	<5	<5	<5
	C	320	<10	<5	<5	<5	<5	<5
10595	A	<10	<20	<20	<20	5	<5	<5
	C	80	20	20	40	5	<5	<5
10629	A	160	20	40	40	40	<5	<5
	C	160	5	160	40	≥640	<5	<5
10751	A	<10	-	-	-	<5	<5	<5
	C	40	20	20	40	<5	<5	<5
10787	A	<10	-	<40	<4	<5	<5	<5
	C	80	-	80	80	20	<5	<5
10788	A	<10	-	<40	<40	<4	<5	<5
	C	20	-	<40	<40	<4	<5	<5

Samples of the patients' paired sera were tested by HI for evidence of infection with mumps and measles virus. Fifteen (41%) of 37 patients tested showed evidence of a diagnostic rise antibody titer for measles virus. Four (11%) of 37 patients showed evidence of infection with mumps virus. Further testing with varicella is planned.

Measurement of IgM class antibody for rapid diagnosis of dengue infection (J.G. Olson). A series of 10 isolation confirmed dengue patients from Indonesia were tested for evidence of IgM class antibody. The first objective was to determine if a diagnosis could be made by testing a single serum specimen and the second was to evaluate whether the identity of the serotype could be determined.

Affinity chromatography purified goat anti-human IgM obtained from TAGO Inc, Burlingame,CA was diluted in PBS (pH 7.4) and allowed to incubate overnight at 4°C in 96 - well Immunolon II immunoassay plates obtained from Dynatech Inc. After 3x washing in PBS-Tween, dilutions of convalescent sera from isolation confirmed dengue patients were added and allowed to incubate 2 hours at 37°C. Controls included sera from individuals without evidence of previous dengue infection and sera from dengue IgG positive persons based on neutralization studies. After 3x washing in PBS, suckling mouse brain hemagglutinating antigen (HA) prepared to all 4 serotypes of dengue and normal mouse brain were diluted in PBS-Tween and 5% horse serum and 1% dextran sulfate and incubated 1 hour at 37°C. After 3x washing with PBS-Tween a mouse monoclonal antibody to dengue viral serotypes (WRAIR #4G-2-4-15) was added and incubated for 1 hour at 37°C. After 3x washing with PBS-Tween, an affinity chromatography purified goat anti-mouse IgG conjugated with peroxidase obtained from Tago,Inc. was incubated for 1 hour at 37°C. After 3x washing with PBS-Tween the substrate ABTS was added and a color change observed and measured by Titertek Multiskan. Titers were given as the highest dilution which differed significantly from the mean and 3 S.D. of the negative control sera.

IgM was detected in all 10 of patient convalescent sera. In 7 of 10 the reaction was specific to the serotype isolated. In the remaining 3 the isolated serotype gave an equal response to another serotype (in all 3 cases DEN-4). Additional details are discussed in the original antigenic sin section (below).

Clearly, the IgM capture EIA provides a sensitive means of diagnosing dengue infections and may be useful in determining the infecting serotype in the absence of an isolate.

Original antigenic sin in dengue fever patients (J.G. Olson). Acute and convalescent phase blood specimens were examined from 10 Indonesian subjects with uncomplicated dengue fever. Dengue virus (DEN) was recovered from the acute phase serum of each patient by mosquito inoculation and the virus identified by complement fixation test. Acute phase sera were tested by hemagglutination inhibition (HI) and microneutralization (NT) to determine if patients had been previously infected with dengue virus (Table 39). Patients who had dengue HI or NT antibody in their acute phase sera were classified as reinfections. Convalescent phase sera were assayed for antibodies by NT, HI, indirect immunofluorescence (IFA), and enzyme immunoassay (EIA).

Figure 5 shows that each of the 4 subjects classifed as experiencing a reinfection, based on preexisting antibody in the acute phase serum, had highest

TABLE 39

Acute Phase Antibody Titers

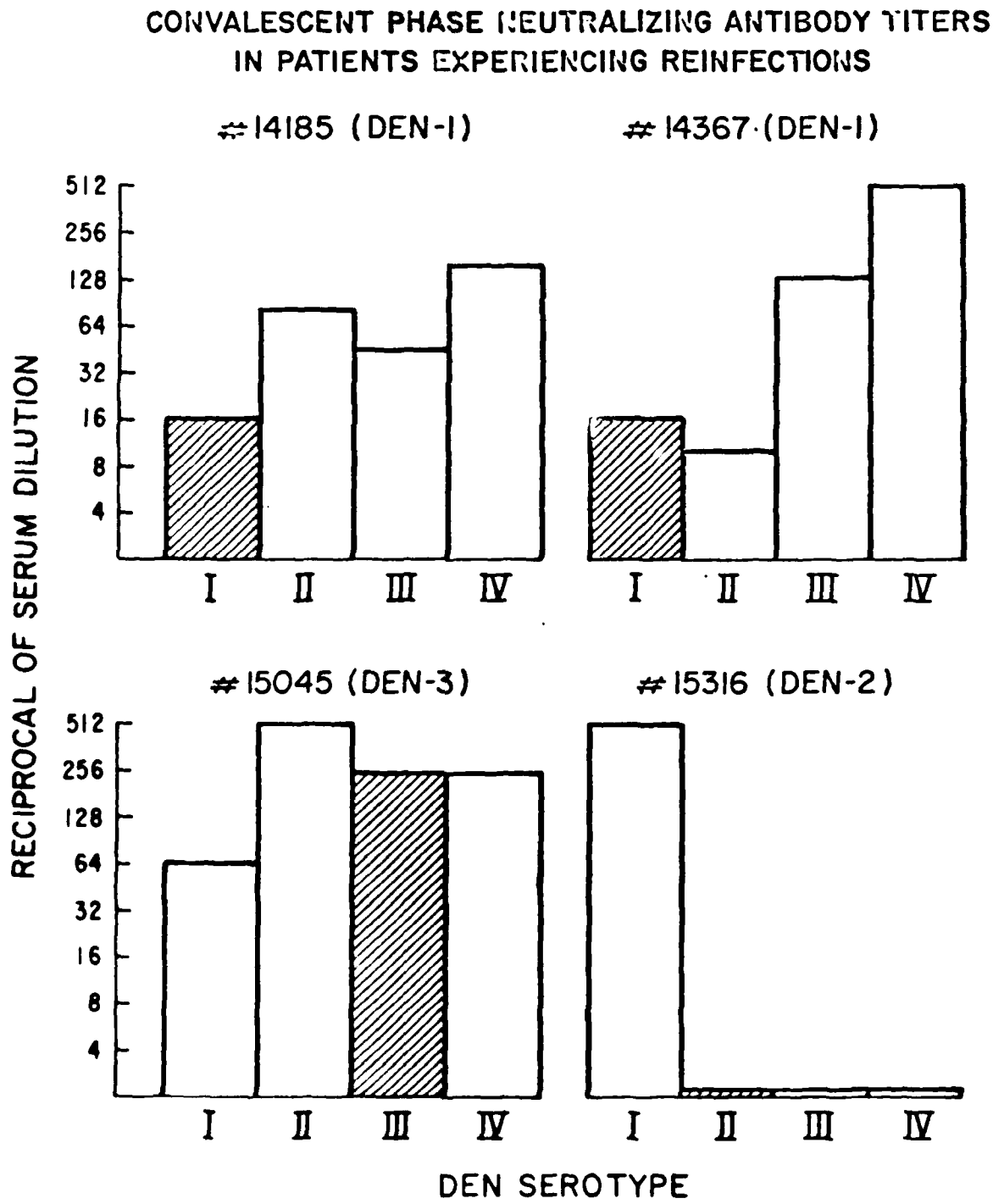
	Hemagglutination inhibition				Neutralization			
	DEN-1	DEN-2	DEN-3	DEN-4	DEN-1	DEN-2	DEN-3	DEN-4
Serum#								
13928	<10*	<10	<10	<10	<4	<5	<10	<10
14185	<10	10	10	10	ND	<5	<10	<10
14367	>40	20	<10	10	<4	<5	<10	80
14759	<10	<10	<10	<10	<4	<5	<10	<10
14760	<10	<10	<10	<10	<4	<5	<10	<10
15045	<10	<10	<10	<10	<4	16	<4	<4
15296	<10	<10	<10	<10	<4	<4	<4	<4
15316	<10	<10	<10	<10	8	<4	<4	<4
15353	<10	<10	<10	<10	<4	<4	<4	<4
16012	<10	<10	<10	<10	<4	<4	<4	<4

Conclusion: 14185, 14367, 15045 and 15316 are reinfections. The others are primary.

* reciprocal of highest dilution inhibiting 8 HAU.

ND - not done

Figure 5


CONVALESCENT PHASE NEUTRALIZING ANTIBODY TITERS
IN PATIENTS EXPERIENCING REINFECTIONS
#14185 (DEN-1)
#14367 (DEN-1)
#15045 (DEN-3)
#15316 (DEN-2)
RECIPROCAL OF SERUM DILUTION
512
256
128
64
32
16
8
4
I
II
III
IV
DEN SEROTYPE

neutralizing antibody response to a dengue serotype other than the one isolated. Each of 3 patients for whom the primary infecting serotype could be determined, had highest neutralizing antibody titers to the primary infecting serotype. The identity of the primary dengue serotype infecting patient 14185 could not be determined.

Data presented in figure 6 represent convalescent antibody titrations measured by the HI test. Each of the same 4 subjects responded with highest antibody titers to a serotype other than the one isolated (indicated by cross hatching). Unlike the neutralizing antibody data, there was little association between the dengue serotype which had highest titers and the primary infecting serotype. Only subject 14367 had the highest antibody titer to the primary infecting serotype.

Figure 7 shows convalescent antibody titer measured by IFA. In 3 of 4 subjects the highest antibody titers were for serotypes other than the isolated virus. Subject 15316 responded with equal titers for all 4 serotypes. The highest IFA titers did not identify the primary infecting serotype.

Five of the 6 subjects classified as experiencing primary infections showed highest IgM titers measured by EIA to the infecting dengue serotype (figure 8). In subject 15353 the IgM antibody titers were equal for DEN-4 as well as DEN-3, the infecting serotype.

Finally, figure 9 shows that in 2 of the 4 subjects experiencing reinfections with DEN, the highest IgM antibody titer correctly identified the infecting serotype. In both of the other reinfections, the IgM response was equal to but not higher than one other serotype.

Patients who experience mild disease as a result of reinfection with DEN show evidence of the doctrine of original antigenic sin. The measurement of IgM class antibody may be a rapid and useful means to determine the infecting DEN serotype when a virus can not be recovered.

Diagnosis of chikungunya infection in Indonesia (J.G. Olson). An isolate suspected of being an alphavirus was submitted by Dr. R. Slemons, NAMRU-2, Jakarta. Neutralization tests confirmed the identity as chikungunya virus (Table 40).

IV. SEROLOGIC SURVEYS

ELISA to detect antibodies to CCHF (L. Lee, J. Meegan, R. Shope, A. Antoniadis). An ELISA was developed to detect antibodies to CCHF virus in human and domestic animal sera. The ELISA was evaluated using goat and human sera collected at a CCHF enzootic site in northern Greece by Dr. A. Antoniadis.

Two methods of attachment of antigen to the solid phase were evaluated. The first was to purify the virus by gradient centrifugation and adsorb it directly on the polystyrene plates. The second adsorbed on the plates partiallly purified mouse anti-CCHF antibody which then trapped CCHF antigen from a semi-crude antigen preparation. In each case the starting material was a 30% sucrose-acetone extracted mouse brain suspension which was inactivated by beta-propiolactone. Both methods worked, but the antigen trapping method was substantially easier (since only antibody had to be purified) and allowed the

Figure 6

CONVALESCENT PHASE HEMAGGLUTINATION INHIBITING ANTIBODY TITERS IN PATIENTS EXPERIENCING REINFECTIONS

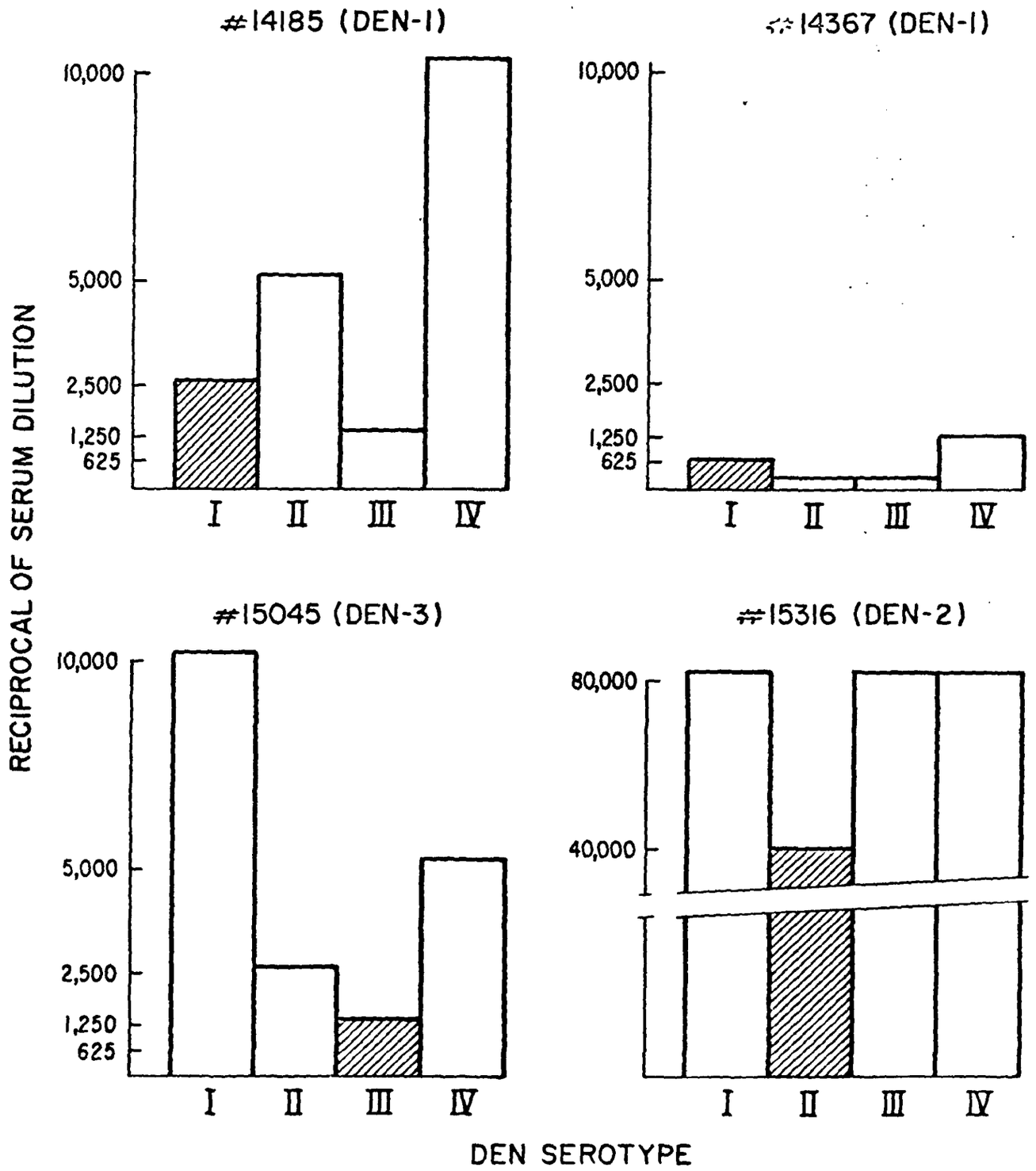


Figure 7

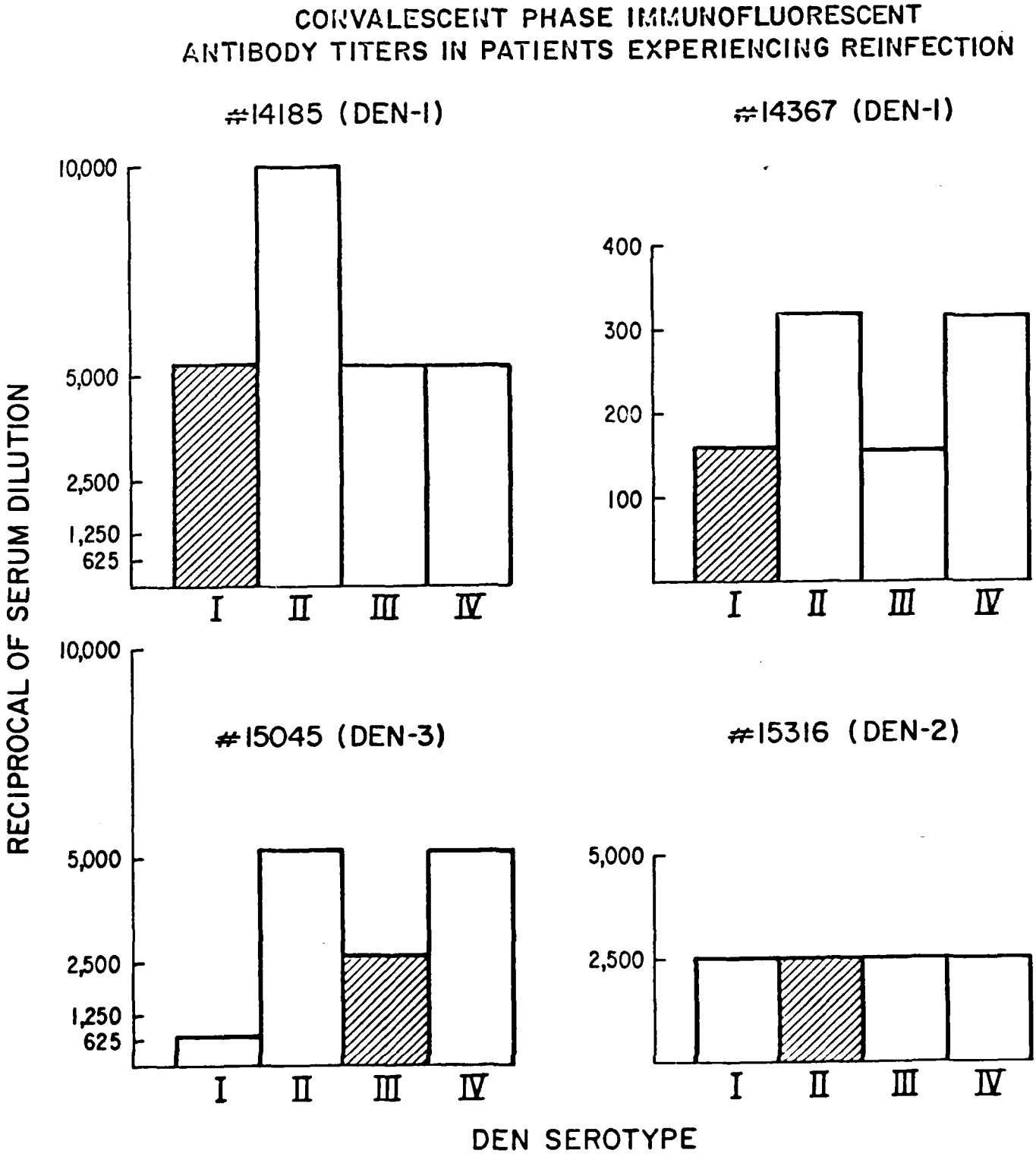

CONVALESCENT PHASE IMMUNOFLUORESCENT
ANTIBODY TITERS IN PATIENTS EXPERIENCING REINFECTION
#14185 (DEN-1)
#14367 (DEN-1)
#15045 (DEN-3)
#15316 (DEN-2)
RECIPROCAL OF SERUM DILUTION
10,000
5,000
2,500
1,250
625
400
300
200
100
I
II
III
IV
DEN SEROTYPE

Figure 8

CONVALESCENT PHASE IgM CLASS ANTIBODY TITERS
MEASURED BY ENZYME IMMUNOASSAY
IN PATIENTS EXPERIENCING PRIMARY INFECTION

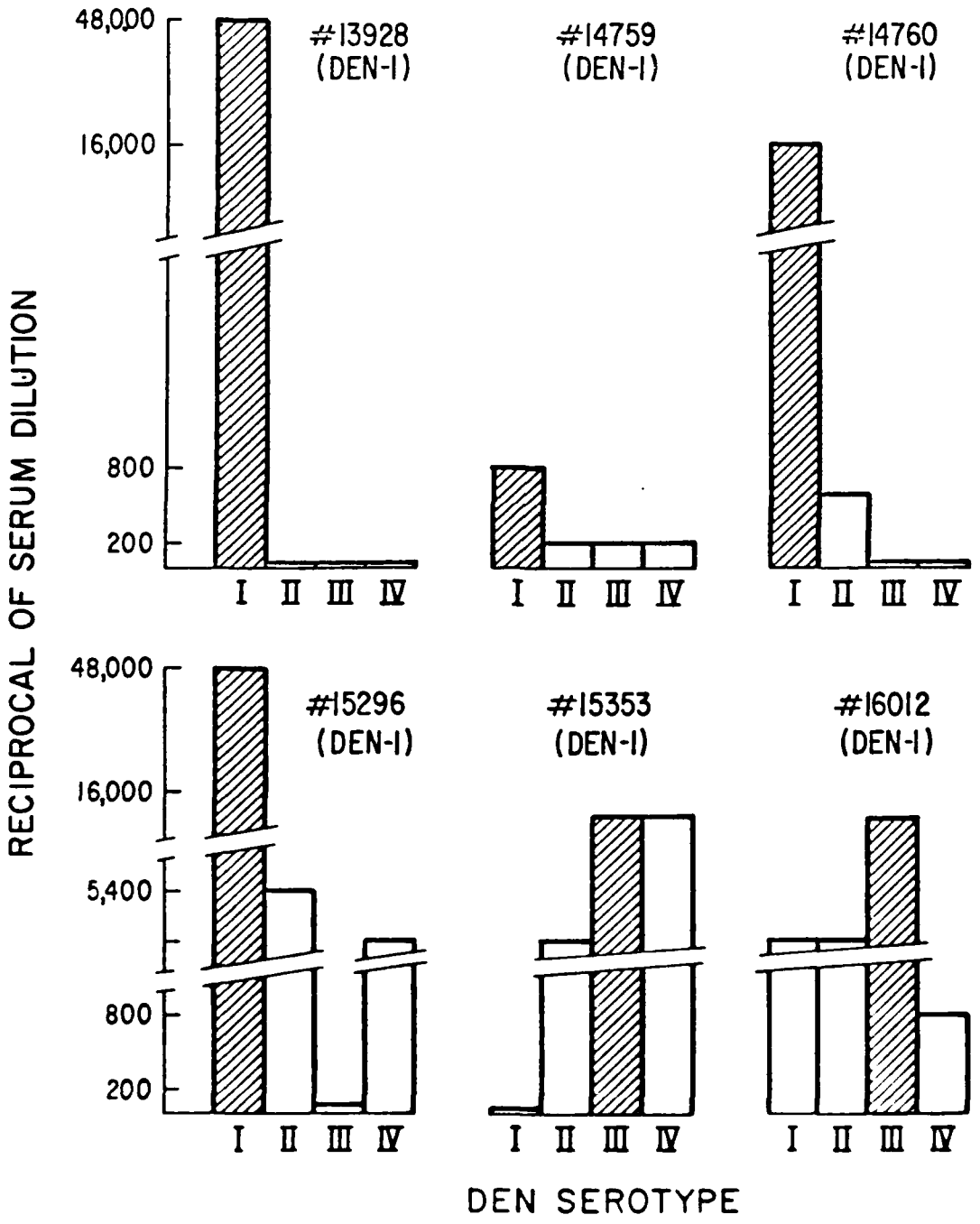
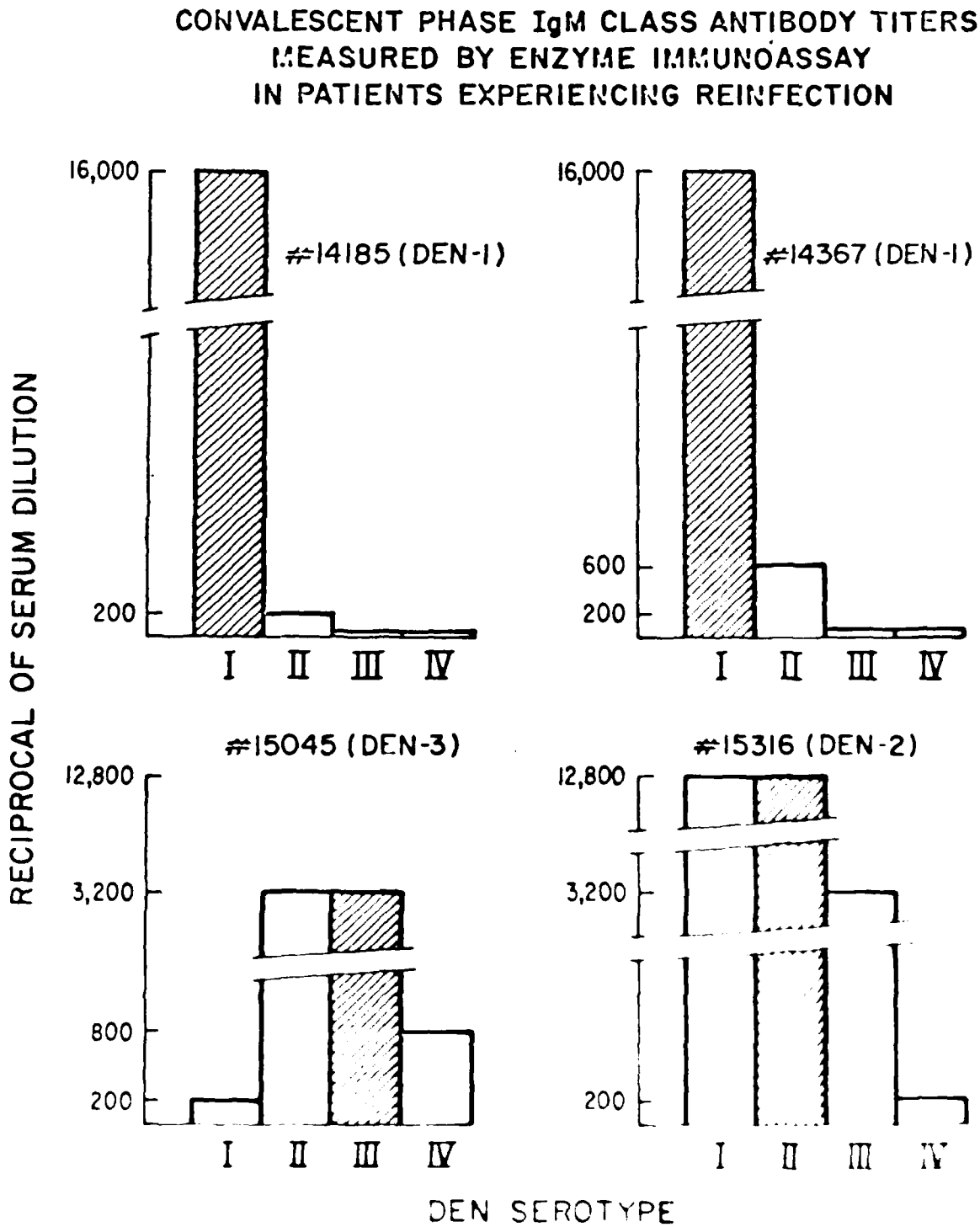


Figure 9


CONVALESCENT PHASE IgM CLASS ANTIBODY TITERS
MEASURED BY ENZYME IMMUNOASSAY
IN PATIENTS EXPERIENCING REINFECTION
RECIPROCAL OF SERUM DILUTION
16,000
200
#14185 (DEN-1)
16,000
600
200
#14367 (DEN-1)
I II III IV
#15045 (DEN-3)
12,800
3,200
800
200
#15316 (DEN-2)
12,800
3,200
200
I II III IV
DEN SEROTYPE

antigen to be used more dilute. This is a variation of a method which was previously shown effective for testing human sera for CCHF antibody (Donets et al., Am. J. Trop. Med. Hyg. 31:156, 1982).

We compared the antigen-detection ELISA to standard serological tests including: HI, IFA, and immunodiffusion (ID). All tests at Yale used the beta-propiolactone-inactivated antigen.

Preliminary trials established that domestic animal sera required 0.5% Tween-20, 500 microgram dextran sulfate per ml buffer, and a final concentration of 5.0% normal horse serum in the standard ELISA diluting buffer for the ELISA accurately to detect antibodies. Using this procedure, a good correlation was found with 58 goat sera tested by HI, and the ELISA and ID (Table 41). The ID is known to be a less sensitive test than HI, but has the advantage (in our hands) of being specific for CCHF while the HI is cross-reactive with other nairoviruses. Preliminary experiments indicated that the ELISA was also broadly cross-reactive, detecting cross-reactive antibodies to other members of the Nairovirus genus.

Table 42 shows a comparison of titers obtained by HI and ELISA. There was excellent correlation. There were no ELISA false negatives but three ELISA false positives, which might represent an increased sensitivity of the ELISA. Sufficient sera were available to compare the ELISA to the IF test for detection of human antibodies. Table 43 reveals an 85.2% agreement between the two tests. In general, ELISA was sensitive, rapid, and reproducible when compared to currently available standard tests. This technology was transferred to the Aristotelian University of Thessaloniki, Greece in June, 1983.

Serosurvey of Sudanese recruits (J. Meegan, G. Tignor). In January, 1979, the U.S. Naval Medical Research Unit Number 3 (NAMRU-3) embarked on a series of collaborative studies on infectious diseases in the Sudan. The initial phase of the program involved the collection of sera and clinical specimens from humans and animals from representative geographical areas to determine which bacteria, parasites, and viruses were major causes of disease.

The preliminary work centered on determination of antibody prevalence rates for selected viruses. More detailed research was completed this year in two areas: a. the determination of antibody prevalence rates for arthropod-borne viruses, and b. the determination of antibody prevalence rates for the hemorrhagic fever viruses - Lassa, Marburg, Rift Valley fever (RVF), Crimean-Congo hemorrhagic fever (CCHF), and Ebola viruses, Zaire and Sudan strains (EBO-Zaire and EBO-Sudan). This latter portion of the project was supported by a contract from the US Army MR&D Command in addition to Reference Center support.

The large size of the collection of sera, and the young age and limited travel history of most of the soldiers allowed the selection of separate populations representing a. soldiers who were stationed (and bled) near their home of origin, and b. soldiers who were moved to new areas for their military service. Prevalence rates on the former sample should establish the geographic distribution of the viral agents. The latter group could provide data on the rate of movement of susceptible individuals into an endemic area. Since very little was known about the distribution of most of the viruses under investigation (especially the hemorrhagic fever viruses), it was hoped these studies would lead to new insights into the ecology/epidemiology of these diseases and to the discovery of endemic areas where future in depth virological, entomological, and clinical studies could be undertaken.

Table 40

Microneutralization testing of two virus strains recovered from febrile humans during an epidemic in Jogjakarta, Indonesia, 1983

Hyperimmune mouse ascitic fluids	JKT Arbo #23575	JKT Arbo #23590	titer with homologous virus
JKT #23574	*160	40	
Bebaru (BEB)	5	5	20
Chikungunya(CHIK)	80	10	80
Getah (GET)	5	5	640
Mayaro (MAY)	5	5	160
Ross River (RR)	5	5	40
Sagiyama(SAG)	5	5	320
Semliki forest (SFV)	5	5	5
Sindbis (SIN)	5	5	80

*reciprocal of dilution which protected equal or greater than 50% of Vero cells from 100 TCID 50 virus dose.

Table 41

Comparison of CCHF ELISA and ID to the HI test for goat sera

	Positive HI test N = 45		Negative HI test N = 13		% agreement with HI test
	+	-	+	-	
ID	33	12	1	12	77.6
	+	-	+	-	
ELISA	45	0	3	10	94.8

Table 42

Comparison of CCHF HI and ELISA antibody titers in goat sera

ELISA titer	HI titer 0	10	20	40	80	160	Total
0*	10						10
400	1						1
800	1						1
1600		2	1				3
3200		8	2	1			11
6400	1	6	6	2			15
12800		1	4	1	1		7
25600		1	3	2			6
51200			2		2		4
	13	18	18	6	3	0	58

*0 = less than 100

Table 43

Comparison of CCHF ELISA to the FA test with human sera

	Positive FA test N = 11		Negative FA test N = 16		% agreement with FA test
	+	-	+	-	
ELISA	11	0	4	12	85.2

For the hemorrhagic disease viruses, the IFA technique was used throughout the study. Antigen slides for the hemorrhagic fever viruses were prepared and inactivated at the U.S. Centers for Disease Control. They were graciously supplied by Drs. K. Johnson and J. McCormick.

For the arthropod-borne viruses, an initial IFA screening of all sera was followed by microneutralization tests on a smaller geographically stratified sample. Viruses for study were selected because of their pathogenicity for man or animals, their distribution in Africa or specifically Sudan, and/or the lack of knowledge about their role in disease.

The majority of sera were from military recruits, 18 to 28 years old. The age, military identification number, camp where based for training, and home village are known for each soldier. Soldiers were bled at camps near or in: 1. Khartoum, 2. El Fasher, 3. Wau, 4. Maridi, 5. Juba, 6. Bor, 7. Muglad, 8. El Gadaref, and 9. Port Sudan. Sera from residents of the Nile Delta in Egypt were tested as controls.

Ebola (EBO) and Rift Valley fever (RVF) viruses have been documented in Sudan; Lassa, Marburg, and Crimean-Congo hemorrhagic fever viruses (CCHF) have not been reported from Sudan. Our objective was to study these viruses to determine if they circulate in this part of sub-Saharan Africa, and to map their distribution in Sudan.

Twenty to forty sera from soldiers native to villages near each base where sera were collected, were tested on polyvalent CRE2LM slides (CCHF, RVF, Ebola both Zaire and Sudan strains, Lassa and Marburg). Since these soldiers grew up and were stationed in the same area, we used them as an indication of which areas in Sudan were endemic for hemorrhagic fever viruses. Interestingly, only one sera of 55 collected near Khartoum was positive (for RVF virus) while rates of greater than 25% were seen in southern provinces. Since soldiers stationed in and around Khartoum had little chance of contracting the diseases during military training, we considered sera from these soldiers as representive of the antibody prevalence rates in their native governorates, and included them in our studies of the distribution of these viruses in Sudan. Over 580 sera have been screened on polyvalent CRE2LM slides and retested on monovalent slides. Table 6 gives the prevalence data for antibodies to each virus. Significant geographic clustering of antibody positive sera occurred for Lassa, EBO-Z and EBO-S viruses. For these viruses, the northern provinces had little or no evidence of antibody, but the southern and southwestern provinces (bordering Central African Republic, Zaire, Chad and Uganda) had significantly higher rates. The provinces located in central Sudan had varied prevalence rates. The majority of endemic areas have a savanna type of vegetation; the major economic activity is grazing.

In the central province of Southern Kordofan, we have located one village (Muglad) with a high antibody prevalence rate for Ebola virus. But villages in the same ecological zone within 100 miles of Muglad show low antibody prevalence rates. If the prevalence rates do not change as we test additional sera from the control villages, this area might be an excellent future study site for Ebola virus.

We studied the EBO antibody prevalence rates for troops native to a non-endemic area but stationed in either endemic or non-endemic areas. The study is ongoing and the sample size is still small, but it appears that these are

significantly higher antibody prevalence rates in the group of soldiers who trained in the endemic region.

Currently, we are increasing the number of tested sera from each province. We will attempt to confirm the specificity of sera positive for Lassa, RVF, EBO, or CCHF by neutralization studies (in collaboration with the U.S. Army Medical Research Institute of Infectious Diseases)

Our current effort is to confirm our earlier reported IFA results by neutralization tests. Table 44 summarizes the neutralization results to date. It is evident that neutralizing antibody to alphaviruses, flaviviruses, bunyaviruses and phleboviruses is prevalent in these populations, with great variation depending on site of residence.

Palyam group viral antibodies in Indonesia (J.G. Olson and T. Thirkill). A serologic study of domestic animal sera from Lombok Island, Indonesia was completed to determine the prevalence of antibody to 4 Australian Palyam group viruses. Each was recovered in Australia from Culicoides spp. which are represented in Lombok Island. Table 45 shows the results of these tests together with those of JE, SEP and BAT. Convalescent sera, from 19 human patients from Lombok Island who had febrile illnesses were tested for evidence of Palyam group antibody by immune fluorescence and found negative.

Serologic survey of U.S. naval personnel resident in Cuba for evidence of dengue virus infections (J.G. Olson). During 1981 an epidemic of dengue fever occurred in Cuba and was associated with frequent incidence of dengue hemorrhagic fever and shock syndrome. DEN 2, the serotype which was responsible for the epidemic, had not been transmitted in Cuba previous to 1981. More than 300,000 cases occurred in Cuba but no cases were reported among the residents of the U. S. Naval Station, Guantanamo Bay, Cuba. The island base is arid and does not provide natural breeding places for Aedes aegypti but breeding in man-made containers is a potential problem. Daily surveillance for breeding has been continuous since 1960 and has never recorded Aedes aegypti on the station.

No clinical cases of dengue fever were reported by the U. S. Naval Hospital, Guantanamo Bay during 1981. Further, careful examination of both in-patient and out-patient case reports showed no unusual increases over those of previous years.

Our objective was to determine whether undetected dengue viral infections may have occurred in the station population during the 1981 Cuban epidemic. We selected 4 populations to study serologically for evidence of DEN-2 infection. The highest risk population sampled was comprised of local nationals who live outside the station and commute daily to their jobs. Persons who had history of a febrile illness during June or July (the peak period of the epidemic) were sampled as well as those whose duties occupationally exposed them to biting mosquitoes. Finally, a control population was comprised of persons whose exposure to biting mosquitoes was relatively slight and who had no history of illness during the epidemic. A 10 ml venous blood specimen was collected from volunteers who gave informed consent during February-April 1982. Blood specimens were centrifuged and serum tested for evidence of anti-dengue antibodies by hemagglutination-inhibition (HI) testing.

Table44. Neutralization test results, Sudan Recruit Survey

PERCENT ANTIBODY POSITIVE

AREA	#SERA	SIND	CHIK	ONY-ONY	WN	ZIKA	YF	DENG-2	BUNY	GERM	ILESHA	BWAMBA	RYF	SFS	SFN	ARU	GOR	STFL	GF
KHARTOUM	28	-	-	-	40	-	14	-	4	4	.	4	-	11	4	-	-	4	7
PORT SUDAN	19	-	5	-	32	5	37	5	11	10	.	5	5	11	-	-	5	5	11
GEDAREF/KASSALA	30	-	-	3	33	3	10	-	3	3	.	-	-	-	3	-	17	-	13
FASHER	27	-	-	-	22	-	11	-	19	17	.	-	-	-	-	-	11	-	11
MUGLUD	32	-	19	3	56	41	47	-	47	42	.	9	-	-	6	-	6	6	22
WAU	50	-	24	10	54	26	44	14	96	92	.	88	10	4	-	-	2	2	2
BOR	38	3	8	18	84	21	21	18	100	90	.	61	26	5	3	29	11	3	16
JUBA/TORIT	64	-	19	19	33	22	20	8	61	58	.	86	5	3	2	2	2	-	8
MARIDI	48	-	17	33	6	13	17	-	73	65	.	94	13	2	-	-	2	-	2
EGYPT - NILE DELTA	43	2	-	NT	54	-	2	2	-	NT	.	NT	36	21	6	-	-	-	-

- = NONE SEROPOSITIVE

NT = NOT TESTED

. = IN PROGRESS

Table 45

Results of serum microneutralization tests of Indonesian domestic animals

Number positive*/number tested (% positive)

Flavivirus	Horse	Cow	Carabau	Goat	Sheep
JE	34/52 (65)	2/51 (4)	0/19	2/33 (6)	0/7
SEP	39/52 (75)	24/51 (47)	3/19 (16)	9/33 (27)	0/7
Bunyavirus					
BAT	3/52 (6)	15/51 (29)	6/19 (32)	1/33 (3)	0/7
Orbivirus (Palyam serogroup)					
D'Aguilar	0/51	34/52 (65)	12/21 (57)	1/33 (3)	0/7
CSIRO Village	0/51	37/52 (71)	14/21 (67)	1/33 (3)	0/7
Bunyip Creek	0/51	34/52 (65)	10/21 (48)	0/33	0/7
Marrakai	0/51	37/52 (71)	9/21 (43)	1/33 (3)	0/7

*Titers equal to or greater than 8 were considered positive.

Table 46

Serologic evidence of dengue infection in Guantanamo Bay, Cuba, 1982

Population	No. positive* / No. Tested (percent positive) hemagglutination inhibition test	neutralization test
Cuban commuters	74/77 (96%)	73/77 (95%)
U.S. personnel with history of illness during epidemic	8/25 (32%)	6/25 (24%)
U.S. personnel occupationally exposed to mosquito bites	1/25 (4%)	0/25
U. S. personnel without occupational exposure	3/62 (5%)	2/62 (3)

*Antibody titers 1:10 were considered evidence of previous infection.

Each serum which had an HI antibody titer equal to or greater than 1:10 was tested by microneutralization (Kaiazek and Liu, 1980) for antibodies to DEN-3. Results of neutralization testing and HI testing are shown in Table 46. Six (24%) of the sera from 25 persons tested who had experienced febrile illness in June or July 1981 had evidence of DEN N antibody. Two of the 62 subjects who made the control population without occupational exposure to mosquitoes had NT. Further investigation of the 8 subjects for whom evidence suggested DEN viral infections revealed that all 6 subjects who had experience febrile illnesses were Jamaica Nationals employed by the United States Navy and recent travel history back to Jamaica was established. Both control subjects were U.S. military personnel of Filipino extraction who had been born and lived in the Philippines.

Antibody Response of Humans to Yellow Fever Vaccination (R.B.Tesh, J. Boshell, A.P.A. Travassos da Rosa and F. Pinheiro). Acute and convalescent sera from approximately 76 Brazilian subjects, living in a yellow fever-free zone, were sent to us by Dr. Francisco Pinheiro, Pan American Health Organization, Washington. Fifty-one of these persons had been vaccinated with the 17-D strain of yellow fever virus 40 years before (Table 47). In May 1981, these people were bled to determine their immune status and then were given another injection of the 17-D vaccine. A follow-up serum was obtained from the subjects one month later (June 1981). A second group of 27 individuals who had never previously received yellow fever vaccine served as controls (Table 48). The latter group was also vaccinated in June 1981.

The paired sera on each subject were examined by hemagglutination-inhibition (HI) test in Brazil against 3 yellow fever strains (FA Hill, 17-D and French neurotropic), Ilheus, St. Louis encephalitis and Rocio viral antigens. Plaque reduction neutralization tests (PRNT) were done at Yale on the same specimens using the French neurotropic strain (SV46) of yellow fever virus. The latter virus strain was selected for use in PRNT because of its large sharp plaques. Initially all sera examined by PRNT were screened at a 1:4 dilution. Specimens which were positive at this dilution were then titrated at dilutions from 1:8 to 1:16,384, using two microplate wells per dilution. Specimens producing equal or greater than 90% plaque reduction were recorded as positive.

Results of HI and PRN tests are shown in Tables 47 and 48. In general, results of the two tests were in agreement. Interestingly, many of the people vaccinated 40 years previously with yellow fever vaccine still had detectable levels of antibodies (Table 47). Subsequent revaccination with the 17-D vaccine resulted in a significant increase in antibody titer in most of these individuals. A few of the subjects listed in Table 47 had higher levels of HI antibodies to Ilheus or SLE than to yellow fever in their May 1981 sera. It is possible that these people may have been naturally infected with one of these viruses which also occur in Brazil. Serologic response of the control group (Table 48) was unremarkable except that several people apparently failed to develop antibodies after vaccination and several individuals had yellow fever neutralizing antibodies in their pre-vaccination sera.

Table 47

Pre- and post- immunization antibody titers in Brazilian subjects receiving the 17-D vaccine

Serum Number		PRNT May 1981	PRNT June 1981	HI Test May 1981 FA Hill	17D	SV46	ILH	SLE	ROC	HI Test June 1981 FA Hill	17D	SV46	ILH	SLE	ROC
ETA	6	4	4,096	0	0	0	0	0	0	20	40	40	0	0	0
ETA	11	16		0	0	0	0	0	0	0	0	0	0	0	0
ETA	13	32	16	0	0	0	0	0	0	0	20	20	0	0	0
ETA	14	8		0	0	0	0	0	0	0	20	20	0	0	0
ETA	16	8	256	0	0	20	0	0	0	20	20	40	20	0	0
ETA	23	64	4,096	0	0	0	0	0	0	40	40	80	20	0	0
ETA	27	16	128	0	0	0	0	0	0	20	20	40	20	20	0
ETA	28	64	256	0	0	0	0	0	0	0	0	20	0	0	0
ETA	32	4	128	0	0	20	0	0	0	0	20	40	0	0	0
ETA	41	4	256	0	20	20	40	40	0	40	40	40	40	40	0
ETA	42	4	1,024	0	0	0	0	0	0	40	40	40	0	0	0
ETA	70	4	1,024	0	0	0	0	0	0	40	40	80	0	0	0
ETA	73	64		0	0	20	20	0	0	20	20	20	20	0	0
ETA	113	8	256	0	0	0	0	0	0	20	20	40	20	0	0
CGL	16	32	128	20	40	40	20	40	0	20	40	40	40	40	0
CGL	24	4	64	0	0	0	0	0	0	20	20	20	20	20	0
CGL	31	64	32	0	20	20	20	80	0	0	20	20	20	80	0
PSA	51	64	128	0	0	0	0	0	0	0	20	20	0	0	0
PSA	52	32	64	0	0	20	0	0	0	0	20	20	0	0	0
PSA	53	4	128	0	20	20	0	0	0	20	20	20	20	20	0
PSA	54	4	2,048	0	0	0	0	20	0	80	80	160	20	20	0
PSA	55	16	128	0	0	0	0	0	0	20	40	40	20	0	0
PSA	56	≥128	256	0	0	0	0	0	0	0	20	20	0	0	0
PSA	68	8	128	20	20	20	20	20	0	20	40	40	20	20	0
ETA	1	<4													
ETA	5	<4	512	0	0	0	0	0	0	20	40	40	0	0	0
ETA	15	4	≥8,192	0	0	0	20	0	0	80	160	≥320	20	0	0
ETA	30	<4	64	0	0	0	0	0	0	20	20	20	0	0	0
ETA	35	8	512	0	0	0	0	0	0	20	40	40	20	0	0
ETA	37	4		0	0	0	0	0	0	20	20	20	0	0	0
ETA	53	<4	512	0	0	0	0	0	0	20	40	40	0	0	0
ETA	61	<4	64	0	0	0	0	0	0	0	0	20	0	0	0
ETA	76	<4	128	0	0	0	0	0	0	20	20	0	0	0	0
ETA	77	<4	256	0	0	0	0	0	0	40	40	80	0	0	0
CGL	4	16	≥16,384	0	0	0	0	0	0	40	80	≥320	80	80	0
CGL	8	<4	128	0	0	0	0	0	0	20	40	40	40	40	0
CGL	17	4	256	0	0	0	20	0	0	20	40	80	40	40	0
CGL	19	4	2,048	0	20	20	20	40	0	80	80	80	40	40	0
CGL	23	8		0	0	0	0	0	0	40	40	80	0	0	0
CGL	29	16	128	0	0	0	0	0	0	0	20	20	0	0	0
CGL	34	32	512	0	0	0	0	0	0	0	20	20	0	0	0
PSA	50	32	1,024	0	0	0	0	0	0	80	80	80	40	40	0
PSA	59	<4	16	0	0	0	0	0	0	0	0	20	0	0	0

cont.

Table 47 (continued)

Serum Number	PRNT May 1981	PRNT June 1981	HI Test May 1981 FA Hill	17D	SV46	ILH	SLE	ROC	HI Test June 1981 FA Hill	17D	SV46	ILH	SLE	ROC
PSA 62	16	1,024	0	0	0	0	0	0	40	80	80	20	0	20
PSA 63	32	512	0	0	20	0	0	0	40	40	80	0	0	0
ETA 8	<4	4	0	0	0	0	0	0	0	0	20	0	0	0
ETA 10	<4	8,192	0	0	0	0	0	0	40	40	160	0	0	0
ETA 26	<4		0	0	0	0	0	0	20	40	40	0	0	0
ETA 36	<4	2,048	0	0	0	0	0	0	80	80	160	20	0	0
ETA 44	16	2,048	0	0	0	0	0	0	40	40	80	20	0	0
ETA 45	<4	64	0	0	0	0	0	0	0	20	20	20	0	0
ETA 46	<4	1,024	0	0	0	0	0	0	40	80	80	0	0	0
ETA 57	<4	1,024	0	0	0	0	0	0	20	20	80	0	0	0
ETA 75	<4	1,024	0	0	0	0	0	0	40	80	80	0	0	0
ETA 143	8	8,192	0	0	0	0	0	0	40	80	160	0	0	0
CGL 1	<4	512	0	0	0	0	0	0	20	40	80	0	0	0
CGL 7	<4	64	0	0	0	0	0	0	0	0	20	0	0	0
CGL 9	<4	512	0	0	0	0	0	0	40	80	160	0	0	0
PSA 57	8	512	0	0	0	0	0	0	40	40	40	0	0	0
PSA 64	<4	512	0	0	0	0	0	0	40	40	80	0	0	0
PSA 71	<4	64	0	0	0	0	0	0	20	20	20	20	0	0

*Reciprocal of highest serum dilution producing ≥90% plaque reduction.

Table 48

CONTROLS

Serum Number	PRNT May 1981	PRNT June 1981	HI Test May 1981 FA H111	17D	SV46	ILH	SLE	ROC	HI Test June 1981 FA H111	17D	SV46	ILH	SLE	ROC
PSA 1	<4	64	0	0	0	0	0	0	20	40	40	0	0	0
PSA 6	<4	<4	0	0	0	0	0	0	0	0	0	20	0	0
PSA 16	<4	8	0	0	0	0	0	0	0	0	0	0	0	0
PSA 74	<4	128	0	0	0	0	0	0	20	20	20	20	0	0
PSA 75	<4	8	0	0	0	0	0	0	0	20	20	20	0	0
PSA 76	64	256	0	0	0	0	0	0	0	0	0	0	0	0
PSA 77	<4	<4	0	0	0	0	0	0	20	20	160	0	0	0
PSA 78	<4													
PSA 79		<4	0	0	0	0	0	0	0	0	0	0	0	0
PSA 80	<4	<4	0	0	0	0	0	0	0	0	0	0	0	0
PSA 81	<4	64	0	0	0	0	0	0	0	0	20	0	0	0
PSA 82		<4	0	0	0	0	0	0	0	0	0	0	0	0
PSA 83	<4	16	0	0	0	0	0	0	0	20	20	0	0	0
PSA 84	<4	1,024	0	0	0	0	0	0	40	20	80	0	0	0
PSA 85	<4	256	0	0	0	0	0	0	40	20	40	0	0	0
PSA 86	<4	<4	0	0	0	0	0	0	0	0	20	0	0	0
PSA 87	8	256	0	0	0	0	0	0	40	40	40	0	0	0
PSA 88	<4	<4	0	0	0	0	0	0	0	0	20	0	0	0
PSA 89	8	1,024	0	0	0	0	0	0	20	40	40	0	0	0
PSA 90		<4	0	0	0	0	0	0	0	0	0	0	0	0
PSA 91	4	64	0	0	0	0	0	0	0	0	20	0	0	0
PSA 92	<4	8	0	0	0	0	0	0	0	20	20	0	0	0
PSA 93	<4		0	0	0	0	0	0	40	160	160	0	0	0
PSA 94	16	4	0	0	0	0	0	0	0	20	40	0	0	0
PSA 95	<4	<4	0	0	0	0	0	0	0	0	0	0	0	0
PSA 96	4	64	0	0	0	0	0	0	20	40	40	0	0	0
PSA 97	<4	16	0	0	0	0	0	0	0	0	20	0	0	0

V. DEVELOPMENT OF NEW TECHNIQUES

Characterization of monoclonal antibodies to RVF virus (J. Meegan, C. Peters). Monoclonal antibodies produced in past contract years were characterized. Studies included: determination of proteins to which each monoclonal binds; reactivity to RVF virus in fluorescent antibody (FA), hemagglutination-inhibition (HI), complement fixation (CF), enzyme-linked immunosorbent assay (ELISA), and plaque reduction neutralization (PRNT) tests; cross-reactivity with other phleboviruses; and competition studies to determine topological arrangement of antigens on the virion. These studies were performed on a collaborative basis with the Medical Division at the U.S. Army Medical Research Institute of Infectious Diseases. Studies were also initiated to separate epidemiologically distinct strains of RVF virus using the currently available bank of monoclonals.

To date, although most of the studies have been successful, a lingering problem has been the difficulty in clearly separating G1 from G2 proteins in immunoprecipitation studies. Hopefully this problem has been solved by the introduction of new polyacrylamide gel techniques. Despite this problem, we can combine our results from each study to identify at least seven antigenic sites. Table 49 summarizes the results to date. There are monoclonal antibodies which identified two nucleoprotein sites, three G2, and 2 G1 sites. One of the nucleocapsid sites was more cross-reactive than the other. As would be expected, both nucleocapsid sites were reactive in the CF test. The G2 sites were reactive in the HI and N tests. Both G1 and G2 sites were specific for RVF.

Detection of RVF virus antigen using polyclonal and monoclonal antibodies in ELISA systems (J. Meegan). During past years, we have developed antigen detection ELISA systems for RVF virus using purified polyclonal anti-RVF sera. Others have recently published on similar systems, and in all cases the sensitivity has been lower than desired. One problem has been that polyclonal sera appear to have unequal amounts of antibody to all viral proteins. For example, in phlebovirus systems where reference antibody is generated by live virus infection or injection of killed mouse brain stock, the resulting antiserum sensitively detects antigen in preparations with high amounts of nucleocapsid antigen but is far less sensitive in preparations of whole virions. This presumably reflects a disproportionately large population of antibodies to nucleocapsid protein at the expense of antibodies to the external virion glycoproteins.

We compared in ELISA antigen detection tests a mixture of three monoclonal antibodies (one each against G1, G2, and N proteins) and a polyclonal serum. Although both appeared to detect antigen equally in RVF virus preparations containing large amounts of N protein (e.g. infected liver), the ELISA using the mixture of monoclonal sera was 100-fold more sensitive than the polyclonal assay when testing antigen preparations rich in whole virions (e.g. vaccine). Further studies are exploring additional monoclonal antibody preparations to determine the effect, on antigen detection, of antibody specificity and avidity.

Use of ELISA to detect IgG and IgM antibodies to RVF virus in sera and CSF (J. Meegan). Studies in previous contract years led to the development of IgG and IgM ELISA systems for detection of antibodies after RVF virus infection. IgG and IgM assays of sequential bleedings from patients infected with RVF virus

Table 40

Antigenic sites defined by RVF monoclonal antibodies

Prototype monoclonal (of those identifying site)	Antigenic binding sites		Characteristics
	Protein	Site designation	
1-4-3PA	N	N-A	Negative in NT and HI; positive in CF; cross-reacts with Aguacate, Belterra, Munguba, and Chagres viruses
203-6	N	N-B	Same as N-A except cross-reacts only with Chagres and Aguacate
1-25-6A	G2	G2-A	Negative in CF; positive in HI and NT; no cross-reactions
4-10-3C	G2	G2-B	Same as G2-A but does not overlap
4-39-CC	G2	G2-C	Same as G2-A except very low NT activity
4-8-11B	G1 (?)	G1-A	Negative in all but ELISA and FA tests; no cross-reactions
1-29-5B	G1	G1-B	Negative in CF and NT but low titer in HI; otherwise same as G1-A

revealed IgG and IgM titers rising at seven [illegible] days post [illegible]. IgG titers remained elevated for greater than four years, while IgM titers were [illegible] after three to six months. However, low level IgM titers remained detectable for one to two years post infection.

We have extended this work to the detection of antibodies in CSF from patients experiencing RVF encephalitis. CSF samples were available from [illegible] RVF encephalitis patients studied during the [illegible] Egyptian outbreak. The ELISA systems were modified so that a specific amount of IgG and IgM [illegible] test for its specificity to RVF virus. In this way, a predetermined microgram amount of IgG or IgM could be trapped from either serum or CSF and the amount of specific RVF antibody per microgram of Ig could be calculated. If antibody present in the CSF was not synthesized locally but instead represented leakage of serum antibody across the damaged blood brain barrier, the amount of RVF specific antibody per microgram of IgG or IgM should be equal in CSF and serum. In [illegible] of [illegible] patients, the specific amount of anti-RVF virus IgG and IgM per microgram of immunoglobulin was far greater in CSF than serum, indicating that local CSF antibody synthesis appears to be a common response in RVF encephalitis.

Further studies are ongoing and will attempt to correlate the presence of IgM or IgG in CSF with the clinical course of disease. However, as an early means to diagnose RVF encephalitis, these ELISA systems appear to be valuable.

Use of ELISA to detect antibodies to RVF virus in domestic animals (J. Meegan, R. Shope, R. Yedloutschnig, J. Walker). Although ELISA has become popular for detecting antibodies in human sera, the assay has been used on a limited basis in veterinary medicine. Problems have included the lack of specific and sensitive enzyme conjugated second antibodies, and the apparent non-specific "stickiness" of domestic animal sera. In collaboration with the U.S. Department of Agriculture's laboratory at Plum Island, we have evaluated an ELISA for detection of antibodies to RVF virus in sheep and goat sera.

We have determined that several companies produce good horseradish peroxidase antisheep and antibovine conjugates, although there is considerable lot-to-lot variation in our tests. We have reduced non-specific reactions by altering the standard ELISA diluting buffer to include: 0.5% Tween 20, 5.0% normal horse serum, and 500 microgram dextran sulfate per ml buffer.

Results (Table 50) show that ELISA has specificity similar to HI and PRNT with sheep and bovine sera and that it is somewhat more sensitive than either of the standard techniques.

Combining ELISA and filter paper collection of blood for serological studies (M. Fletcher, J. Meegan). The collection of blood samples by venipuncture for serological surveys is complicated by cultural resistance to venipuncture, cost of needles and syringes, difficulty in processing and refrigerating samples appropriately under field conditions, and difficulty in transport. An alternative collection method, by absorbing drops of blood obtained by fingerprick onto filter paper, is in use in several diagnostic laboratories (Mathews, H.M., 1981. Parasitic disease: testing with filter-paper blood spots. Lab. Management 19:55-62; Nakano et al., 1983. Microtiter determination of measles hemagglutination inhibition antibody with filter

papers (Am. J. Trop. Med. 17:860-863). Advantages include greater ease of collection, transport, and storage. A disadvantage is in the relatively small volume of blood collected, which limits the number of different tests that can be performed. This technique could be very useful in serosurveys evaluating vaccine effectiveness, for instance, and samples can be conveniently obtained in conjunction with blood slides for malaria and trypanosomiasis surveys.

We are presently evaluating this technique for detecting antibody to yellow fever by capture ELISA, for eventual use in field survey work. We sought to determine stability of antibody under various storage conditions, optimal conditions for elution, comparability of results obtained with serum, and concentration of filter paper eluate needed to approximate results obtained with serum. Storage of blood dried on filter paper at 37°C rather than at room temperature resulted in an initial two- to four-fold drop in titer, but thereafter titers remained stable within a two-fold dilution over a period of three weeks at 37°C. Best results were obtained with samples eluted for 45 min in PBS, as opposed to elution overnight. Two discs of 6.5 mm in diamenter (each containing about 20 microliter of whole blood) punched out with a standard paper punch, eluted in 0.2 ml of PBS and further diluted 1:5 (resulting in a final dilution of about 1:25) were equivalent to a 1:100 dilution of serum. Results obtained using the filter paper technique compared favorably with results obtained using serum. From four discs (representing four drops of blood), a volume of 0.4 ml at a 1:5 dilution was obtained and used for ELISA or HI tests. Further evaluation of this method is being carried out, including stability of antibody in the filter paper eluate stored at 4°C.

Rapid diagnosis of dengue virus infections in humans (J.G. Olson). Laboratory confirmation of the diagnosis of dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) currently depends on being able to isolate virus from the patient or to demonstrate an increase in serum antibodies during the course of the illness. In endemic areas for dengue virus (DEN) where most clinically diganosed patients are experiencing a second exposure to DEN, serologic testing can usually show four-fold increases in antibody titer between serum specimens collected early in the acute phase and sera taken 5 days following the initial specimens. Thus, using hemagglutination-inhibition (HI) or immunofluorescence (FA) testing, patients' DEN infections can be documented in a majority of cases. When individuals who are likely experiencing their initial infection with a DEN serotype are being tested, the paired sera collected with an interval of 5 days between them may not show a four-fold diagnostic rise in antibody titer. Since the patients are not showing an anamnestic antibody response, diagnosis depends on collection of blood specimen as close to onset as possible and taking a second [illegible] later. Thus, the serologic laboratory confirmation in primary dengue [illegible] be delayed to 10 or more days.

Virus isolation using extremely sensitive techniques has been [illegible] can begin as soon as a DEN infection is suspected. The intrathoracic inoculation of mosquitoes or inoculation of the C6/36 clone of Aedes cells with acute phase sera requires processing of specimens at the time of collection or the maintenance of specimens at or below [illegible]. The minimum time for isolations is 14 days for inoculated mosquitoes and for C6/36 cells.

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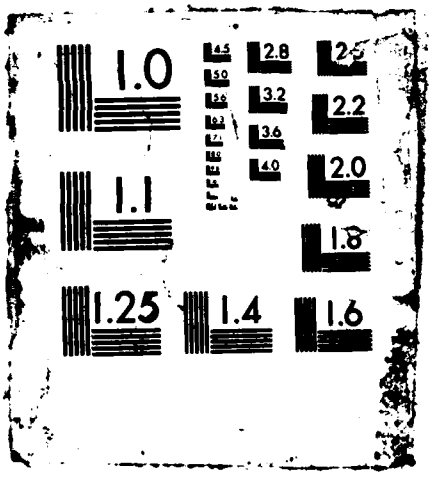
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1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3

We have attempted to develop techniques which will increase the rapidity with which a laboratory diagnosis may be made. The first technique is a modification of the isolation techniques already in practice. We use vertebrate cells (Vero) to recover DEN from acute phase serum and detect its presence by indirect immunofluorescence (IFA).

As reported last year, mouse brain suspensions of all four serotypes of DEN were titrated in C6/36 cells on tissue culture 8 chamber/slides (Lab Tek) using fluorescent foci as evidence of infection. The highest 10-fold dilution of virus which showed fluorescence after 7 days incubation was considered the endpoint. Monolayers of Vero cells were grown in Lab Tek 8 chamber/slides and inoculated with DEN virus, incubated for 24, 48 and 72 hours and tested for evidence of indirect immunofluorescence. The sensitivity of the technique was greatest at 72 hours after inoculation. At 24 hours, all 4 serotypes were detectable but sensitivity was increased 10-fold by waiting until 72 hours. The sensitivity of this technique compares closely with titration of DEN serotypes in Vero cells in 96-well microtiter plates using cytopathic effect as an endpoint.

A modification of the fluorescent focus inhibition test (FFIT) for measurement of neutralizing antibodies has been developed this year for use with 96-well flat-bottomed microtiter trays. The test has all the advantage of the microneutralization test and can be read before cells show cytopathic effect. Further studies are under way to reduce the period between inoculation and successful endpoint of the test.

An Igm capture system for assaying human sera for the presence of dengue specific Igm antibody has also been developed. Preliminary data suggest that the test is capable of determining the infecting strain of dengue virus in a patient experiencing a secondary infection; however, further studies are necessary.

Detection of dengue viral antigen in infected cell culture fluids and in suckling mouse brain suspensions by the modified double antibody sandwich enzyme linked immunosorbent assay. (J.G. Olson and T.L. Thirkill) An antigen detection enzyme linked immunosorbent assay (ELISA) for dengue virus (DEN) was developed for virus stocks prepared in Aedes albopictus clone C6/36 cells and in suckling mouse brains. A pool of human convalescent sera from laboratory confirmed dengue fever patients was coated on 96-well flat bottomed microtiter plates. The virus stocks, previously titrated by either hemagglutination (HA) or by cell culture, and uninfected control antigens were added after the coating step. Mouse DEN immune ascitic fluids were added next followed by goat anti-mouse immunoglobulin conjugated with alkaline phosphatase. The enzyme substrate, p-nitrophenyl phosphate added in the final step, is colorimetrically altered in the presence of the bound enzyme. The subsequent reactions were read spectrophotometrically on a Titer-Tek Multiscan plate reader at 405 nm. Absorbance values exceeding the mean of multiple replicates of the uninfected control antigens plus three times their standard deviation were recorded as positive.

As reported last year, using a combination of hyperimmune mouse ascitic fluids and mouse ascitic fluids which contained monoclonal DEN antibodies, we were able to detect and identify all 4 serotypes of DEN. ELISA was a slightly less sensitive technique than cell culture and more sensitive than HA for

detecting DEN antigen. The ELISA was capable of detecting antigen at levels below those normally found in human patients with DEN infections.

Attempts to detect DEN antigen in coded acute phase sera provided by Dr. Duane Gubler of the CDC laboratory in San Juan, Puerto Rico failed. In order to determine whether the failure of the detection system was due to a lack of sensitivity or some interfering substance in human sera, we tested human serum specimens with measured amounts of DEN antigen added. Our preliminary results suggest that human serum which lacks detectable anti-DEN antibody enables the detection of DEN antigen. Further when pooled convalescent sera from DEN patients were combined with DEN antigen and complex formation allowed to occur (60° for 1 hr), DEN antigen was not detected. These data suggest that the presence of anti-DEN antibody in the acute phase serum may be responsible for interfering with antigen detection. The presence of circulating immune complexes in dengue patients is well documented (Ruangjirachuporn, W. et al., 1979. Clin. Exp. Immunol. 36:46-53). Studies are underway to determine whether acute phase sera tested have evidence of complexes and whether the complex can be used to identify the cause of illness.

Development of CCHF monoclonal antibody. (L. Lee, J. Meegan, R.Shope, and J. Olson). C57 BL/6 mice were immunized with a BPL inactivated mouse brain stock of CCHF virus The mice were given 2 inoculations of virus mixed with Freund's complete adjuvant about 2 weeks apart and an IV boost 3 days before fusion. The spleens were removed and the cells fused with NS-1 cells. The resulting fusion products were plated into 18 24-well dishes of which 249 wells (57.6%) had growth. Of these 249 hybridomas, 43 (13.2%) were found to be positive against CCHF by EIA. Twenty-two hybridomas were sucessfully grown to sufficient cell number and frozen to be cloned at a later date. Attempts to clone on soft agar were not sucessful. One hybridoma is being cloned and subcloned by limiting dilutions. The antibody producing hybridomas were tested against nine different strains of Congo virus HA antigen by EIA and 4 distinct reaction patterns of the monoclonals were apparent.

Detection of Eastern Equine Encephalitis (EEE) Viral Antigen in Avian Sera (J.G. Olson). An antigen detection enzyme immunoassay (EIA) was developed for chicken sera employing hyperimmune mouse ascitic fluid and rabbit antisera against EEE virus prepared by Dr. Steve Hildreth. Chicks were inoculated intramuscularly with EEE virus and exsanguinated after 24 hours incubation. Virus titrations were done in BHK-21 cells using 96-well microtiter plates. The pooled chick sera contained more than 10.0 log10 TCID50 per ml virus. We were able to detect EEE antigen in diluted serum specimens which contained less than 3.0 log 10 TCID50 per ml EEE virus.

In order to determine whether the EIA had potential for field application, we tested chicks (Gallus domesticus) and house sparrows (Passer domesticus) inoculated with EEE virus and serially bled them at 24 hours intervals. Venous blood specimens were collected from the jugular vein and added to phosphate buffered saline (PBS) to make a 1:10 dilution. After centrifugation EEE virus titrations were done in BHK-21 cells in microtitration. Specimens were coded and retested by EIA for EEE antigen. All of the 26 specimens which contained infectious virus were positive by EIA for a sensitivity of 100%. Of 28 specimens which contained no infectious virus, 6 (21%) gave a false positive EIA

result for a specificity of 79%. Antigen was detected by EIA in three blood specimens from house sparrows collected after viremia could no longer be detected (day 6 and day 7 for one bird and day 4 for another). Although these 3 discrepant findings were counted as false positives, they may represent the ability of the EIA to detect EEE antigen in the absence of detectable infectious virus. These data suggest that EIA is a sensitive means for assaying for EEE antigen in avian sera and may have potential for rapidly screening large numbers of avian sera for EEE virus.

Effect of hibernation on EEE virus circulation and antibody in turtles (A.J. Main and M. Fletcher). In order to interpret positive serological reactions detected in serosurveys in New Jersey and Connecticut during 1981 and 1983, pilot studies were done with musk turtles. Over half of the field collected musk and mud turtles (family Kinosternidae) were HI positive for EEE virus (Table 51). To study this phenomenon further, 3 field collected musk turtles (Sternotherus odoratus) were inoculated with EEE virus; a fourth specimen was held as control. All four turtles were placed in an environmental chamber under conditions simulating fall and winter temperatures and photoperiods. Virus was not detected in preinoculation blood samples although HI "antibody" was detected in one of the three inoculated turtles. Postinoculation/prehibernation blood samples have not been completely tested.

Development of techniques for experimental transmission of arboviruses by ticks (A.J. Main and L. Lorenz). With completion of a new tick-holding facility for work with arboviruses, techniques are being evolved for transmission of viruses by ticks. Yellow fever is being used as a model. Yellow fever virus has been recovered from naturally infected ticks (Amblyomma) in both the New and Old Worlds, including isolations from the progeny of field infected ticks. Early laboratory experiments have shown that both hard (Amblyomma) and soft (Ornithodoros) ticks are capable of transmitting yellow fever virus. To pursue this phenomenon further, pilot studies tracing yellow fever virus in experimentally infected ticks of these two genera are underway. So far, second stage nymphal O. parkeri were fed through a mouse skin membrane on virus-infected Guinea pig blood.

VI. COLLECTION OF LOW PASSAGE ARBOVIRUS REFERENCE STRAINS (R.B. Tesh and A.J. Main)

In collaboration with the Subcommittee for the Collection of Low Passage Arbovirus Strains (SCLAS) of the American Committee on Arthropod-Borne Viruses (ACAV), an attempt has been made to establish a collection of low passage strains of selected arboviruses of public health importance. Additional information about the collection was given in our 1981 and 1982 Annual Reports. During 1983, stocks of 23 more virus strains were prepared and lyophilized. These agents as well as their origin and passage history are listed in Table 52.

It is hoped that interested persons working in arbovirology will continue to submit samples of low passage virus strains from different geographic locations and time periods. We intend to create a data file with pertinent information on each virus in the collection. This information as well as the lyophilized virus stocks will be available to interested investigators at no cost. It is anticipated that this collection will prove to be an invaluable reference resource for future comparative studies of viral genetics, biochemistry, pathogenicity and antigenic relationships.

Table 50

Comparison of ELISA to HI and PRNT with sheep and bovine sera

Sera	Titer		
	HI	PRNT	ELISA
Sheep			
1	2560	2560+	12800
2	320	640	1600
3	5120	2560+	12800
4	2560	2560+	12800
5	5120	2560+	12800
6	0*	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
Bovine			
1	2560	2560+	3200+
2	640	1280	1600
3	1280	1280	1600
4	1280	1280	1600
5	320	1280	400
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0

*0 = less than 10 for HI and PRNT; less than 50 for ELISA

Table 51

EEE hemaggutination-inhibiting activity in turtles of the family kinosternidae in New Jersey (1981, 1983) and Connecticut (1983).

	New Jersey	Connecticut	Totals
Mud Turtles *Kinosternon subrubrum*	15/28*	-	15/28 (53.6%)
Musk Turtles *Sternotherus odoratus*	-	2/5	2/5 (40.0%)
TOTALS	15/28	2/5	17/33 (51.5%)

*number positive/number tested

Table 52. Low Passage Virus Strains Collected and Lyophilized in 1983

Virus identification	Strain	Passage	Source	Locality	Date isolated
WEE	BFS-1428	C6/36 #1	Culex tarsalis	Kern Co., California	June 1952
WEE	BFS-4143	C6/36 #1	Culex tarsalis	Kern Co., California	July 1962
WEE	BFN-3258	C6/36 #1	Culex tarsalis	Glenn Co., California	Aug. 1971
WEE	DLAN-23-82	C6/36 #1	Culex tarsalis	Tulane Co., California	July 1982
SLE	BFS-508	C6/36 #1	Culex tarsalis	Kern Co., California	Aug. 1950
SLE	BFS-2874	C6/36 #1	Culex tarsalis	Kern Co., California	Sept. 1960
SLE	BFN-1324	C6/36 #1	Culex tarsalis	Butte Co., California	July 1970
SLE	E-2819	C6/36 #1	Culex tarsalis	Riverside Co., California	July 1980
California encephal.	BFN-2130	C6/36 #1	Aedes melanimon	Butte Co., California	May 1970
California encephal.	BFN-3931	C6/36 #1	Aedes melanimon	Butte Co., California	Aug. 1971
California encephal.	E-19032	C6/36 #1	Aedes melanimon	Kern Co., California	Sept. 1981
California encephal.	Kern 175-82	C6/36 #1	Aedes melanimon	Kern Co., California	Sept. 1982
EEE	DV-260-82	C6/36 #1	Parus bicolor	Dennisville, New Jersey	July 1982
Highlands J	WC-431	C6/36 #1	Dumetella carolinensis	West Creek, New Jersey	Sept. 1981
Cache Valley	RU-68	Vero #1	Aedes sollicitans	Dennisville, New Jersey	Sept. 1982
Sicilian	91045-I	Vero #2	Phlebotomus papatasi	Isfahan Prov., Iran	Aug. 1975
VSV-New Jersey	82A175	Vero #1	Equus caballus	Loveland, Colorado	Sept. 1982
Keystone	FD-BHK2	BHK #2, C6/36 #1	Aedes atlanticus	Pocomoke Swamp, Maryland	1975

Table 52 (continued). Low passage strains - 1983

Virus	Strain	Passage	Source	Locality	Date
SLE	Ft. Wash. - 4	C6/36 #1	Culex pipiens	Pocomoke Swamp, Maryland	Jan. 1977
Jamestown Canyon	MP-935	C6/36 #1	Aedes canadensis	" " "	1979
EEE	MP-9	C6/36 #1	Culiseta melanura	" " "	1979
Punta Toro	Adames	Vero #3	man	Darien Pr., Panama	Apr. 1972
EEE	M-210-83A	original brain	horse	Colchester,Connecticut	Sept. 1983

VII. DISTRIBUTION OF REAGENTS, WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH (R.E. Shope, A. Main, R.B. Tesh, S. Buckley, J. Olson, G.H. Tignor, J. Meegan). The equivalent of 1,008 ampoules of arbovirus reagents were distributed from the WHO Centre to laboratories in 21 countries during 1983. This total consisted of 442 ampoules of virus stock, 339 ampoules of virus antigen, and 227 ampoules of mouse immune ascitic fluid or sera. Of the viruses and antibody distributed, there were represented 222 different arboviruses.

During 1983, the equivalent of 554 ampoules of arbovirus reagents was referred to this Centre from laboratories in 14 different countries. The referrals consisted of 372 viruses (Table 53) and 1,186 sera received for diagnosis and arbovirus antibody survey testing.

Six different cell lines and colonized insects were distributed in 1983 to 15 laboratories..

Table 53

Viruses referred to YARU for identification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
Brazil			
SP An 47817		Itimirim	
Be Ar 407981			
Be Ar 408005			
Be Ar 411391			
Be Ar 303197		Sena Madureira, Changuinola	Changuinola gp.
Be Ar 397956		Changuinola group	Changuinola gp.
Be Ar 397370		Changuinola group	Changuinola gp.
Be Ar 397374		Changuinola group	Changuinola gp.
Be Ar 411459		VSV group	VSV group
Be Ar 413570		VSV group	VSV group
China			
BA 68038		XHF (CCHF)	not viable
C 68031	domestic animal	XHF (CCHF)	CCHF
HY 13	tick	XHF (CCHF)	CCHF
France			
Brest Ar 221	tick		
Brest Ar 219	tick		
Indonesia			
H 23574	human blood	alphavirus	chikungunya
143 isolates	mosquitoes	isolated in mosquito cells	JE, orbiviruses plus many unidentified
Israel			
EO 239			
OFB 95			
EO 226			
NT 192			

Table 53 (continued)

Viruses referred to YARU for identification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
Nigeria			
Ib An 57245		Ife (orbivirus)	
Panama			
Jiminez	human blood	yellow fever	
7 VEE strains			
Sweden			
Okelbo	mosquitoes	alphavirus	Sindbis
Thailand			
KP 0039-235	mosquitoes		
-79	mosquitoes		
-44	mosquitoes		
-66	mosquitoes		
-216	mosquitoes		
USA			
BFN 3112		Llano Seco (orbivirus)	
4 strains WEE			
4 strains SLE			
4 strains CE			
SAW			
HLP 7421			
MP 935		Jamestown Canyon	
FW 4		SLE	
MP9		EEE	
Key BHK2		Keystone	
3 VSV-NJ strains		VSV-NJ	
USSR			
Leiv 3641A		Kismayo	

END

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