ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 29 September 1975

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Roy W. Chamberlain, Sc.D., Editor
Bette Ann Brenneman, Secretary

COMMENTS FROM THE EDITOR

I wish to apologize for the Info-Exchange being a week or so later than usual. The Third International Congress of Virology in Madrid, September 10-17, intervened and put its assembly and distribution a bit behind schedule. It was good to be able to meet many of you. A summary of some of the reports and discussions presented at the open meeting of the American Committee on Arthropod-borne Viruses is included in this issue of the Info-Exchange.

Let me remind you that the deadline for receipt of reports for issue No. 30 of the Info-Exchange is March 1, 1976. Please mark your calendars. Send your reports to:

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Report on a number of viruses in the Bunyamwera supergroup registered as of 31 December, 1974

Several antigenic groups were examined by the Subcommittee in order to determine whether, from a serologic point of view, there were any duplicate registrations and to classify them within each group. The following groups were examined: Bwamba, Capim, Koongol, Olinfantsvlei, Patois and Tete. In addition, unassigned viruses were also considered. For the majority of these viruses there is little information available in the literature; the evaluation of their antigenic relationships is based mainly on the results given in the Catalogue and in a number of annual reports from various laboratories. It is evident that in order to complete the establishment of the interrelationships among these agents additional work must be done with neutralization and possibly also cross-protection tests, the latter based perhaps on presence or absence of viremia.

Serologic intergroups relationships - the basis for considering all these agents as part of the Bunyamwera supergroup - are sufficient for all with the exception of the Koongol serogroup; the only published evidence of a tie between the Koongol group and other groups of the supergroup is given in Casals, J., Anais de Microbiologia 11: Parte A, 13-34, 1963. SIRACA is of the opinion that available data showing the quantitative links between serogroups in the supergroup should be added to the catalogue cards.

The available evidence shows that there are no duplicate registrations of these viruses; the results of routinely used tests, CF, HI (when possible) and NT tests, indicate that the viruses are unique and easily or relatively easily separable from one another.

Bwamba group. Consists of two viruses, Bwamba and Pongola. Easily separable by NT test; it is reported that Pongola antiserum does not neutralize Bwamba virus. Separable, although to a lesser extent, by CF, with homologous titers 2 to 4 times greater than heterologous ones. Little information by HI; antigens are not easily prepared.

Capim group. Consists of 6 viruses, distinct by CF, HI and NT tests: Acara, Bushbush, Capim, Guajara, Juan Diaz and Moriche. Another virus, Gamboa, was at first placed in this group on the basis of some cross by HI and of a CF cross-reaction between Bush bush serum and Gamboa antigen only, at a heterologous/homologous ratio of 1/16. Due to this meager relationship, it seems advisable to place Gamboa among the unassigned viruses.

The viruses in this group can be assembled as follows:

Group	Complex	Virus		
Capim	Capim	Capim	Bushbush	Juan Diaz
		Guajara	Moriche	Acara

Although the 6 viruses are distinct, the last three-Moriche, Juan Diaz and Acara - seem to be closer among themselves and to Bushbush than to the other two viruses. Additional work is needed to decide whether there is in this group a second complex - Bushbush - consisting of 4 viruses.

Koongol group. Two viruses, Koongol and Wongal. The relationship between the two viruses by CF, HI and NT tests has been well investigated, and shows that they are different and related. There is extensive screening by CF to show that the viruses are unrelated to any other catalogued agents. For the reason for being included in the supergroup, see par 2 of preceding page.

Olifantsvlei group. Two viruses, Bobia (Dak Ar 1569, Senegal) and Olifanstvlei (SA Ar 5133, South Africa) are antigenically related and show distant relationships to other members of the supergroup. These two agents have been extensively investigated at YARU and at the Pasteur Institute, Dakar; for this reason, although not registered, they are included in this report.

Patois group. Consists of 4 viruses: Pahayokee, Patois, Shark River and Zegla. They are grouped by the CF test, but easily separable by HI and virus-dilution neutralization tests. The coexistence of Patois and Zegla in the same habitats supports the view that they lack cross protection. Similarly, coexistence of Shark River and Pahaoykee supports their difference. It is recommended that cross challenge tests be done with these two sets of agents.

Tete group. Four viruses in the group: Bahig, Matruh, Tete and Tsuruse. The strains of these viruses studied so far by CF and HI are distinguishable; but as NT and crosschallenge tests are lacking, the final characterization of these agents is not complete.

Unassigned viruses. Seven registered viruses (including Gamboa: see above, Capim group) are placed, at least for the time being, in this category on the grounds that they have shown distant serologic relationships with other members or groups of the Bunyamwera supergroup, but none strong enough to justify inclusion of the individual unassigned virus in a given group rather than in another group. In other words, the position of the unassigned viruses with respect to the individual serogroups of the Bunyamwera supergroup is ambiguous; however, there is nothing ambiguous about the fact that they belong, serologically, in the supergroup.

The unassigned viruses and some of their reported relationships are:

a. Botembi: crossreacts with Koongol-Wongal and Olifantsvlei-Bobia.

b. Gamboa: crossreactions with Bushbush virus only.

c. Guaratuba: has crossreacted with CF and NT test with Mirim and Bertioga viruses (the latter, for the Guama group).

d. Jurona: crossreactions by HI test with both Oropouche serum (Simbu group) and by a Bunyamwera group polyvalent serum, at similar titers 1:20 and 1:40.

e. Kaeng Khoi: extensive serological studies show complete lack of crossing by CF test between an immune ascitic fluid for this virus and nearly 250 antigens from various groups; it has been placed in the Bunyamwera supergroup, without assigning it to any group; it may well be that this virus is a Bunyavirus not necessarily belonging to the supergroup.

f. Minatitlan: cross-CF tests involving immune ascitic fluid for this virus and 159 YARU reference antigens, and 20 polyvalent immune ascitic fluids and Minatitlan antigen, failed to show any cross-reactions. Only the group C polyvalent fluid reacted reproducibly, but at very low titer, with this virus' antigen.

g. Mirim: crossreactions only with Guama and Moju viruses (Guama group) and only by HI test. Not sufficiently close to the Guama group to consider it part of the group.

This report reviews a total of 27 registered viruses of the Bunyamwera supergroup. SIRACA welcomes comments and contributions not only on points of detail but also, and particularly, on the overall approach to the problem.

JC:cb

New Haven, 19 August, 1975

For SIRACA:

Dr. Jordi Casals, Chairman
Dr. Charles H. Calisher
Dr. Joel Dalrymple
Dr. Frederick A. Murphy
Dr. William F. Scherer

REPORT FROM THE CHAIRMAN OF THE SUBCOMMITTEE ON EVALUATION OF ARTHROPOD-BORNE STATUS (SEAS) FOR 1975

The 1975 virus evaluation activities of the SEAS subcommittee have been minimal. This inactivity can be attributed partly to the low number of newly submitted viruses, to the Catalogue Committee's preoccupation with the publishing of the new Arbovirus Catalogue (1), and to this individual's responsibility concerning the finalizing of the revised new virus registration forms.

Only nine newly registered viruses were submitted to the subcommittee during the year. Two tick-associated agents were from the Australian area and the remainder came from African mosquitoes and vertebrates. Because so few supporting data accompanied the registrations, one agent could be considered only a "probable arbovirus" and eight were rated "possible arboviruses". In addition to the new agents, the subcommittee reevaluated three previously registered viruses, raising one to full arbovirus status and two to the probable category.

Two years ago at the Athens Congresses of Tropical Medicine and Malaria, this subcommittee's report called attention to the large number of agents rated no more than"possible arboviruses" - 48% of 280 evaluated viruses. At that time various suggestions were made to stimulate interest in this area of investigation(2). The newly published Catalogue of 359 viruses listed 185 in the possible category, 52% of the total. This is a little improvement over the 1973 picture but far from satisfactory; and note that only three viruses were upgraded during 1975. For many agents the mere demonstration of viremia sufficiently potent to infect a feeding arthropod would be sufficient evidence to raise an arthropod virus to the "probable" category. Demonstration of vertebrate infectivity by means of the subcutaneous portal of entry would be still another simple but important contribution to our knowledge.

A quick review of the new catalogue reveals that 90 of the 359 registered viruses have been isolated from humans. Excluding arena viruses and other agents which probably have no arthropod cycle, one is left with 85 agents of which 59 (69%) are considered true arboviruses, 10 (12%) are "probable arbos" and 16 (19%) are "possible arbos". Thus present knowledge indicates that approximately 81% of these agents are probably arthropod borne. Only about 26 of these viruses are responsible for causing significant disease in man. All 26 are recnized as true arboviruses except for Oropouche which undoubtedly is an arbovirus but so far has not yielded to successful arthropod transmission studies. Considering the 26 viruses isolated from domestic animals, 16 or 61% are rated true arboviruses, one is a "probable arbovirus" and 9 (35%) are still in the "possible" category. Twelve of the 26 domestic animal agents (46%) are responsible for serious disease epidemics. All 12 are considered true arboviruses except for Vesicular Stomatitis-New Jersey type. Thus it is evident that where a pathogen is recognized as an important agent, adequate knowledge to elucidate its biology is usually forthcoming. For a large number of viruses, about half of the known agents,

little information is available other than the original source of isolation or series of isolations. Who is to judge when one or more of these agents is to suddenly exploit a favorable niche when there is a break in the ecological balance of a particular environment produced by land development, colonization, disruption of climate, etc. Only through continued study of the lesser known agents can we hope to be prepared for future emergencies.

One additional matter of business concerns the appointment of Dr. James Hardy to the subcommittee as replacement for Dr. Rudnick who is on duty in Malaysia.

References

(1). International Catalogue of Arboviruses including certain other viruses of vertebrates. Second edition. U.S. Department Health, Education, & Welfare DHEW Pubn. No. (CDC) 75-8301. 1975.

(2). Arthropod-Borne Virus Information Exchange, No. 26, March, 1974, p. 2.

Thomas H.G. Aitken, Chairman

Roy W. Chamberlain
James L. Hardy
D. Bruce Francy
Donald M. McLean
Albert Rudnick (In absentia)
John P. Woodall
Nick Karabatsos

Report dtd August 29, 1975

ARBOVIRUSES CURRENTLY AVAILABLE FOR DISTRIBUTION

October 1975

Distributed by:

Research Resources Branch
National Institute of Allergy
and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20014
Phone: AC 301-496-5937

NOTE: Requests for the reagents listed should be submitted on form NIH-381-2, "Requisition for Research Materials" and forwarded to the Research Resources Branch, NIAID, NIH.

Some of the seed virus materials require PHS and/or USDA permits before the reagents can be distributed. Please see numerical notation beside each seed virus listing for permit requirement. If a permit is required, please obtain the permit from either USDA or PHS before submitting your request for reagents to the Research Resources Branch.

USDA (United States Department of Agriculture) permits are obtained from the Chief Staff Veterinarian, Organisms and Vectors, APHIS, USDA, Hyattsville, Maryland 20782. PHS (Public Health Service) permits are obtained from the Office of Biohazards, Center for Disease Control, Atlanta, Georgia 30333.

ARBOVIRUSES CURRENTLY AVAILABLE FOR DISTRIBUTION

Catalog Number	Arbovirus Type	Strain	Volume
V-517-701-562	Anopheles A-IAF*	Original	0.5, 1.0 ml
V-546-001-522	Anopheles B-Seed virus(3)	Original	0.5 ml
V-546-701-562	Anopheles B-IAF	Original	1.0 ml
V-569-001-522	Bebaru-seed virus(2)	MM 2354	0.5 ml
V-569-701-562	Bebaru-IAF	MM 2354	1.0 ml
V-527-002-522	Bimiti-seed virus(3)	TRVL 8362	0.5 ml
V-527-702-562	Bimiti-IAF	TRVL 8362	1.0 ml
V-519-001-522	Bluetongue-seed virus(1)	8	0.5 ml
V-565-001-522	Bunyamwera-seed virus(3)	Original	0.5 ml
V-561-001-522	Bussuquara-seed virus(3)	BeAn 4073	0.5 ml
V-561-701-562	Bussuquara-IAF	BeAn 4073	1.0 ml
V-534-001-522	Buttonwillow-seed virus (1)	A 7956	0.5 ml
V-534-701-562	Buttonwillow-IAF	A 7956	0.5, 2.0, 5.0 ml
V-501-001-522	Bwamba-seed virus(3)	M459	0.5 ml
V-501-701-562	Bwamba-IAF	M459	0.5,2.0 ml
V-502-001-522	Calif. Encep.-seed virus(1)	BFS 283	0.5 ml
V-502-701-562	Calif. Encep.-IAF	BFS 283	0.5,2.0 ml
V-555-001-522	Candiru-seed virus(3)	BeH 22511	0.5 ml
V-555-701-562	Candiru-IAF	BeH 22511	1.0 ml
V-543-001-522	Caraparu-seed virus(3)	BeAn 3994	0.5 ml
V-528-001-522	Catu-seed virus(3)	TRVL 32046	0.5 ml
V-528-701-562	Catu-IAF	TRVL 32046	1.0 ml
V-523-001-522	Chagres-seed virus (3)	JW-10	0.5 ml
V-523-701-562	Chagres-IAF	JW-10	0.5,1.0 ml
V-529-001-522	Changuinola-seed virus(3)	BT 436	0.5 ml
V-529-701-562	Changuinola-IAF	BT 436	1.0 ml
V-548-001-522	Chikungunya-seed virus(3)	S 27	0.5 ml
V-548-701-562	Chikungunya-IAF	S 27	1.0 ml
V-514-701-562	Cocal-IAF	TRVL 40233	1.0 ml
V-506-001-522	Col. Tick Fev.-seed virus(1)	Florio	0.5 ml
V-506-701-562	Col. Tick Fev.-IAF	Florio	0.5,2.0,5.0 ml

Catalog Number	Arbovirus Type	Strain	Volume
V-533-001-522	Cowbone Ridge-seed virus(0)	W-10986	0.5 ml
V-533-701-562	Cowbone Ridge-IAF	W-10986	1.0 ml
V-575-001-522	Dengue type 2-seed virus(2)	New Guinea C	1.0 ml
V-515-001-522	EEE-seed virus(3)	Massachusetts	0.5 ml
V-515-701-562	EEE-IAF	Massachusetts	1.0,2.0,5.0 ml
V-535-001-522	EHD-seed virus(1)	New Jersey-55	0.5 ml
V-568-001-522	Getah-seed virus(3)	AMM 2021	0.5 ml
V-568-701-562	Getah-IAF	AMM 2021	1.0 ml
V-510-001-522	Guama-seed virus(3)	TRVL 33579	0.5 ml
V-510-701-562	Guama-IAF	TRVL 33579	0.5,2.0,5.0 ml
V-531-001-522	Guaroa-seed virus(3)	31498	0.5 ml
V-531-701-562	Guaroa-IAF	31498	0.5,1.0 ml
V-516-001-522	Gumbo Limbo-seed virus(0)	Fe-371H	0.5 ml
V-539-001-522	Hart Park-seed virus(1)	Ar 70	0.5 ml
V-539-701-562	Hart Park-IAF	Ar 70	0.5,1.0 ml
V-513-001-022	Hughes-seed virus(1)	DT-1	0.5 ml
V-513-701-562	Hughes-IAF	DT-1	0.5,1.0 ml
V-509-001-522	Ilheus-seed virus(3)	TRVL 5800	0.5 ml
V-509-701-562	Ilheus-IAF	TRVL 5800	0.5,2.0,5.0 ml
V-537-001-522	JBE-seed virus(3)	Nakayama	0.5 ml
V-557-001-522	Junin-seed virus(3)	XJ	0.5 ml
V-557-701-562	Junin-IAF	XJ	1.0 ml
V-552-001-522	Kern Canyon-seed virus(0)	M-206	0.5 ml
V-552-701-562	Kern Canyon-IAF	M-206	1.0,2.0,5.0 ml
V-551-001-522	Klamath(Microtus)-seed virus(0)	M-1056	0.5 ml
V-551-701-562	Klamath(Microtus)-IAF	M-1056	1.0 ml
V-570-001-522	Lokern-seed virus(0)	ATCC VR 620	0.5 ml
V-571-001-522	Lonestar-seed virus(0)	TMA 1381	0.5 ml
V-571-701-562	Lonestar-IAF	TMA 1381	1.0 ml
V-564-701-562	Main Drain-IAF	BFS 5015	1.0 ml
V-540-001-522	Manzanilla-seed virus(3)	TRVL 3587	0.5 ml
V-540-701-562	Manzanilla-IAF	TRVL 3587	0.5,1.0 ml

Catalog Number	Arbovirus Type	Strain	Volume
V-507-001-522	Mayaro-seed virus(3)	TRVL 15537	0.5 ml
V-507-701-562	Mayaro-IAF	TRVL 15537	0.5,2.0,5.0 ml
V-511-701-562	Melao-IAF	TRVL 9375	0.5,2.0,5.0 ml
V-538-001-522	Modoc-seed virus(1)	M544	0.5 ml
V-538-701-562	Modoc-IAF	M544	0.5,1.0 ml
V-541-001-522	MML(1)	B310-A564	0.5 ml
V-541-701-562	MML-IAF	B310-A564	0.5,1.0 ml
V-503-011-522	Naples-seed virus(3)	Sabin	0.5 ml
V-503-711-562	Naples-IAF	Sabin	0.5,2.0,5.0 ml
V-526-001-522	Nepuyo-seed virus(3)	TRVL 18462	0.5 ml
V-526-701-562	Nepuyo-IAF	TRVL 18462	1.0 ml
V-542-001-522	Oriboca-seed virus(3)	TRVL 47827	0.5 ml
V-505-001-522	Oropouche-seed virus(3)	TR 9760	0.5 ml
V-505-701-562	Oropouche-IAF	TR 9760	0.5,2.0 ml
V-547-001-522	Patois-seed virus(3)	BT 4971	0.5 ml
V-547-701-562	Patois-IAF	BT 4971	1.0,2.0,5.0 ml
V-566-001-522	Piry-seed virus	BeAn 24232	0.5 ml
V-573-001-522	Pixuna-seed virus(3)	BeAr 35645	0.5 ml
V-573-701-562	Pixuna-IAF	BeAr 35645	1.0 ml
V-518-001-522	Powassan-seed virus	Byers	0.5 ml
V-562-001-522	Punta Toro-seed virus(2)	Balliet	0.5 ml
V-562-701-562	Punta Toro-IAF	Balliet	1.0 ml
V-536-001-522	Rio Bravo-seed virus(1)	M64	0.5 ml
V-536-701-562	Rio Bravo-IAF	M64	1.0 ml
V-559-001-522	Ross River-seed virus(3)	T-48	0.5 ml
V-559-701-562	Ross River-IAF	T-48	1.0 ml
V-558-001-522	RSSE-seed virus(3)	Moscow B-4	0.5 ml
V-549-701-562	Sawgrass-seed virus(0)	B64A-1247	0.5 ml
V-549-701-562	Sawgrass-IAF	B64A-1247	1.0 ml
V-545-001-522	Semliki Forest-seed virus(3)	Original	0.5 ml
V-503-001-522	Sicilian-seed virus(3)	Sabin	0.5 ml
V-503-701-562	Sicilian-IAF	Sabin	0.5,2.0,5.0 ml

Catalog Number	Arbovirus Type	Strain	Volume
V-512-001-522	Silverwater-seed virus(3)	131	0.5 ml
V-512-701-562	Silverwater-IAF	131	1.0,2.0,5.0 ml
V-560-001-522	Sindbis-seed virus(3)	Ar 339	0.5 ml
V-560-701-562	Sindbis-IAF	Ar 339	1.0 ml
V-524-001-522	SLE-seed virus(0)	Parton	0.5 ml
V-544-001-522	Tacaribe-seed virus(3)	TRVL 11537	0.5 ml
V-544-701-562	Tacaribe-IAF	TRVL 11537	1.0 ml
V-550-701-562	Tamiami-IAF	W-1077	1.0 ml
V-553-001-522	Tembusu-seed virus(3)	MM 1775	0.5 ml
V-553-701-562	Tembusu-IAF	MM 1775	1.0 ml
V-530-001-522	Tensaw-seed virus(0)	A9-171B	0.5 ml
V-522-001-522	Trivittatus-seed virus(0)	4124-48	0.5 ml
V-522-701-562	Trivittatus-IAF	4124-48	1.0 ml
V-504-002-522	Turlock-seed virus(0)	847-32	0.5 ml
V-504-702-562	Turlock-IAF	847-32	0.5,1.0 ml
V-532-001-522	VEE-seed virus(1)	TC 83	0.5 ml
V-532-701-562	VEE-IAF	TC 83	1.0 ml
V-520-001-522	VSV(1)	Indiana	0.5 ml
V-520-701-562	VSV-IAF	Indiana	0.5,2.0,5.0 ml
V-520-011-522	VSV(1)	New Jersey	0.5 ml
V-520-711-562	VSV-IAF	New Jersey	1.0,2.0,5.0 ml
V-554-001-522	West Nile-seed virus(3)	B956	0.5 ml
V-554-701-562	West Nile-IAF	B956	1.0 ml
V-521-001-522	WEE-seed virus(0)	Fleming	0.5 ml
V-521-701-562	WEE-IAF	Fleming	1.0 ml
V-521-011-522	WEE-seed virus(0)	Highlands J	0.5 ml
V-521-711-562	WEE-IAF	Highlands J	1.0 ml
V-508-011-522	Wyeomyia-seed virus(3)	Original	0.5 ml
V-508-711-562	Wyeomyia-IAF	Original	1.0,2.0,5.0 ml
V-525-001-522	Yellow Fever-seed virus(3)	17D	0.5 ml
V-525-701-562	Yellow Fever-IAF	17D	1.0,2.0,5.0 ml

Catalog Number	Arbovirus Type	Strain	Volume
V-556-001-522	Zegla-seed virus(2)	BT 5012	0.5 ml
V-556-701-562	Zegla-IAF	BT 5012	1.0 ml

* IAF - immune ascitic fluid
0 - No permit required
1 - USDA permit required
2 - PHS permit required
3 - Both USDA and PHS permits required

ARBOVIRUS GROUPING FLUIDS CURRENTLY AVAILABLE FOR DISTRIBUTION

RRB Catalog #	Volume	Type	Viruses Used in Preparation of Grouping Fluids
G-201-701-567	1.0,2.0 ml	Group C	Marituba Apeu Madrid Ossa Nepuyo Caraparu Murutucu Itaqui Oriboca
G-202-701-567	1.0 ml	Group Guama	Guama Catu Moju Bimiti
G-203-701-567	1.0,2.0 ml	Group Simbu	Simbu Ingwavuma Akabane Manzanilla Oropouche Utinga Sathuperi Yaba-7 Buttonwillow
G-204-701-567	1.0,2.0 ml	Group VSV	VSV-Indiana VSV-New Jersey Cocal
G-205-701-567	1.0,2.0 ml	Group Bunyamwera	Bunyamwera Germiston Kairi Taiassui Ilesha Cache Valley Tensaw Sororoca Batai Guaroa Maguari Wyeomyia

ARBOVIRUS GROUPING FLUIDS (Continued)

RRB Catalog #	Volume	Type	Viruses Used in Preparation of Grouping Fluids
G-206-701-567	1.0,2.0 ml	Group California	California Trivittatus Melao Be AR 103645 Tahyna
G-207-701-567	1.0,2.0 ml	Group Tacaribe	Tacaribe Amapari Junin Pichinde Tamiami
G-208-701-567	1.0,2.0 ml	Group Phlebotomus	Anhanga Be An 100049 Bujaru Candiru Chagres Co Ar 3319 En An 754-61 Karimibad Icoaraci Itaporanga Naples Punta Toro Sicilian Arumowot
G-209-701-567	1.0,2.0 ml	Group A	EEE WEE VEE Pixuna Una Mucambo Y-62-33 Semliki Forest O'nyong-nyong Chikungunya Ross River Getah Bebaru Sindbis Mayaro Middleburg Nduma Aura

ARBOVIRUS GROUPING FLUIDS (Continued)

RRB Catalog #	Volume	Type	Viruses Used in Preparation of Grouping Fluids
G-210-601-567	1.0,2.0 ml	Polyvalent Quaranfil	Quaranfil Kaisodi Bandia Johnston Atoll Qalyub Silverwater Lanjan
G-211-601-567	1.0,2.0 ml	Poly. Anopheles A	Anopheles A Lukuni Anopheles B Boracea Tacaiuma CoAr 1071 CoAr 3624 Turlock Umbre M'Poko
G-212-601-567	1.0,2.0 ml	Polyvalent Bwamba	Bwamba Pongola Mossuril Kamese Eretmapodites 147 Nyando
G-213-601-567	1.0,2.0 ml	Polyvalent Patois	Patois Zegla Shark River Mirim Bertioga
G-215-701-567	1.0,2.0 ml	Group Capim	Capim Guajara Bushbush Acara BeAn 84381
G-216-701-567	1.0,2.0 ml	Group B	Bussuquara Dengue-1 Dengue-2 Dengue-3 Dengue-4 Edge Hill Ilheus Israel Turkey ME JBE

ARBOVIRUS GROUPING FLUIDS (Continued)

RRB Catalog #	Volume	Type	Viruses Used in Preparation of Grouping Fluids
G-210-601-567	1.0,2.0 ml	Group B (Con't)	Kokobera Kunjin MVE Ntaya SLE Spondweni Stratford Tembusu Uganda S Usutu Wesselsbron WN Yellow Fever Zika Powassan Louping Ill Negishi Langat Modoc Cowbone Ridge USBSG MML Entebbe bat SG Bukalesa bat Dakar bat Apoi
G-219-601-567	1.0,2.0 ml	Polyvalent Polyam	Palyam Vellore Kasbe Corriparta Acado Eubenangee Pata D'Aguilar
G-220-501-567	1.0,2.0 ml	Group Kemerovo	Kemerovo Chenuda Mono Lake Wad Medani Tribee Huacho

ARBOVIRUS GROUPING FLUIDS (Continued)

RRB Catalog #	Volume	Type	Viruses Used in Preparation of Grouping Fluids
G-221-601-567	1.0,2.0 ml	Polyvalent Congo	Congo Hazara Ganjam Dugbe Bhanja
G-222-601-567	1.0,2.0 ml	Polyvalent 1	Bahig Tete Matruh Matariya Eg An 1398-61 Burg el Arab
G-223-601-567	1.0,2.0 ml	Polyvalent 2	Jurona Minatitlan MARU 11079 Gamboa Be An 141106
G-224-601-567	1.0,2.0 ml	Polyvalent 3	Koongal Wongal Bakau Ketapang Mapputa Trubanaman Maprik
G-225-601-567	1.0,2.0 ml	Polyvalent 4	Nyamanini Uukuniemi Grand Arbaud Thogoto
G-226-601-567	1.0,2.0 ml	Polyvalent 5	Hughes Sawgrass Matucare Lonestar Soldado
G-227-601-567	1.0, 2.0 ml	Polyvalent 6	Marco Timbo Chaco Pacui

ARBOVIRUS GROUPING FLUIDS (Continued)

RRB Catalog #	Volume	Type	Viruses Used in Preparation of Grouping Fluids
G-228-601-567	1.0,2.0 ml	Polyvalent 7	Hart Park Flanders Kern Canyon Klamath Mt. Elgon bat
G-229-601-567	1.0,2.0 ml	Polyvalent 8	Bluetongue EHD Ib Ar 22619 Changuinola Irituia Colorado tick fever
G-230-601-567	1.0,2.0 ml	Polyalent 9	Navarro Triniti Aruac Pacora
G-231-601-567	1.0,2.0 ml	Polyvalent 10	Upolu DGK Wanowrie Dhori
G-233-601-567	1.0,2.0 ml	Polyvalent 12	Okola Olifantsvlei Witwatersrand Bobia Tataguine
G-236-601-567	1.0,2.0 ml	Polyvalent Rabies	Rabies LCM Vaccinia Newcastle disease Herpes

BPL INACTIVATED OF ANTIGENS

Catalog Number	Arbovirus Type	Volume
G-201-101-549	Caraparu	0.5 ml
G-202-101-522	Bimiti	0.5 ml
G-203-101-522	Mermet	0.5 ml
G-204-101-522	Cocal	0.5 ml
G-205-101-522	Tensaw	0.5 ml
G-206-101-522	California Encephalitis	0.5 ml
G-207-101-522	Tacaribe	0.5 ml
G-208-101-522	Sicilian	0.5 ml
G-209-101-522	Semliki Forest	0.5 ml
G-210-101-522	Lanjan	0.5 ml
G-212-101-522	Bwamba	0.5 ml
G-215-101-522	Capim	0.5 ml
G-216-101-522	Yellow Fever	0.5 ml
G-218-101-522	Turlock	0.5 ml
G-219-101-522	D'Aguilar	0.5 ml
G-220-101-522	Chenuda	0.5 ml
G-222-101-522	Miram	0.5 ml
G-223-101-522	Ganjam	0.5 ml
G-224-101-522	Bahig	0.5 ml
G-225-101-522	Jurona	0.5 ml
G-226-101-522	Mapputta	0.5 ml
G-227-101-522	Uukuniemi	0.5 ml
G-228-101-522	Lonestar	0.5 ml
G-229-101-522	Marco	0.5 ml
G-230-101-522	Kern Canyon	0.5 ml
G-231-101-522	Colorado Tick Fever	0.5 ml

BPL INACTIVATED OF ANTIGENS (Continued)

Catalog Number	Arbovirus Type	Volume
G-232-101-522	Navarro	0.5 ml
G-233-101-522	Dhori	0.5 ml
G-234-101-522	Withwatersrand	0.5 ml
G-235-101-522	LCM	0.5 ml

SUMMARY REPORT OF THE OPEN MEETING OF THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES MADRID, SPAIN, SEPTEMBER 12, 1975

An open meeting of the ACAV was held from 8:00-10:00 p.m. on September 12, 1975, at Madrid's beautiful Palace of Congresses. Sixty-three people from 28 countries signed the circulated attendance sheet:

Australia	2	Japan	5
Belgium	1	Kenya	1
Canada	1	Netherlands	1
Cuba	1	Norway	1
Czechoslovakia	3	Panama	1
Egypt	2	Portugal	1
Ethiopia	1	Romania	1
Finland	2	South Africa	1
France	2	Switzerland	1
French Guyana	1	Taiwan	1
Germany	2	United Kingdom	3
Israel	2	USSR	4
Italy	1	USA	19
Ivory Coast	1	Venezuela	1

AGENDA

1. Brief introduction of activities and purposes of ACAV with short historical comment - F.A. Murphy

2. Introduction of International Advisors

3. Subcommittee reports by Chairmen:

   a. Subcommittee on Information Exchange - R.W. Chamberlain (catalogue, information exchange, abstracting service)

   b. Subcommittee on Evaluation of Arthropod-borne Status - R.W. Chamberlain as attending subcommittee member for T.H.G. Aitken, chairman

   c. Subcommittee on Interrelationships Among Catalogued Arboviruses - J. Casals

   d. Subcommittee on Arbovirus Laboratory Safety - W.F. Scherer

   e. Subcommittee on Applied Molecular Arbovirology - P.K. Russell

4. Scientific Program - Scott B. Halstead, Chairman

   a. Topic is "The Allocation of Scientific Resources in Combating Arbovirus Disease Problems"

b. Identification of present shortcomings in the allocation of resources, talent and money in worldwide efforts to combat and control arbovirus diseases; particular comment on Southeast Asia - S.B. Halstead

c. Resource allocation by WHO for the control of arboviral diseases; particular comment on WHO Arbovirus Reference Center programs and diseases of Africa - P. Bres

d. Resource allocation by PAHO for the control of arboviral diseases; particular comment on PAHO/WHO Laboratory programs and diseases of Central and South America - J. Bond

e. Comment by each of International Advisors:

C. Hannoun
P. Galindo
D. Lvov
R. Doherty (not expected to be present)

f. Contributions expected of the ACAV in the future; interaction of ACAV with other agencies working on arboviral diseases - ACAV Subcommittee Chairmen and Open Discussion

REPORT OF THE CHAIRMAN AND SUBCOMMITTEE REPORTS

F.A. Murphy, the present chairman of the Executive Committee of ACAV, briefly presented the history of the ACAV, its goals and its achievements.

R.W. Chamberlain, new chairman of the Information Exchange Subcommittee, replacing Dr. T.O. Berge who retired in June, 1975, expressed the gratitude of the entire ACAV community to Dr. Berge for his excellent service since 1968. Chamberlain then described the Working Catalogue of Arboviruses (at present provided to over 160 laboratories throughout the world) and the Arthropod-borne Virus Information Exchange newsletter (with 250 recipients.) Dr. Nick Karabatsos, CDC, Ft. Collins, Colorado, is now editor of the Catalogue and Abstract Service; Chamberlain remains editor of the newsletter.

The new published International Catalogue of Arboviruses, edited by Dr. Berge before his retirement and including 359 viruses registered as of December 31, 1974, was described and offered to meeting attendants who did not already have one. New workers in the field of arboviruses were also invited to become Information Exchange collaborators.

Thomas H.G. Aitken, Chairman of the Subcommittee on Evaluation of Arthropod-borne Status (SEAS) was unable to attend. His report was given by Chamberlain. He emphasized the need for more complete experimental data to prove the arbovirus status of many of the registered arboviruses. Approximately half of the 359 viruses in the new catalogue still bear the weak rating of "possible arbovirus" because of lack of data to confirm vertebrate viremia and arthropod transmission by bite. Many of these viruses may

eventually assume medical importance, and a greater knowledge of their ecology could assume great practical as well as academic importance. On the other hand, he points out that if a virus is known to be important, it has generally been well studied. Most of the viruses of known significance to man or domestic animals are already rated as proven arboviruses.

J. Casals, Chairman of the Subcommittee on Interrelationships among Catalogued Arboviruses (SIRACA) review the subcommittee's operations and the present status of the Bunyaviridae. (See also his report in this issue of the Information Exchange).

W.F. Scherer described the newly formed Subcommittee on Arbovirus Laboratory Safety, and P.K. Russell described the new Subcommittee on Applied Molecular Virology. Both of these subcommittees were formed too recently to have accomplishments to report.

SCIENTIFIC PROGRAM

The main thrust of the "scientific" program was to review the current status of important arbovirus problems, point out shortcomings, indicate needs and guidelines for the future and discuss means of allocation of available resources for most effective disease prevention and control. There are some countries with fewer workers in arbovirus health problems now than 10 years ago, and major disease problems are greater rather than less.

S.B. Halstead emphasized the increasing problem of hemorrhagic dengue in Southeast Asia and expressed concern over the shrinking resources to combat it. In the short span of 20 years since its discovery, DHF has emerged as a major human scourge.

For over a decade, WHO has been increasingly active in DHF, and for the past 4 years has supplied diagnostic dengue antigens to national laboratories free of charge. In 1974 and 1975, an interregional Technical Advisory Committee on DHF met to develop guides for diagnosis, treatment, test methods, vector control and epidemiological surveillance. Furthermore, WHO has supplied consultants and supported study tours. Also, the Southeast Asia Ministers of Education Organization has sponsored scientific forums and now provides small research grants.

The laboratory is crucial to the management of DHF. It also plays a central role in monitoring the amount and type of dengue infections in populations; from such efforts predictive clues may emerge which could assist in epidemic prevention. However, in the 14 nations or territories in the dengue endemic area of tropical Asia, there are only 8 diagnostic laboratories. Entomological resources are even scarcer; only Singapore has a national program with adequate entomological staff to sustain Aedes control.

At a minimum, teaching hospitals and key provincial laboratories should develop dengue HI test capability. This would provide more than 100 laboratories able to perform dengue serology. A large training program is needed to attain this goal.

Dr. Bres, Chief Medical Officer, Virus Diseases, WHO, reviewed the development of a network of reference centers (WHO Collaborating Centres) to provide reference services and reagents to laboratories engaged in research on virus diseases. As part of this program, a network of WHO Collaborating Centres for Arbovirus Reference and Research has been developed. WHO also allocates resources to field studies through its Vector Biology and Control Unit; research is done on the ecology, biology and control of vectors of yellow fever, dengue, chikungunya and Japanese encephalitis. Field research units in Thailand, Korea, Tanzania, Nigeria, Indonesia and Venezuela have developed control methods and trained local specialists.

Regional centres have been designated in Australia, Czechoslovakia, France, India, Japan, Senegal, Uganda, USSR and the USA. They identify arbovirus strains submitted by laboratories in their regions and provide reagents as needed. They also give assistance at time of epidemics, provide training and develop epidemiological information for WHO. Other laboratories may be designated for specific tasks, such as preparation of antigens or testing of vaccines: examples, the Virus Collaborating Centres in Brazil, Trinidad, UK and the USSR.

The Yale Arbovirus Research Unit (YARU), New Haven, Connecticut, USA, is the WHO World Centre for Arboviruses. It receives all strains which cannot be identified by regional centres or national laboratories. It also has training responsibilities and conducts its own research program.

The directors of Collaborating Centres meet every 4 to 5 years to advise WHO on policy in arbovirus diseases. The last meeting was in 1974. Symposia or workshops were held by regional centres in USA, Czechoslovakia, Senegal and Japan.

The World Health Assembly in 1974 and 1975 recommended a program to coordinate biomedical research in developing countries, using local resources and bringing in external expertise when necessary. This program is being started in Africa as the Special Programme for Research and Training in Tropical Diseases. Efforts will first be centered on the immunopathology of parasitic diseases, but later on will probably also incorporate study and control of arboviruses.

Dr. Bond, Regional Advisor on Virus Diseases, PAHO, stated that in the western hemisphere the important arbovirus diseases are yellow fever, dengue, Venezuelan equine encephalitis, eastern equine encephalitis, St. Louis encephalitis and California encephalitis; he also included the arenavirus-caused diseases of Argentinian hemorrhagic fever and Bolivian hemorrhagic fever. PAHO has supported research on such of these problems as occur in Latin America, has developed arbovirus control programs, sponsored vaccine production and convened scientific meetings and symposia.

Jungle yellow fever has been of considerable concern over the last 20 years. Urban yellow fever cases have been few; however, the presence of large Aedes aegypti infestations in susceptible urban communities poses a constant threat. Bolivia, Brazil, Colombia and Peru, on the basis of sylvatic YF case reports, appear to have a high chronic risk. Colombia, with high

A. aegypti indexes, faces more risk than the others.

With the widespread occurrence of A. aegypti, dengue is also a constant threat. Since 1960 dengue outbreaks have been limited to the Caribbean area, but attack rates were high, sometimes exceeding 70-80%. Only serotypes 2 and 3 are currently recognized in the Americas, and the dengue shock syndrome or DHF have not yet been identified, except possibly in Curacao in 1968.

Venezuelan equine encephalitis is of great concern to the Americas. In addition to the thousands of human cases in the last large outbreak between 1969-1973, 150,000 or more equine deaths were also recorded, a serious economic loss to countries dependent on these animals.

Eastern equine encephalitis is of less regional importance but of local significance. Human cases are generally few, but horse losses can be great. St. Louis encephalitis is more of a human health problem, particularly in central and southern United States. In recent years, however, it is recognized with increasing frequency in large areas of Latin America and the Caribbean. It is known to have been active in Argentina, Brazil, Peru, Ecuador, French Guyana, Venezuela, Colombia, Panama and Mexico.

Argentinian Hemorrhagic Fever is an important disease in the Province of Buenos Aires, with about 16,000 cases reported in the last 15 years. Bolivian Hemorrhagic Fever, with fatality rates of 30-100%, has produced at least 1,000 cases in the last 10 years, mostly in rural areas of Bolivia.

Compared to many other regions of the world, the Western Hemisphere is wealthy in laboratory and epidemiological surveillance resources for control and investigation of arboviruses. It has the International Arbovirus Collaborating Reference Centre at Yale and the Regional Arbovirus Collaborating Reference Centre at Fort Collins, Colorado. In addition, there are 22 national virus laboratories in Central and South America and the Caribbean region, supplemented by 4 veterinary public health laboratories. Also, PAHO administrates 3 large research centres: in Buenos Aires (CEPANZO); in Maracay, Venezuela (Venezuelan Equine Research Centre); and in Port of Spain, Trinidad (Caribbean Epidemiology Centre-CAREC). Besides these, there are a large number of public and private research institutions in Latin America, including such examples as the Gorgas Memorial Laboratory, Panama; IVIC, Caracas; Institut Pasteur, Cayenne; Evandro Chagas Institute, Belem; Oswaldo Cruz Foundation, Rio de Janeiro; Adolfo Lutz Institute, Sao Paul; Instituto Malbran, Buenos Aires; National Institute of Health, Bogota; Institute of Hygiene, Havana, and the Institute of Virus Diagnosis and Reference, Mexico City. Also there are well-known arbovirus centres in the Caribbean, U.S. and Canada.

PAHO plays a coordinating role; a Scientific Advisory Committee for dengue has been established since 1970, which now also embraces yellow fever and Aedes aegypti. Still other advisory committees devote some of their activities to arboviruses.

PAHO has sponsored research on VEE in Mexico, Central America, Ecuador and Peru, and on other emerging new arbovirus disease problems in the Amazon

River Basin of Brazil. Symposia have been sponsored, as on VEE in 1972 and VEE vaccines in 1974, and a workshop on vector control, 1972. A major effort is made to disseminate information on important arboviral diseases.

The major unmet needs are application of effective control measures against yellow fever, VEE and dengue. Logistic problems of YF vaccination in difficultly accessible areas must be solved. PAHO intends to develop a centre to study VEE at Maracay, Venezuela. It also proposes to establish an International Centre for Investigation of Hemorrhagic Fevers.

It is clear that at this time of rising costs, it is more important than ever to promptly identify the most important problems and to orient available resources to solve them. Resources devoted to areas of study no longer considered of major importance should be reallocated.

Comments by International Advisors

C. Hannoun, Paris, was optimistic. He saw no immediate problems in Europe which would demand emergency action or major reassessment of goals. However, he did not specifically discuss the tick-borne encephalitis problem in Central Europe, which continues to be significant.

D. Lvov, Moscow, looks to the future for further elucidation of arboviruses in the arctic and sub-arctic regions, and indicates that many answers to basic questions of arbovirus ecology are to be found there. He feels particularly that certain viruses believed to be solely tick-borne may be shown to be basically mosquito-borne.

P. Galindo, Panama, says that one of the greatest problems may be in understanding the changing ecology of *Aedes aegypti* in the new world. His recent observations in Panama indicate that the strains there may breed in tree holes at considerable distance from the ground. *Aedes aegypti* control, basic to prevention of both yellow fever and dengue, will require a much greater knowledge of the habits and behavior of this basic vector than we now have. He also points out that we must remain flexible enough to quickly meet new major problems as they arise.

REVISED CATALOGUE REGISTRATION FORM

Last year an ad hoc subcommittee, consisting of Thomas H.G. Aitken (Chairman), Robert W. Dickerman, Frederick A. Murphy and John P. Woodall was appointed to revise the form used for registering viruses in the Catalogue with the purpose of permitting inclusion of more information on physicochemical properties, simplifying recording of data, permitting easier coding for computerization and improving the general readability.

The new form, consisting of four separate sheets, is now ready for distribution. A sample set is included for your information with this mailing of the Info-Exchange. Also, a reduced facsimile of each of the four sheets is included on the following pages, together with a set of detailed instructions.

You will note that the new registration form is large (12x17 inches), mainly due to broad margins. This particular format was designed to simplify lay-outs in the printshop for photographic reproductions and will reduce cost of operation. Despite the 12-inch width, the form will fit into a standard typewriter.

Those of you who have new viruses to register and require a supply of these new forms should request them of:

Dr. Nick Karabatsos
Editor, Arbovirus Catalogue
Center for Disease Control
P.O. Box 2087
Fort Collins, Colorado 80522

Virus name	Abbreviation
Arbovirus status	Antigenic group Taxonomic status

I. (1) FULL VIRUS NAME AND PROTOTYPE NUMBER
(2) Information from: Day Mon. Year
(3) Address:

II. (1) ORIGINAL SOURCE: Isolated by at
(2) Genus and species: Sentinel
(3) Age/Stage Sex: M F ? Isolated from: Whole blood Clot
(4) Serum/plasma Other fluids. Organs/Tissues:
(5) Signs and symptoms of illness:
(6) Arthropod engorged depleted gravid Time held alive before inoculation.
(7) Collection: Day Mon. Year Method
(8) Place collected Lat. ° Long. °
(9) Macrohabitat
(10) Microhabitat.
(11) Method of storage until inoculated

III. (1) METHOD OF ISOLATION AND VALIDITY: Inoculation: Day Month Year
(2) Animal Embryonated egg Tissue culture (Details next page, Par. VI).
(3) Route inoc.: Reisolation: Yes No Not tried Other reasons:
(4)
(5) Homologous antibody formation by *source animal*: (See II (2)]: Yes No Not tested
(6) Test used: HI CF NT Other

IV. (1) VIRUS PROPERTIES: Physicochemical: RNA , DNA , Single Strand , or Double
(2) Pieces . Infectivity . Sedimentation coefficient(s) S.
(3) Percentage wt. of virion protein , lipid , carbohydrate
(4) Virion polypeptides: Number. Details.
(5)
(6)
(7)
(8) Non-virion polypeptides: Number. Details:
(9)
(10) Virion density: in Sedimentation coefficient S.
(11) Nucleocapsid density in Sedimentation coefficient S.
(12) *Stability of infectivity* (effects). pH
(13) Lipid solvent: (ether) After treatment titer Control titer.
(14) (chloroform) After treatment titer Control titer.
(15) Detergent: (deoxycholate) After treatment titer Control titer.
(16) Other (formalin, radiation)
(17) *Virion morphology*: Shape: Dimensions:
(18) Mean. nm; range nm; how measured
(19) Surface projections, envelope.
(20) Nucleocapsid dimensions, symmetry
(21) *Morphogenesis:* site of constituent formation in cell.
(22) Site of virion assembly
(23) Site of virion accumulation
(24) Inclusion bodies .Other
(25) *Hemagglutination:* Yes , No , Not tried . Antigen source
(26) Erythrocytes ; pH range ; pH optimum
(27) Temperature optimum .range remarks
(28)
(29) Serologic methods recommended

V. ANTIGENIC RELATIONSHIP AND LACK OF RELATIONSHIP TO OTHER VIRUSES:

VI. (1) BIOLOGIC CHARACTERISTICS: Virus source (all VERTEBRATE isolates):
(2) .....
(3) .....
(4) Lab Methods of Virus Recovery (ALL ISOLATIONS) .....
(5) .....
(6) Susceptibility of Cell Culture Systems:

Cell system (a)	Virus passage history (b)	Evidence of Infection						
		CPE			PLAQUES			Growth Without CPE
		Day (c)	Extent (d)	Titer TCD_{50}/ml (e)	Day (c)	Size (f)	Titer PFU/ml (e)	+/– (g)

VII. NATURAL HOST RANGE

Vertebrate (species and organ) and arthropod	No. isolations/ No. tested	No. positive/ No. tested. Tes. used	Country and region

VIII. SUSCEPTIBILITY TO EXPERIMENTAL INFECTION (RECORD VIREMIA)

Experimental host and age	Passage history and strain	Inoculation Route-Dose	Evidence of infection	AST (days)	Titer log_{10}/ml
mice (nb)		ic			
" (nb)		ip			
" (nb)		sc			
" (wn)		ic			
" (vn)		ip			

IX. (1) EXPERIMENTAL ARTHROPOD INFECTION AND TRANSMISSION:

Arthropod species & virus source(a)	Method of Infection log_{10}/ml (b)		Incubation period (c)		Transmission by bite (d)		Assay of arthropod, log_{10}/ml (e)		
	Feeding	Injected	Days	°C	Host	Ratio	Whole	Organ	System

X. (1) HISTOPATHOLOGY: Character of lesions. . . . . .
(2) . . . . . .
(3) . . . . . .
(4) Inclusion bodies: Cytoplasmic: (M). . . . . (LV) . . . . . .Intranuclear: (M) . . . . . (LV) . . . . . .
(5) Organs-tissues affected: . . . . . .
(6) . . . . . .
(7) . . . . . .
(8) Category of tropism: . . . . . .

XI. (1) HUMAN DISEASE: In nature: (S) . . . . . (R) . . . . . , Death: (S) . . . . . (R) . . . . . , Residua: (S) . . . . . (R). . . . . .
(2) Laboratory infection: Subclinical: (S) . . . . . .(R). . . . . , Overt Disease: (S) . . . . . .(R) . . . . . .
(3) Clinical manifestations: . . . . . .
(4) . . . . . .
(5) . . . . . .
(6) Category . . . . . .No. of cases . . . . . .

XII. (1) GEOGRAPHIC DISTRIBUTION:
(a) Known (virus). . . . . .
. . . . . .
(b) Suspected (antibody) . . . . . .
. . . . . .

XIII. (1) REFERENCES:

XIV. (1) Remarks:

INSTRUCTIONS AND SUGGESTIONS FOR COMPLETING REGISTRATION FORM

This form is for the purpose of registering animal viruses that might be biologically transmitted by arthropods and are actually or potentially infectious for man or domestic animals. Use typewriter. In answering questions place a capital "X" in appropriate space. Retain a copy of completed registration form for reference. Refer to published articles or "personal communication" by number and list references by number on page 4. If data are too extensive or detailed to record, give references. Use metric measurements.

Page 1

Leave top two lines of boxes blank for ACAV use.

I. (1) VIRUS NAME AND PROTOTYPE STRAIN NUMBER: If a description of the virus has been published, give reference to initial publication and use the name or designation that was employed in the publication. If no publication has been made, designate the virus by name and number for at least temporary reference. If the virus is given a name and if more than one strain has been isolated, it is essential to identify the prototype strain. While the naming of a "new" virus will be left to the person making the registration, it is the opinion of the ACAV that naming a virus after a person or the symptomatology of the disease produced should be avoided. The most common usage is to name a virus after the place or area where it was initially found.

(2) Name of responder supplying the current data.

II. (1) ORIGINAL SOURCE: This refers to the original isolation of prototype strain.

(2) Genus and species: For arthropods, these names should be given. For vertebrates a common name may be used, but follow with the scientific designation and indicate if "bird", "mammal", etc., except for the common domestic animals. Note if the virus was recovered from a sentinel animal.

(3) Age or stage: Age: Applies to vertebrates, but except for man and sentinel animals, exact age may not be known. If unknown, use terms such as infant, nestling, immature, young adult, adult, etc. Stage: Applies to arthropods, especially ticks; e.g., egg, larva, nymph, adult and sex.

(4) Identify fluids, organs, or tissues.

(5) Signs and symptoms of illness: If the isolation was made from man or lower vertebrate, give signs or symptoms of illness if known. If from autopsy material, so state. More complete information on nature of the disease is recorded on page 4.

(6) Self explanatory.

(7) Methods of collection: For wild vertebrates, indicate shot, trapped, netted. For arthropods, caught by hand, trapped, kind of bait. For ectoparasite, give host. For man indicate if autopsy specimen or other.

(8) Place collected: Refers to country and political subdivision within the country such as state, county, city, town.

(9) Macrohabitat: Physiographic type: altitude, terrain, vegetation character, climate.

(10) Microhabitat: Refers to the more exact place of collection such as indoors, outdoors, elevation or ground level, in sun or shade, if parasite (on host), etc.

(11) This applies only if the specimen was held for some time after it was taken.

III.(1-2) METHOD OF ISOLATION AND VALIDITY. (From original source only).

(3-4) If reisolation not done, give clear reasons why isolation is valid (repeated isolation from similar or other sources and presence of specific antibody in associated vertebrate).

(5) Refers to vertebrate source animal only (retained or recaptured, sentinel or man).

IV. (1) VIRUS PROPERTIES: Physicochemical: Nucleic acid DNA or RNA. Single or double stranded.

(2) Pieces of nucleic acid stated as 1, known number, or S for segmented. Infectivity (+ or -). Sedimentation coefficient(s) of nucleic acid in Svedberg units.

(3) Self explanatory.

(4-7) Virion polypeptides in descending size order giving (a) letter or number designation, (b) molecular weight, (c) biological activity or function. Add "G" for glycosylation and "P" for phosphorylation of polypeptide.

(8-9) Same details as for virion polypeptides.

(10) Virion density with gradient material given (CsCl, sucrose etc.). Sedimentation coefficient in same gradient material expressed in Svedberg units.

(11) Same details as in line (10).

(12) Stability of infectivity: pH - generally stated as pH 3 stable (S) or resistant (R).

(13-14) Lipid solvent effects: If either or both ether and chloroform were tested, give concentrations of each in percent, the titer following treatment and the titer of a "control" preparation.

(15) Same details as under (13-14). State concentration of detergent in terms of dilution used.

(16) Other chemical effects: describe briefly relative inactivation with useful chemicals.

(17) Virion morphology: Typical particle shape (spherical, bullet-shaped, etc.). Dimensions.

(18) Expressed as the mean and range of statistically determined numbers of particles measured from described preparation (how measured) - negative contrast, thin section, etc.

(19) Surface projections, envelope - give presence or absence, dimensions and characteristics of surface structures.

(20) Nucleocapsid-give dimensions (mean size and range) and symmetry (T number etc.) (Express all sizes in nm).

(21) Morphogenesis: express site of formation of polypeptide and nucleic acid constituents in infected cell.

(22) Continue details of (21) with site of virion assembly (nucleus, cytoplasm-endoplasmic reticulum, Golgi, etc.) and (23) virion accumulation (reticulum lumen, extracellularly, etc.).

(24) Inclusion body site, constitution and characteristics. Other: add distinguishing morphogenetic characteristics helpful in identification.

(25-27) Hemagglutinating properties: demonstration of valid hemagglutinin (yes or no) with conditions for formation as indicated.

(27-28) Remarks: State if on account of low titer or for other reasons, the HA has not proven useful for performing HI tests. If no HA has been demonstrated, give methods tried.

(29) Methods preferred: HI, CF, NT, etc.

V. ANTIGENIC RELATIONSHIP AND LACK OF RELATIONSHIP TO OTHER VIRUSES: In recording relationship or lack of relationship to other viruses, be as specific as space and data permit. Be sure to specify method (HI: hemagglutination-inhibition; CF: complement-fixation; NT: neutralization; PC: protection challenge, that is, immunization by live or killed vaccine and subsequent challenge with live virus;

other methods). Also, information should be given on the animal source and method of preparation of the immune sera employed, i.e., schedule of inoculation and bleeding in abbreviated form. The antigenic information given should apply to the reference strain. If considerable antigenic variations have been observed in different strains of the same virus in nature or as a result of laboratory manipulation, this should be noted.

If the agent has not been assigned to an antigenic group: a) list all antigens against which an immune serum (state its titer) for the agent has been tested and given negative reactions; b) list all sera with their homologous titers expressed, or, if polyvalent, giving the titer-range against which an antigen for the agent has been tested and given negative reactions.

If the agent has been assigned to an antigenic group, give in tabular form the results of all cross-tests when dealing with a small antigenic group, or those with other members of a complex when the agent belongs in a large group. In the second case, stating the results of cross-tests with all the members in the group may take more space than is available in the registration form.

For the results of HI tests give the titers of the sera against the number of units used. For the NT in mice or plaque reduction tests in cell culture, give the (a) log N indices when using constant serum and virus dilutions; (b) the serum titers when using constant virus and serum dilutions. The results of the CF tests should be expressed by giving for each serum-antigen combination the titer of the serum and that of the antigen. To save space, the titers can be given in the form of a fraction, of which the numerator is the reciprocal of the serum titer, the denominator the titer of the antigen: for example, 128/64 means that the titer of the serum was 1:128, the titer of the antigen 1:64.

The results of other pertinent tests, such as agar gel diffusion and precipitation and fluorescent antibody techniques should be given, if available. Use tabular form, otherwise report the results as a statement.

In all cases, the recorder is at liberty to choose the method he desires in presenting the data.

VI. (1-3) BIOLOGIC CHARACTERISTICS: Virus source. Write the source (blood, CSF, CNS, heart, lung, liver, spleen, kidney, skin lesions, nasopharyngeal, milk, urine, faeces, salivary gland, mammary gland, lymph node, skeletal muscle, etc.) of all primary isolations made by anyone from naturally infected vertebrates. Specify if the source was from man (M) and/or lower vertebrates (LV).

(4-5) Lab Methods of Virus Recovery: This applies to all isolations from invertebrates and vertebrates including sentinel animals. Write the name(s) of the animal (mouse, chick, hamster, rabbit, guinea pig, primate, etc.) that has been used successfully for virus isolation and indicate whether the animal was infant or weanling, and in the case of chicks whether embryo or baby.

(6) Susceptibility of Cell Culture Systems: The information requested in the table is meant to be representative of virus behavior in particular cell systems. Describe the best cell system first. Include cell culture used in initial field isolation (if pertinent) and cultures used with adapted strains. In Column (a) note cell system used and indicate if primary culture (PC) or cell line (CL); Col. (b) passage history is self evident; Col. (c) Day (CPE or PLAQUES) means day post inoculation when effect is first clear; Col. (d) Extent (CPE) means ultimate effect on scale of 1+ to 4+ (using standard definitions); Col. (e) Titer should indicate ultimate growth of virus and reflect the relative sensitivity of the system (specify system); Col. (f) Plaque size may be expressed numerically (mm) or as "small, medium, variable, etc."; Col. (g) as invertebrate cells usually will only show virus growth (indirectly) mark the result + or -. Any special, useful culture condition factors should be noted.

VII. NATURAL HOST RANGE: Here should be listed in the appropriate column all vertebrates and arthropods from which the virus has been recovered in nature, as well as results of antibody surveys, giving number tested and number positive. Be sure to specify source of virus (e.g., blood, brain, unengorged mosquitoes, etc.). Give scientific name and in case of vertebrates include common name for clarity. The preferred sequence in listing is man, lower primates, other vertebrates, and arthropods. Give reference to source of data. Also indicate place (country and region) where virus isolations were made and serological surveys conducted. If field source was sentinel animal, be sure to specify. In recording serological surveys, give test used (NT, HI, or CF). For viruses extensively studied general statements on frequency of virus isolations, as well as percentage range of antibodies, may be given instead of exact numbers.

VIII. SUSCEPTIBILITY TO EXPERIMENTAL INFECTION: List experimental hosts (animals, embryonated eggs, etc.) that have been tested for susceptibility to infection with the virus. Give passage history of strain used in test, i.e., number of passages in animal (species) and route. Give evidence of infection in animals such as viremia, paralysis, death, etc., and AST (average survival time) for a particular dose. Since it is frequently useful in classifying the viruses, behavior in newborn (nb), weanling (wn), and adult mice should be recorded. If the information cannot be recorded in the limited space, give references to publications or other reports. Titer refers to the infectious titer in the animal designated, preferably expressed as $\log_{10}$ per ml.

IX. (1) EXPERIMENTAL ARTHROPOD INFECTION AND TRANSMISSION: Summarize experiments indicating in Column (a) species of arthropod used and virus source, e.g. viremic vertebrate, artificial "blood" meal, mouse brain or serum, etc.: Col. (b) titer of virus offered to arthropod; Col. (c) duration of (in days) and temperature during extrinsic incubation period; Col. (d) indicate virus transmission by host bitten (mouse, chick, etc.) and the transmission ratio, i.e. the number of individual arthropods transmitting (numerator) over the number tested (denominator); in the event of pool feeding, state size and number of pools; Col. (e) express titer of transmitting arthropod as $\log_{50}$ of one whole body or organ (salivary glands, etc.) in 1 ml of diluent as assayed (state system). Workers are urged to terminate infectivity experiments by allowing the test arthropod to bite a susceptible host. It is recommended that if identity of the test arthropod species is in question, sample specimens be preserved for future confirmation of identity.

X. (1-3) HISTOPATHOLOGY: State briefly general character of lesions in man (M) if known, and/or in lower vertebrates (LV) either naturally or experimentally infected. Give name of animal in which lesions are described and, whether naturally or experimentally infected (specify route). Give references.

(4) Inclusion bodies in man (M) or lower vertebrate (LV).

(5-7) Organs-tissues affected: Specify tissues affected and indicate if from man (M) or lower vertebrate (LV). Specify species of animal.

(8) Category of Tropism: Refers to system of organs and tissues particularly and characteristically involved. This applies mainly to the disease in man or in animals naturally infected.

XI. (1) HUMAN DISEASE: To simplify codification of the usual and the unusual, the registrant is requested to indicate by "X" whether human disease as observed in nature is common or significant (S) or uncommon but reported (R), likewise for death and residual manifestations.

(2) Laboratory infections: Mark by "X" if subclinical manifestations are significant (S) or reported (R), likewise for overt disease.

(3-5) Indicate clinical manifestations such as: fever, headache, prostration, conjunctival inflammation, stiff neck, myalgia, arthralgia, hemorrhagic signs, CNS signs (including encephalitis), respiratory involvement, leukopenia, CNS pleocytosis, rash, lymphadenopathy, jaundice, vomiting, other significant symptoms. Indicate each if significant (S) or reported (R).

(6) Specify if disease manifestation is one of the following categories: a) febrile illness, b) febrile illness with rash, c) hemorrhagic fever, d) polyarthritis and rash (no fever), e) encephalitis. If only a few cases have been observed, the actual number should be given, otherwise estimates will suffice. List references.

XII. (1) GEOGRAPHIC DISTRIBUTION: Designate after "Known" (virus recovery) or "Suspected" (serological survey antibodies) as may apply, the countries involved. Give reference for each country.

XIII. (1) REFERENCES: List references as previously referred to by number. Save space by not giving title of published articles and give only the name of first or possibly second author. When space does not permit listing of all important references and when pertinent abstracts and personal communications are in the ACAV Abstract File, reference to the file may be made by number and under "References" state "see Abstract File" following the corresponding number. Permission to make reference to information in unprinted annual reports or the Information Exchange should be obtained from the proper person and should be referred to under his name as "personal communication"; give page number of such reports.

XIV. (1) REMARKS: Space is left here for additional information of importance not covered in previous questions or where space was not sufficient for recording. But, do not use this space unless necessary, as it may also be employed for approved additions to the preceding records. Virus synonyms can be included here.

REPORT FROM THE ARBOVIRUS LABORATORY, INSTITUTO ADOLFO LUTZ, SAO PAULO, BRAZIL

On April 3, 1975, we received information that several cases of a disease attacking the central nervous system were occurring in a few small counties in the South sea coast of Sao Paulo State. The physicians thought they were dealing with meningococcic meningitis, as we had been having an extensive epidemic of this disease since 1972. However, the observed cases in that region had a distinct clinical picture and the cerebro-spinal fluid examinations were consistently negative for any bacteria.

Based on this information we visited the region on April 7 and verified that most of the cases were occurring in Peruibe and Itanhaem counties. Peruibe is a quite large county (23°20'S and 47°01'W) with an area of 339 square kilometers and a population of about 10,000 inhabitants, mostly city dwellers. The region is a sea level plain which finishes in a mountain range called Serra do Mar. The average temperature is 21°C and the total annual rainfall is about 2,000 mm, which makes the place a very humid one. The same description could be applied to Itanhaem, located at about 30 kilometers from Peruibe. Both places are two of the sea resorts for our State and they received many tourists each weekend and during the vacation seasons. Most of the people living there work for tourists. Other occupations are wood-cutting, clearing and cleaning plots of land, planting bananas, rice and a very few other fruits.

During our first visit, which lasted until April 10, we observed many cases of a severe disease attacking the central nervous system, causing encephalitis or a meningo-encephalitis with several deaths. The major symptoms were high fever (about 39°C or 102°F) headache, tremors, paralysis, muscular weakness, difficulty of speech, and coma in the more severe cases. The coma appeared to be lethargic and some died due to stopping of the heart or of the respiration. No hemorrhagic symptoms or jaundice were observed. The examination of cerebro-spinal fluid showed pleocytosis (around 200 WBC), with lymphocytes predominating.

The outbreak started suddenly in the middle of March and lasted until the end of June. From Peruibe a total of 216 persons needed to be hospitalized (see figure). The great majority of cases occurred among people living in rural areas of the counties and almost no disease was observed from those living inside the cities. A few encephalitis cases appeared among inhabitants of Sao Paulo city who had been in the Peruibe area. It seems that, judging by their exposure, the incubation period was about 12 days. All age groups were attacked but it seems that those from 15 to 30 years of age were the most attacked. However, these data should be estimated better to verify a significative difference, as there are no reliable statistics about the region. Males were more attacked than females but no colour differences were observed.

Virus Work

During our first trip (April 7 to 10) we collected anal and oral swabs and blood from the hospitalized acute cases in order to try to isolate the agent causing the outbreak. Field work was started by collecting mosquitoes using human bait and light traps, netting wild birds and trapping mammals; a number of domestic animals were also bled. We exposed sentinel animals (mice and hamsters) and several virus isolations were made from them. Seven were of a very fast agent which kills 2-day-old mice in 1.5 days. It was found to belong to the C group, being closely related if not identical to SPAn 26 550, a virus close to Caraparu and isolated by us in January 1974, in the Rio Guaratuba field station (23°45'S and 45°55'W). Another isolation also came from a sentinel mouse. It was a slow virus, killing suckling mice in 6 days and by CF testing it seemed to be a strain of Cotia virus, a poxvirus isolated many times from sentinel animals in several of our field stations. This isolation was quite a surprise, as we had not isolated Cotia virus since 1965. No other virus has yet been recovered from any source, including all human material. The majority of the field materials (blood from wild birds, bats and domestic animals and the mosquitoes), are stored at -60°C awaiting inoculation. It is interesting to note that the wild birds were scarce, judging by our previous experience in other field stations. Net captures average about 18 birds per 100 net hours and in Peruibe we obtained 9 birds per 100 net hours. Also we sere unable to trap wild mice in a reasonable number, in spite of running 16 traps for 8 capture nights. We could not find any explanation to this fact, except for the human invasion and the indiscriminate use of agricultural inseticides there.

Entomological Studies

We tried to collect blood-sucking arthropods in the region. The mosquito population was high and very active. We captured at least 8 different species, the most frequent being those of the genus *Culex* (with 3 sub-genera: *Culex*, *Melanoconion* and *Microculex*), *Aedes serratus* and *Aedes scapularis*, *Mansonia albifera* and *Psorophora cingulata* and *Psorophora ferox*. The Anophelini and Sabethini were scarcer than the Culicini mosquitoes. Several larvae collections were made to help in the identification of adults and most of this material will be sent to Dr. J. Belkin from University of California to correct or confirm our identifications. Up to now no virus was isolated from 8 mosquito pools. We were unable to collect a reasonable number of any other blood-sucking arthropods, such as horse flies or black flies. The tick population was not at its peak, which occurs in our dry-cold season, that is, during the winter. A few ticks identified as *Boophilus microplus* were collected on domestic animals and no virus isolations were obtained from them.

Human Studies

We are trying to isolate virus from blood and cerebro-spinal fluid taken from patients in the acute phase who sought medical care in beginning of the neurological manifestations. Part of the blood was saved for serology and was considered as the first sample, waiting for convalescent serum. No virus

isolation was obtained from blood examined up to now and it seems to us that if there is any viremia, it is prodromic and very short, as we have finished the inoculation of over 80 acute blood samples. We obtained viscera from 5 post-mortem examinations a few hours after the patients' death. From one of them we isolated a virus, DCA resistant, which titered low in baby mice brain (10^{-2} - 10^{-3}). The patient from whom it was isolated did not show any CF antibodies for this virus. Ten pairs of sera from encephalitis patients did not show any serological response, in CF tests, to this virus. It seems to us that this agent, which needs to be bettered studied, did not play a significant role in the outbreak.

Serology of People

During our first stay in Peruibe we collected sera from 150 people from the local rural population to try to find, by serological tests, which viruses were circulating at that time. For the Arbovirus group A we found few sera able to inhibit Mucambo (BeAn 8) and VEE (Donkey 1) agglutinins. These inhibitions could be explained by the fact that we have isolated Mucambo virus in 1969 at Casa Grande field station, which is not far from there. Their titer was low (up to 1/40) and those for Mucambo were always higher. No antibodies were found to WEE, EEE, Mayaro and Pixuna. The absence of antibodies for EEE virus was interesting as we isolated this virus several times at the Guaratuba field station, which is at the northern part of the same sea-level plain.

For Arbovirus group B tests, all of the viruses used had been isolated in Brazil, except for Yellow Fever, where we are using the Asibi strain which was described to be more sensitive than the JSS strain isolated in Brazil. Bussuquara is represented by BeAn 4511, Ilheus by the original isolation and SLE by SPAn 11916 strain, which was isolated by us and found to be identical to Parton and Tr 9464 in CF, HI and N tests.

From patients with encephalitis we have already tested 91 paired sera with more than 20 days between the bleedings. We observed that 49 (54%) showed sero-conversions resembling primary reactions for at least one of the B group viruses, with more being reactive to Ilheus and the fewest reactive to Bussuquara. We have also observed that 21 (23%) had secondary group B reactions. Both groups together accounted for 77% of the total paired sera. Fourteen (15%) of the paired sera did not show a rise in antibodies and 6 (7%) were negative. Table I shows the total results and Table II shows some of the reactions observed with HI and CF tests. Table III presents results of a few other HI tests. The paired sera that converted in HI and CF tests with a primary reaction had a constant pattern. The acute sera showed no antibodies or had them in a very low titer (1/10; 1/20) and in convalescent sera the antibodies raised to 1/40 in 1/160 for one or all 4 antigens, with almost identical titers. This pattern has suggested to us that we probably were picking up heterologous reactions and that the virus responsible for the conversions was not present. This kind of reaction appeared mostly in children and young adults.

Concerning the C group virus isolated by us in the region, we observed

only very few conversions among the 91 paired sera tested up to now. The same applies to Cotia virus which was tested in CF against 10 pairs of sera with negative results. It seems to us that both viruses were circulating in the region but did not play any role in the outbreak.

Serology of Domestic and Wild Vertebrates

We collected sera from several domestic and wild vertebrates and the results for B group virus obtained up to now are shown in Table IV. The serological pattern resembled very closely that obtained from the human population. From our data it seems that they were infected by this B group agent and played a very important role in the outbreak.

We have received the visit of Drs. F. Pinheiro, A.H.P. Andrade da Rosa and Jorge T. da Rosa from the Belem Virus Laboratory. They tried to test 2 other strains of SLE virus (one of them isolated from a human being), a strain of Ilheus virus and another of Yellow Fever, all viruses isolated at the Belem Virus Laboratory. They have a hypothesis that the SLE virus that we were using may behave like the M 1/111 strain of JBE (see Information Exchange nº 28, pag. 133). Paired sera by CF and HI tests showed similar titers with all strains demonstrating that we have been used equally sensitive strains.

Comments

The epidemiological pattern of the disease suggested to us that we are dealing with an agent which has caused the most important human arbovirus epidemic which has occurred in Brazil, other than Yellow Fever. Our assumption is based on several facts. The disease occurred in the rural areas where the houses and huts are built deeply in contact with the forest, which, in that region, is one of secondary growth. The cases were scattered all over and we have not observed any evidence of a man-to-man transmission or nothing that could lead us to suspect an enterovirus epidemic. The serological results obtained from the paired sera and from the population helped to built up this hypothesis, which could be proved by the isolation of the virus responsible for the epidemic.

It also seems to us that the virus was only recently introduced because the disease affects all age groups suggesting no previous experience with it. Besides, older inhabitants were unable to recall the occurrence in the past of any similar disease in the region.

Up to now we could not isolate any virus which could be incriminated as the etiologic agent of the outbreak. However, we have several specimens in the freezer waiting to be inoculated into mice and we hope to obtain isolations from the field material. The agents isolated by us (Caraparu, Cotia and the human unidentified virus) do not seem to be responsible for this outbreak.

Our field and human work will continue in the affected area in order to prove our hypothesis, and other lines of study are being developed. For instance, a house-to-house inquiry is being conducted to try to obtain more

precise data on the local population, the number of diseases and deaths, possible relationships among the inhabitants and their way of life, presence of domestic animals, proximity of forests, quantity of blood-sucking arthropods, etc. This inquiry also aims to discover the way of virus transmission and to determine the incidence, prevalence, attack rate, etc.

At the same time, a vigilance program is being set outside of the epidemic region to try to detect the virus if it invades other areas. It will consist of setting sentinel animals and collecting mosquitoes for virus isolation and obtaining blood samples from the human population to verify their antibody levels.

(O.S. Lopes, L.A. Sacchetta, T.L.M. Coimbra)

TABLE I

Results of HI tests of paired sera from patients with encephalitis from Peruibe Region (totals)

Number	Negatives		Primary Conversion		Secondary reaction		No change in titer	
	Nº	%	Nº	%	Nº	%	Nº	%
91	6	7	49	54	21	23	14	15

Monthly distribution of human encephalitis cases from Peruibe, state of São Paulo, Brazil, 1975.

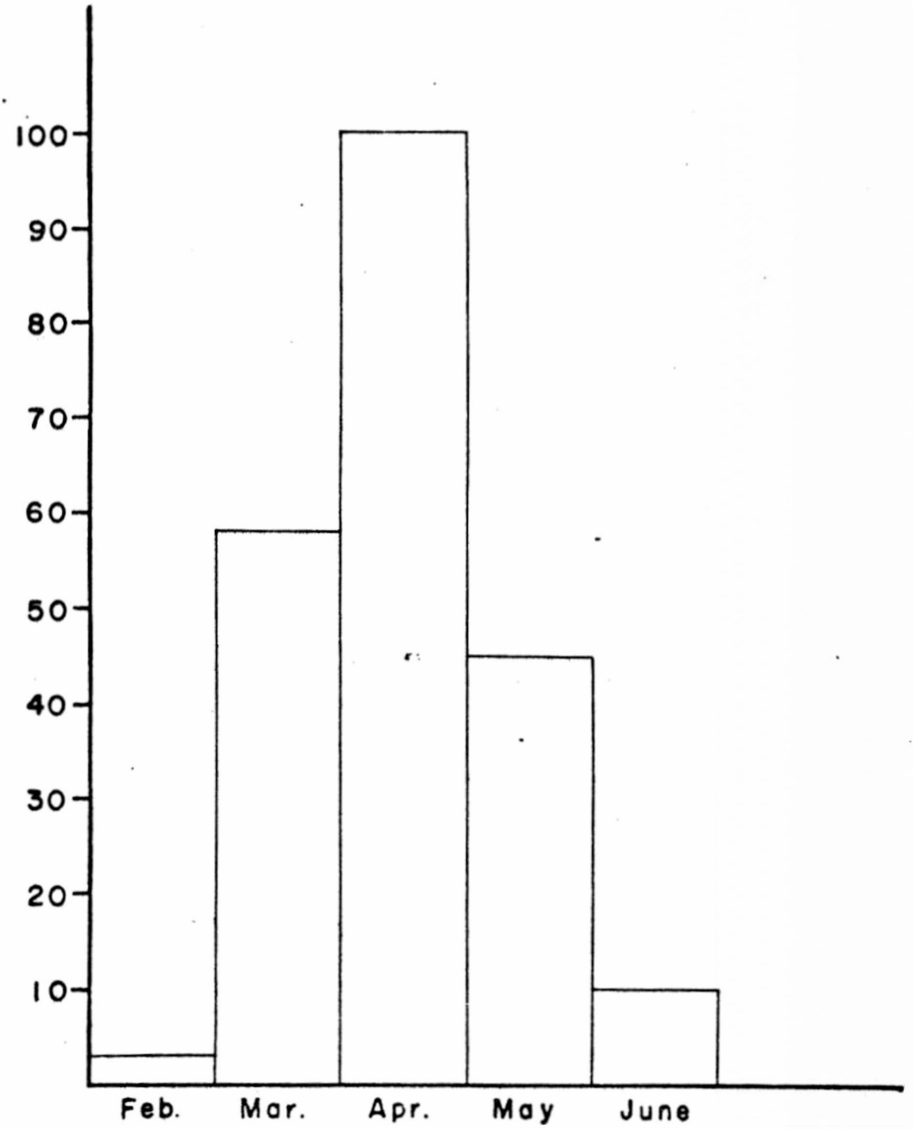


TABLE II

HEMAGGLUTINATION-INHIBITION and COMPLEMENT FIXATION TESTS WITH PAIRED SERA OF 10 PATIENTS WITH ENCEPHALITIS, FROM PERUÍBE REGION, STATE OF SÃO PAULO, BRAZIL.

Patients and Lab nº	Age in years	Date of bleeding	Bleed in days after onset	HI				CF				COMMENTS
				YF	BSQ	ILH	SLE	YF	BSQ	ILH	SLE	
E.S.S. H 30.030	7	5/4/75	5	0	0	40	0	0	0	0	0	Onset of disease 31/3/75 Primary reaction
		28/4/75	29	40	40	80	40	0	0	8/64	8/64	
		3/5/75	33	40	40	80	40	0	0	8/64	8/16	
M.F.F. H 30.029	5	5/4/75	5	0	0	40	0	0	0	0	0	Onset of disease 31/3/75 Primary reaction
		28/4/75	29	0	0	80	40	0	0	8/64	8/16	
		3/5/75	33	40	40	80	40	0	0	8/64	8/64	
A.R. H 30.175	30	9/4/75	6	0	40	40	0	0	0	0	0	Onset of disease 3/4/75 Primary reaction
		3/5/75	28	0	40	40	40	0	0	8/64	8/16	
M.S.S. H 30.266	6	10/4/75	3	0	0	0	0	0	0	0	0	Onset of disease unknown Hospitalised on 7/4/75 Primary reaction
		18/4/75	11	40	0	40	40	0	0	32/64	8/64	
		3/5/75	26	40	0	40	40	0	0	8/64	8/64	
E.F. H 30.028	23	5/4/75	5	640	320	640	320	128/64	128/16	128/64	128/64	Onset of disease 31/3/75 Secondary reaction
		24/4/75	24	320	160	640	320	128/64	32/16	128/64	128/64	
		3/5/75	33	320	160	320	320	32/64	32/16	128/64	128/64	
C.A. H 30.186	17	9/4/75	6	40	40	160	40	8/16	8/16	8/64	8/16	Onset of disease 3/4/75 Secondary reaction
		24/4/75	21	40	40	80	40	8/16	8/16	32/64	8/64	
		3/5/75	30	40	40	160	40	8/16	8/16	8/64	8/64	
J.K.S. H 30.009	45	5/4/75	3	40	320	640	≥1280	128/64	128/64	128/64	128/64	Onset of disease 2/4/75 Secondary reaction
		24/4/75	22	40	320	320	320	128/64	128/64	128/64	128/64	
		3/5/75	31	40	320	640	640	8/64	32/64	32/64	128/64	
N.A.S. H 30.174	22	9/4/75	7	160	40	80	160	32/64	8/16	32/64	32/64	Onset of disease 2/4/75 Secondary reaction
		30/4/75	28	320	40	160	160	32/64	8/64	32/≥256	32/64	
M.C.S. H 30.026	53	5/4/75	10	40	40	160	40	8/64	8/16	8/64	8/64	Onset of disease 25/3/75 Secondary reaction
		28/4/75	33	40	40	160	40	8/64	8/64	8/64	8/64	
A.G.J. H 30.015	43	5/4/75	7	≥1280	≥1280	≥1280	≥1280	128/64	128/64	≥512/256	≥512/64	Onset of disease 29/3/75 Secondary reaction
		28/4/75	29	640	640	≥1280	640	128/64	128/64	512/64	128/64	
		3/5/75	34	640	320	≥1280	640	32/16	32/16	128/64	128/64	

TABLE III

HEMAGGLUTINATION-INHIBITION TESTS WITH 14 PAIRED SERA OF PATIENTS WITH ENCEPHALITIS, FROM PARUIBE REGION, STATE OF SÃO PAULO, BRAZIL

Patients and Lab nº	Age in years	Date of bleed	Bleed in days after onset	HI					COMMENTS
				EF	BSQ	SLE	ILH	An 30.256	
M.S.S. H 30.259	6	10/4/75	3	0	0	0	40	40	Onset of disease unknown Hospitalized on 7/4/75 Primary reaction
		18/4/75	11	0	40	80	80	40	
		3/5/75	26	40	80	40	80	40	
L.T.S. H 30.268	11	10/4/75	5	0	0	0	40	40	Onset of disease 5/4/75 Primary reaction
		24/4/75	19	0	40	0	40	40	
		3/5/75	28	0	40	0	40	40	
M.G.S. H 30.260	14	10/4/75	5	0	0	0	0	0	Onset of disease 5/4/75 Primary reaction
		24/4/75	19	0	40	0	40	0	
		3/5/75	28	40	80	40	80	0	
D.R. H 30.034	31	9/4/75	-7	0	0	0	0	0	First serum obtained for a serological survey Onset of disease 16/4/75 Primary reaction
		24/4/75	8	0	20	0	20	0	
		3/5/75	17	0	40	0	40	0	
E.D. H 30.024	3	5/4/75	3	0	0	0	0	0	Onset of disease 2/4/75 Primary reaction
		3/5/75	31	0	40	0	40	0	
C.C. H 30.014	11	5/4/75	6	40	80	0	40	0	Onset of disease 31/3/75 Primary reaction
		3/5/75	34	160	320	40	80	0	
J.M.N H 30.261	56	10/4/75	7	320	≥1280	320	640	0	Onset of disease 3/4/75 Secondary reaction
		18/4/75	15	640	≥1280	640	≥1280	0	
		3/5/75	30	320	≥1280	≥1280	640	0	
T.C. H 30.069	68	9/4/75	3	320	≥1280	320	320	0	Onset of disease 6/4/75 Secondary reaction
		18/4/75	12	320	≥1280	640	640	0	
J.V.C. H 30.263	49	10/4/75	5	160	320	80	160	0	Onset of disease 5/4/75 Secondary reaction
		18/4/75	13	320	≥1280	160	320	0	
F.F.L. H 30.037	39	9/4/75	1	0	0	0	0	0	Onset of disease unknown Hospitalized on 8/4/75 Secondary reaction
		3/5/75	25	160	320	80	160	80	
A.B.C. H 30.329	29	18/4/75	13	80	320	80	80	80	Onset of disease 5/4/75 Secondary reaction
		3/5/75	28	80	320	80	160	80	
J.J.C. H 30.001	38	9/4/75	14	320	320	80	160	160	Onset of disease 25/3/75 Secondary reaction
		3/5/75	41	160	320	80	80	80	
J.S.S. H 30.176	47	9/4/75	5	40	40	0	40	0	Onset of disease 4/4/75 Cross reaction ?
		3/5/75	29	40	40	0	40	0	
C.T.C. H 30.179	45	9/4/75	10	40	40	40	80	0	Onset of disease 31/3/75 Cross reaction?
		24/4/75	25	40	40	80	40	0	

3 paired sera have not converted by this reaction. The blood samples were collected within intervals of 10, 30 and 35 days after onset, respectively. The patient J.C.S., H 30.023, referred mumps before current disease.

TABLE IV

Serological results with Group B antigens with wild and domestic vertebrates, from Peruibe Region.

Vertebrates		Positives	Total	%
Wild Birds		37	153	24.2
Domestic Birds	Chickens	7	96	7.3
	Ducks	1	4	25.0
	Pidgeons	2	7	28.5
Domestic Animals	Dogs	0	4	-
	Pigs	0	8	-
	Rats	1	14	7.1
Wild Rats		6	8	75.0
Marsupials		3	5	60.0
Bats		6	55	10.9

REPORT FROM THE VIRUS DEPARTMENT
NATIONAL INSTITUTE OF HYGIENE
GUAYAQUIL, ECUADOR

YELLOW FEVER SURVEY. NAPO PROVINCE

In January 1975, two fatal cases of yellow fever were diagnosed by histopathology in the Napo Province of eastern Ecuador, in the Amazonas river basin. Beside surveillance activities, a retrospective sero-survey of humans and wild monkeys was conducted; and mosquitoes were collected in the area of the confirmed cases. This table shows the results of the sero-survey.

LOCALITY	N° Tested	N° POSITIVE (%) ☒ MONOTYPIC OR MIXED HOMOTYPIC				GROUP B REACTION
		YF	SLE	DEN	BUSS	
MAN						
Lumbaqui	127	8 (6%)	0	1 (0.8)	0	21. (16.5)
Lago Agrio	35	4 (11%)	1 (3)	2 (6)	0	11 (31)
Shushufindi (Nueva Esmeraldas)	55	2 (3.6)	2 (3.6)	0	0	1 (1.8)
Other Localities.	21	1 (4.7)	1 (4.7)	1 (4.7)	0	5 (24)
TOTAL =	238	15 (6.3)	4 (1.6)	4 (1.6)	0	38 (15.9)
MONKEYS						
Shushufindi	15	1 (6.6)	0	0	1 (6.6)	3 (20)

☒ CF test ≥ 1:16

VIRUS ISOLATIONS FROM W. ECUADOR

LAB NUMBER	HOST	SPECIMEN	COLL. DATE	COLL LOCATION	TYPE
18315	Sentinel hamster	blood	6-28-74	San Rafael (Tenguel)	CAPIM
19066	bat	blood	8-27-74	Huerta Negra (Tenguel)	VEE
21297	Sentinel hamster	brain	5-2-75	K 32 Duran-Troncal	SLE
21386	cormorant	blood	5-19-75	Abras(Vinces)	EEE
21688	man	throat swab	6-19-75	Salinas(Chone)	VEE

(E. Guitierrez V.)

REPORT FROM CARIBBEAN EPIDEMIOLOGY CENTRE (CAREC) incorporating TRINIDAD REGIONAL VIRUS LABORATORY (TRVL) PORT OF SPAIN, TRINIDAD

During the last months of 1974, the transfer of TRVL from the administration of the University of the West Indies to the Pan American Health Organization was made and completed by 1st January, 1975. The change was made at the request of the Governments of Trinidad and Tobago and the Caribbean Health Ministers' Conference. The new Centre incorporates TRVL and has an expanded role in Epidemiological Surveillance, Training and Research concerning diseases, particularly communicable diseases, of importance in the Caribbean. The first six months of 1975 have allowed re-organization of the Centre to include a surveillance division and improved training facilities.

CAREC continues arbovirus surveillance and plans to develop specific selected arbovirus research studies in association with the surveillance programme.

In Trinidad, patients with jaundice, CNS disease and pyrexias of unknown origin have been monitored for arbovirus infection. The Group B arbovirus serological survey in school children from selected areas has been modified. Group B activity has not been detected.

Plans are currently being made with the Guyanese authorities for a serological survey of equines on the coastal regions of Guyana for the equine encephalitis viruses. In addition, it is planned to monitor arbovirus infections in the interior of Guyana. Both projects are expected to get underway by the end of the year of early in 1976.

(P.J.S. Hamilton)

REPORT FROM FLORIDA DIVISION OF HEALTH, BUREAU OF LABORATORIES
JACKSONVILLE, FLORIDA

As part of general virus diagnostic services provided to the medical community of Florida, we tested 394 patients' sera against a battery of antigens associated with central nervous system diseases during the period January 1975 - June 1975. One case of EEE was detected in a 39 year old male, who was a resident of Georgia and was brought to a Florida hospital where he expired.

It is interesting to note that the 14 horses with EEE and the two horses with WEE were all from the northern part of Florida.

Human and Animal Sera Screened
by the HI Technique with Arbovirus Antigen*
Jan 1975 - June 1975

Species	Number of Sera	Reactors
Human	394	1 EE** 2 EE - constant low titer 6 Group B***
Horses	33	14 EEE 2 WEE
Field Specimens (mammals)	255	None
TOTAL	682	25

* Arbovirus Antigens:
EEE - Eastern Equine Encephalitis
WEE - Western Equine Encephalitis
SLE - St. Louis Encephalitis TBH-28
VEE - Venezuelan Equine Encephalitis TC-83 and/or Fe3-7c

** 39 year old male from Georgia, expired in a Florida hospital.

*** 3 patients' paired sera had constant low level HI antibodies with SLE and Dengue antigens.

(N.J. Schneider and E.E. Buff)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO

Studies on Simian Hemorrhagic Fever (SHF).

Several explosive epizootics of SHF affecting monkeys of the Macaca species have occurred during the past decade in Soviet, American, and British vivaria. The two initial outbreaks were recognized a few weeks apart in 1964 at Sukhumi, U.S.S.R., and the National Institutes of Health (NIH), Bethesda, Maryland among monkeys supplied from India to both institutions by the same shipper. During the late sixties other epizootics in primate colonies were reported -- one in California (1967) and several in Sussex, UK (1966-69). The most recent epizootic, involving over 200 monkeys, occurred in 1972 again at the NIH.

The clinical and pathological features of each SHF outbreak have been similar and the SHF virus strains isolated from the sick monkeys were immunologically related. Early studies with the Bethesda virus suggested that it resembles the arboviruses of group B, but the virus has not been yet classified. Our purpose was to define further the biophysical and biochemical properties of the prototype strain of SHF virus (LVR 42-0/M6941) propagated in MA-104 cells.

We found the virus to have an approximate density of 1.18 g/cm^3 in potassium tartrate. The outer diameters of the particles averaged 48 nm. Most of the virions were spherical with tight-fitting envelopes which masked the ultrastructure of the internal nucleocapsids; some tubular structures, 25 nm in diameter, were occasionally seen. Average diameter of the nucleocapsids of intact particles was 25 nm. The ^{32}P-labeled nucleic acid, extracted from purified SHF virions with phenol-SDS, appeared as a broad peak of ^{32}P activity in polyacrylamide gels. Electropherograms of purified SHF virus doubly labeled with ^{14}C-amino acids and ^{3}H-glucosamine showed four peaks of radioactivity, two of which we identified as glycoproteins by co-electrophoresis. Virions were found to contain a high percentage of phosphatidylcholine, suggesting that the viral envelope originated in the internal membranes of the host cell.

The relevant results of the studies by us and by others can be arranged in the order of virus classification criteria promulgated by the International Committee on Nomenclature of Viruses as follows: (1) the virus nucleic acid core is RNA; (2) the virus capsid symmetry is probably cubic; (3) the virion is enveloped; (4) the site of capsid assembly is in the cytoplasm; (5) the site of nucleocapsid envelopment is at the intracytoplasmic membranes; (6) the virion is sensitive to ether treatment; and (7) the diameter of the virion is between 40-50 nm. This description would place SHF virus into the newly proposed Togavirus family;

furthermore, two of these properties (small size and budding from an internal membrane) suggest that SHF virus may belong to the Flavivirus group.

Reference:

Melnick, J. L. 1973 Classification and Nomenclature of Viruses. Progr. Med. Virol. 16:337-342.

(M. Trousdale, D. Trent, and A. Shelokov)

REPORT FROM THE TEXAS DEPARTMENT OF HEALTH RESOURCES, BUREAU OF LABORATORIES, AUSTIN, TEXAS

Arbovirus activity in Texas remained low during the period of Ja
1975, but there was serological evidence of activity in wildlife

Serology	No. of Sera	Positive
Wildlife	572	45(44 SL 1 WE
Isolation		
Mosquitoes	2,367	0
Horse brains	6	0

(Charles E. Sweet)

DEPARTMENT OF MICROBIOLOGY, SCHOOL OF MEDICINE
UNIVERSITY OF MARYLAND, BALTIMORE, MARYLAND

Solubilization of DEN-2 CF Antigens from Infected Host Cell Membranes with Nonionic Detergents.

Because the yield of dengue virus virions in tissue culture fluids is small, this is a poor source of dengue virus-specified antigens. Larger quantities of dengue-specified proteins and glycoproteins are associated with infected host cell membranes and, hence, these membranes are a potential practical source of such substances. Solubilization of the membrane-associated dengue-specified proteins in a biologically active form is the first step in making them available for further study and purification. We describe here some results on the capacity of a variety of nonionic detergents to solubilize DEN-2 specified proteins with complement fixing activity from DEN-2 infected BHK cell membranes.

A total of 14 detergents was initially screened for their ability to solubilize DEN-2 CF activity using the following procedure. A preparation of cellular membranes isolated from DEN-2 infected BHK cells was suspended in 0.01 M sodium phosphate buffer, pH 7.5, at an initial CF titer of 128 per 0.025 ml. Detergents at final concentrations of 0.1, 0.5, 1.0 and 2.0% were added to the membrane preparation. The mixtures were incubated at 37C for one hour and then centrifuged at 65,000 X g for 30 minutes to remove insoluble material. The supernatants were dialyzed for 48 hours against two changes of 1000 volumes each of 0.01 M sodium phosphate buffer, pH 7.2, at 4 C. The CF activity of the solubilized material is shown in Table 1. These results indicate that a variety of nonionic detergents may be used to solubilize DEN-2 CF activity from cellular membranes and that there was no advantage in using 2% detergent over the 0.1% level. With several of the detergents there was a decrease in CF activity with

an increase in detergent concentration.

Additional screening of detergents utilized comparative checkerboard CF tests of soluble and insoluble material, each at an initial protein concentration of 150 μgm/ml. This procedure was performed to determine if any single detergent was capable of solubilizing all of the DEN-2 CF activity. Table 2 shows that none of the detergents tested solubilized all of the DEN-2 CF activity. In addition, none of the detergents was able to solubilize more than 68% of the total protein from the membranes. Nevertheless, it has been possible to isolate in highly purified form certain of the virus specified proteins from solubilized mixture. Preliminary results suggest that certain fractions exhibit a high degree of serological specificity.

(C.L. Wisseman, Jr., S.A. Stohlman, and O.R. Eylar)

Table 1. The effect of nonionic detergent concentration on the solubilization of type 2 dengue virus CF activity from infected BHK cellular membranes

CF ACTIVITY OF DETERGENT SOLUBILIZED MEMBRANES[1,2,3,4]				
Detergent	Detergent Concentration			
	0.1%	0.5%	1.0%	2.0%
Tween 20	128	64	128	64
Tween 60	16	8	128	32
Tween 80	128	32	128	64
Brij 30	128	32	8	≤2
Brij 35	64	32	128	128
Brij 36T	64	64	≤2	Nd[5]
Brij 58	64	128	128	64
Brij 96	64	≤2	≤2	≤2
Brig 98	64	32	128	64
Triton X 100	64	64	64	2
Triton X 114	64	64	64	128
Luberol PX	16	64	≤2	≤2
Ammonyx	8	64	64	≤2
Zonyl A	8	32	8	4

1. Tested with undilute hyperimmune anti-DEN-2 mouse serum. Serum CF titer was 128 when tested with 75 µg protein/ml of infected BHK cellular membrane.
2. CF activity of untreated membrane preparation = 128
3. CF activity of untreated membranes after dialysis = 128
4. CF activity of untreated membranes after centrifugation at 65,000 x g for 30 minutes = 4
5. Nd = not determined

Table 2. The ability of selected nonionic detergents to solubilize both total protein and CF activity from type 2 dengue virus infected BHK cellular membranes.

DETERGENT	% PROTEIN[1] SOLUBILIZED	% CF ACTIVITY[2] SUPERNATE	PELLET
Nonidet P-40	68	21	22
Triton X 35	11	21	Ac[3]
Triton X 67	53	22	24
Triton X 100	67	23	24
Triton X 114	46	Ac	16
Triton X 305	44	28	30
Triton X 405	52	20	22
Brij 35	50	28	35
Brij 98	64	Ac	25
Tween 60	58	25	29

1. Protein determined by the Folin procedure modified by Wang and Smith.
2. % CF determined by dividing the number of wells having ≤50% hemolysis by the total number of wells (72). Antigen used at 150 µg/ml protein.
3. Anti-complementary.

REPORT FROM THE UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES
FORT DETRICK, FREDERICK, MARYLAND

African green monkeys are susceptible to Machupo virus

Six African green monkeys, (*Cercopithecus aethiops*), were inoculated with 1000 pfu of Machupo virus by subcutaneous injection and observed for clinical signs. Rectal temperatures, hematocrits, viremias, titers of neutralizing antibody and total and differential blood leukocytes were measured.

The African green monkey was highly susceptible to the virus. All monkeys became ill by day 6 to 7, and 5 of 6 died by day 14. One monkey was moderately ill initially, exhibited nearly complete clinical recovery by day 21, became severely depressed on day 22 and died on day 24.

Clinical signs in approximate order of appearance included fever (6/6), depression (6/6), anorexia (6/6), constipation (5/6), nasal exudate (4/6), conjunctivitis (6/6), dehydration (6/6), hemorrhage (4/6) and diarrhea (1/6). Neurologic signs were seen only in the monkey which survived until day 24.

Four monkeys were viremic by day 7 and all were viremic on day 10. The monkey surviving until day 24 developed a neutralizing antibody titer of 1:32 by day 14. Mean hematocrits reached a low of 30 on day 10 postinfection and then began to increase. Mean peripheral lymphocytes and neutrophil values increased on day 3 from day 0 values of 9,000 and 10,000 respectively and then steadily decreased to lows of 2,000 to 3,000 respectively on day 10.

Although histopathologic examination is not complete, necropsy of the first five monkeys to die showed severe liver necrosis and degeneration and hemorrhagic lesions in the small intestine. One of the monkeys had multiple thrombi in the brain, choroid plexus, lung and small intestine. Cause of death in some of the monkeys appeared to be due in part to loss of liver function. Contributing factors were hemorrhage and intravascular clotting.

(Franklin S. Wagner, Charles G. McLeod and Gerald A. Eddy)

REPORT FROM THE STATE OF NEW YORK DEPARTMENT OF HEALTH, DIVISION OF LABORATORIES AND RESEARCH, ALBANY, NEW YORK

Powassan virus was isolated from the brain of an 82-year old man who died on June 21, 1975 one week after onset of aseptic meningitis. He was a rural resident of Madison County, in central New York State. Serum taken the day before death had titers of 1:40 by HI and 1:4 by the CF test. This is the first isolation of POW virus from man since the prototype was isolated from a child in Canada in 1958.

EEE virus was isolated from the brain of a horse that died on July 16 in Oswego County, the same county in which we have confirmed horse deaths from this virus every year beginning in 1971. However, we have never recognized a case earlier than the second week in August before. The blood of a second horse dying on July 28 in the same area had an HI antibody titer of 1:5120 to EEE, indicating current infection. A third horse from the same area died on August 19 and EEE virus was isolated from its brain.

Between July 11 and 18 two sentinel rabbits, exposed in a cage in a wooded area behind a house within the city limits of Albany, converted by neutralization test to California virus. This is the same house where a human case of California encephalitis occurred last year. As of August 25 no current human case of California encephalitis had been seen.

A single isolation of Flanders virus was made from a pool of 100 Culiseta spp collected in Oswego County on July 15. This was the only isolation from over 41,000 mosquitoes tested by mid-August this year.

(John P. Woodall and Margaret A. Grayson)

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE
DEPARTMENT OF MEDICAL MICROBIOLOGY
UNIVERSITY OF TORONTO, TORONTO, ONTARIO, CANADA

Exposure of Canadians Abroad to Arboviruses

1. Survey

During the past 18 months sera were collected by the Reference Service from healthy Canadians returning to Canada after having lived or travelled abroad for a period of at least one year. The sera collected were tested against four Group A (Eastern equine encephalitis, Western equine encephalitis, Sindbis, Chikungunya), and four Group B (Powassan, Dengue, Saint Louis encephalitis, Banzi) antigens in order to monitor for exposure to arboviruses.

Eighty-nine sera were collected from Canadians who had travelled to the following areas: 15 to the Caribbean, Central and South America, 61 to Africa, 7 to the Middle East and 10 to Southeast Asia (4 persons had travelled to more than one area). Sera were kaolin treated and tested by hemagglutination inhibition. All positive sera were acetone treated and retested for confirmation.

Results of the study, presented in Table I, reveal one Group A reaction to Western equine encephalitis which was due possibly to previous exposure in Canada. There were also six low-titred Group B reactors which may have been due to exposure to Group B arboviruses abroad or, less likely, to yellow fever vaccination.

2. Diagnostic Services

Twelve sera were also received by our Reference Service for diagnostic studies on patients who had recently travelled abroad.

Hemagglutination inhibition results, presented in Table 2, two low-titred Group B reactors which were interpreted to b no diagnostic significance.

In addition, however, paired sera received from one patient presented definite evidence of a Group A arbovirus infectio Sera from this patient collected one month apart showed an fold rise to Chikungunya virus by hemagglutination inhibiti testing (acute serum titre 1:10; convalescent serum titre

The patient was a forty-five year old female who had recen returned from a three-week trip to Uganda. Six days after turning to Canada the patient developed a fever and genera rash which lasted for four days. These symptoms, along wi headache and sore joints, were consistent with the serolog diagnosis of a Group A arbovirus infection, possibly Chiku or O'nyong-nyong virus.

H. Artsob and L. Spence)

Table 1. Hemagglutination Inhibition Titres of Survey Sera reacting to Arbovirus Antigens.

Survey Number	Group A Antigen		Group B Antigen				Country to which person travelled
	WEE	Chikungunya	Powassan	Dengue	SLE	Banzi	
9	0*	0	10**	80	20	320	HAITI
18	0	0	10	10	0	20	GUYANA
38	0	0	0	10	0	0	UGANDA
45	0	0	10	0	10	20	AFRICA
51	10	0	0	0	0	0	LEBANON
60	0	0	0	0	0	10	ZAIRE
81	0	0	0	0	0	20	CAMEROONS
83	0	0	0	20	10	40	SAMOA

* Negative reaction at 1:10 dilution.

** Reciprocal of hemagglutination inhibition titre.

Table 2. Hemagglutination Inhibition Titres of Patients' Sera reacting to Arbovirus Antigens.

Patient Number	Group A Antigen		Group B Antigen				Country to which patient travelled
	WEE	Chikungunya	Powassan	Dengue	SLE	Banzi	
25 acute	0*	10**	0	0	0	0	UGANDA
convales.	0	80	0	0	0	0	
53	0	0	0	0	0	10	KENYA AND HAUTE VOLTA
58	0	0	10	0	0	20	AFRICA

* Negative reaction at 1:10 dilution

** Reciprocal of hemagglutination inhibition titre.

REPORT FROM THE UNIVERSITY OF WISCONSIN
DEPARTMENTS OF ENTOMOLOGY AND
VETERINARY SCIENCE
MADISON, WISCONSIN

Field Studies with LaCrosse Virus Vectors:

Studies are continuing during the summer of 1975 to confirm data previously reported (Arthropod-Borne Virus Information Exchange No. 28) on the vertical distribution of A. triseriatus and a non-vector species of LaCrosse (LAC) virus, A. hendersoni. A second years data is also being collected to determine virus incidence in overwintering A. triseriatus eggs.

The relative vector potential of different isolated populations or demes of A. triseriatus colonized from endemic and non-endemic areas of Wisconsin is being determined.

Studies with Variants of LaCrosse Virus:

The apparent selection for plaque size and possibly antigenic variants of prototype LAC virus after subcutaneous inoculation of chipmunks and gray squirrels, previously reported (Arthropod-Borne Virus Information Exchange No. 26) was studied further. In order to better determine the role of these wildlife hosts in selection for variants, a serial biological transmission vector-mammal cycle was established under laboratory conditions. A virus pool containing suckling mouse brain passage 2 material of five LAC field isolates, colonized A. triseriatus mosquitoes in the F5 generation, and chipmunks and squirrels serologically negative for California group viruses were used.

A. triseriatus were allowed to engorge on a membrane feeder containing 3.0 - 3.6 log $SMICLD_{50}$/.025 ml virus - defibrinated guinea pig blood mixture. Each animal was infected by a single transmitting mosquito and uninfected A. triseriatus were allowed to feed on animals at the peak of viremia. After three serial transmission cycles in chipmunks or squirrels, recovered virus was examined for virus variants. Preliminary results indicate no antigenic difference by the plaque reduction neutralization test between virus recovered after passage in either animal species and the original virus pool when compared by homologus and heterologus sera. Examination of plaque size production by these same recovered viruses in Vero cells suggests slight mean plaque size shifts were possibly due to individual animal rather than species selection.

W. Hansen, T. Yuill, G. R. DeFoliart, B. Miller, M. Lisitza, R. Gebhart,
R. P. Hanson and D. Nassif

The tropisms and development of La Crosse (LAC) virus in stages of transovarially infected Aedes triseriatus have been studied with fluorescent antibody (FA) stained dissected organs and by titrations of individual arthropods in suckling mice.

Virus has been isolated from all developmental stages, in 32 of 130 individual arthropods inoculated into suckling mice. Titrations range from less than 1.0 $\log_{10}$ $SMLD_{50}$ for first instar larvae and eggs to 3.0 logs for fourth instar larvae. Adults and pupae average between 2.0 and 3.0 logs.

Increase in titer during maturation was mainly related to increase in size of the organism rather than to an increase in titer per unit volume. Foci of viral fluorescence were as dense in early instars as in later one and in adults, and the $\log_{10}$ volume correlated highly (r=0.832) with titer. The high coefficient of determination (0.692) would indicate approximately 70% of the increase in virus titer was associated with a volume increase in the arthropod.

Virus was detected in 95 of 387 dissected larvae, pupae, and adults. In larvae highest levels of fluorescence were detected in the alimentary tract, especially in stomodeal or proctodeal derived tissues such as pharynx, esophagus, hindgut or ileum, and rectum. Ganglia, malpighian tubules, muscle, salivary glands, and other tissues also contained virus.

In pupae and adults the alimentary tract organs, especially foregut, were again highly infected. High levels of antigen were detected in the salivary glands of male mosquitoes. High levels of antigen were detected in salivary glands in late pupae and adults which would indicate infectivity upon emergence.

The gonadal and associated tissues of both sexes were typically highly infected. In ovaries and oviducts of females were large amounts of virus. In males the testes, vas deferentia, and accessory sex organs contained virus.

No specific tissues or germ layer derived organs appeared to be the sole source of viral replication, a majority of the tissues of Aedes triseriatus are capable of maintaining LAC virus. Antigen was found in these organs immediately upon emergence from the egg.

(Wayne Thompson and Barry Beaty)

Survey Results Indicating Widespread SLE Virus Activity in Southern Illinois and Mississippi

A survey was conducted to define St. Louis encephalitis (SLE) virus activity over a wide area by virologic and serologic testing of wild birds and testing of mosquitoes for virus isolation. Sampling sites in Mississippi included Greenville (the epicenter), Jackson, Indianola, Yazoo City, Vicksburg, Columbus, Peal, and Clarksdale and in southern Illinois the towns of Eldorado, McLeansboro, and Belleville. McLeansboro and Eldorado were sites of SLE epidemics in 1964 and 1968 respectively.

Results of tests for birds are shown in Table 1. A very high antibody prevalence was noted in Greenville itself, with an incidence of infection in juvenile birds during the 1975 summer season of between 54% (based on neutralization antibody) and 72% (based on hemagglutination-inhibition). Antibody prevalence was also high in areas reporting human cases, such as Jackson and Yazoo City. Vicksburg, with 0% hemagglutination-inhibition and neutralization antibody had not reported human disease through August 15.

In Illinois, moderately high SLE activity was indicated by the 18% hemagglutination-inhibition antibody prevalence at Eldorado. (Three human cases have been confirmed from southern Illinois in August by the Illinois Department of Public Health laboratories, Chicago).

Results for mosquitoes tested from Mississippi are summarized in Table 2. Five isolations confirmed as SLE virus were obtained from mosquitoes collected in Greenville, the focus of human SLE cases. This is a minimal infection rate (MIR) of about eight mosquitoes per 1,000. SLE virus was also isolated from mosquito pools collected from three other locations in Mississippi. Eight mosquito pools from the Mississippi State University poultry farm in Starkville yielded SLE virus. Failure to make specific identifications on a number of the mosquitoes collected in Starkville was due to receipt of badly rubbed specimens. Extensive breeding of C. p. quinquefasciatus was found associated with poultry wastes in this area and recommendations were made for immediate adult mosquito control and elimination of sources for breeding. Five virus strains were also recovered from mosquitoes collected in southern Illinois as noted in Table 3. Identification of these strains is in progress; however, all have laboratory characteristics compatible with SLE virus. The data on virus isolations from mosquitoes supplement the serologic and virologic data on avians and provides evidence that SLE virus activity is extensive in these areas.

(Dr. Thomas P. Monath, Dr. G. Stephen Bowen, Dr. D. Bruce Francy, Mr. Werner L. Jakob, Dr. Charles H. Calisher, and Dr. Richard O. Hayes)

Table 1

RESULTS OF TESTS FOR SLE VIRUS AND ANTIBODY, WILD BIRDS
MISSISSIPPI AND ILLINOIS
July 28 - August 1, 1975

Location	Species	Virus	Antibody	
			HI[1]	NT[2]
Greenville, Miss.	House sparrow			
	Adult	0/20[3]	13/20 (65%)[4]	12/20 (60)
	Juvenile	0/36	26/36 (72%)	19/35 (54)
	Cardinal	0/5	5/5	3/5
	Redwing blackbird	0/1	0/1	0/1
	Robin	0/1	1/1	1/1
	Starling	0/2	1/2	1/2
	Total	0/65	46/65 (71)	36/64 (56)
Jackson, Miss.	House sparrow			
	Adult	0/22	8/22 (36)	5/22 (23)
	Juvenile	0/3	1/3 (33)	1/3 (33)
	Mockingbird	0/1	0/1	1/1
	Grackle	0/2	0/2	0/2
	Starling	0/1	0/1	0/1
	Cardinal	0/1	0/1	1/1
	Rock dove	0/21	6/21 (29)	1/21 (5)
	Total	0/51	15/51 (29)	9/51 (18)
Indianola, Miss.	House sparrow Nestlings	2/28	1/28 (3.6)	1/28 (3.6)
Vicksburg, Miss.	House sparrow Adult	0/30	0/30 (0)	0/30 (0)
Yazoo City, Miss.	House sparrow			
	Adult	0/26	4/26 (15)	3/26 (12)
	Mockingbird	0/1	0/1	0/1
	Grackle	0/3	1/3	1/3
	Total	0/30	5/30 (16)	5/30 (13)
Clarksdale, Miss.	House sparrow			
	Adult	0/152	35/152 (23)	27/150 (18)
	Juvenile	0/18	5/18 (28)	2/17 (12)
	Pigeon	0/26	13/26 (50)	2/24 (8)
	Total	0/196	53/196 (27)	31/191 (16)

Table 1 continued

Location	Species	Virus	Antibody HI[1]	Antibody NT[2]
Columbus, Miss.	House sparrow			
	Adult	0/16	1/16 (6)	0/16
	Juvenile	0/19	0/19 (0)	0/12
	Starling	0/3	0/3	0/1
	Barn swallow	0/2	0/2	0/1
	Total	0/40	1/40 (2.5)	0/30 (0)
McLeansboro, Ill.	House sparrow Nestlings	0/14	-	--
Eldorado, Ill.	House sparrow	-	10/58 (17)	-
	Swallow	-	0/1	-
	Robin	-	1/1	-
	Catbird	-	0/1	-
	Total	-	11/61 (18)	-
Belleville, Ill.	House sparrow	-	3/46 (6.5)	-

[1]SLE HI titer $\geq$ 1:20

[2]$\geq$70% plaque reduction of SLE TBH-28 virus in duck embryo cell culture (DECC)

[3]Presumptive SLE isolate in DECC and mice

[4]No. positive/no. tested (5)

Table 2

Summary of Mosquito Collections from Mississippi, 7/28-31, 1975

Collection Area	Mosquito Species	No. Mosq. Tested	No. Pools Tested	No. Pos. Pools	Virus ID
Greenville (26 sites)	Culex p. quinquefasciatus	617	24	5	5 SLE
	Culex salinarius	5	2		
	Culex spp.	12	4		
	Anopheles quadrimaculatus	150	26		
		786	58		
Indianola (8 sites)	C. p. quinquefasciatus	204	7	1	SLE
	An. quadrimaculatus	325	10		
	Aedes aegypti	1	1		
		530	18		
Clarksdale (14 sites)	C. p. quinquefasciatus	101	8		
	Culex spp.	45	5		
	An. quadrimaculatus	327	12		
	Uranotaenia spp.	1	1		
		474	26		
Jackson (4 sites)	C. p. quinquefasciatus	112	7		
	C. salinarius	2	1		
	Culex territans	5	3		
	Culex erraticus	13	6		
	Culex spp.	50	7		
	An. quadrimaculatus	87	9		
	U. sapphirina	1	1		
	Psorophora columbiae	1	1		
		271	40		

Collection Area	Mosquito Species	No. Mosq. Tested	No. Pools Tested	No. Pos. Pools	Virus ID
Pearl (3 sites)	C. p. quinquefasciatus	79	3		
	C. salinarius	3	1		
	C. erraticus	19	3		
	Culex spp.	34	3		
	An. quadrimaculatus	53	3		
	others (5 spp.)	6	5		
		194	18		
Vicksburg (2 sites)	C. p. quinquefasciatus	88	4	1	SLE
	C. erraticus	5	1		
	Culex spp.	11	2		
	An. quadrimaculatus	35	2		
	Anopheles punctipennis	4	1		
	A. aegypti	6	1		
		149	11		
Yazoo City (2 sites)	C. p. quinquefasciatus	81	3	1	
	C. erraticus	2	1		
	Culex spp.	60	2		
	An. quadrimaculatus	58	2		
	P. columbiae	1	1		
		202	9		
Plains (1 site)	C. p. quinquefasciatus	22	1		
	C. erraticus	2	1		
	Culex spp.	10	1		
	An. quadrimaculatus	27	1		
		61	4		
Columbus (4 sites)	C. p. quinquefasciatus	9	3		
	C. erraticus	18	1		
	Culex spp.	1	1		
	An. quadrimaculatus	579	13	3	3 Buny. Gp.
	Coquillettidia perturbans	1	1		
		608	19		
Starkville (2 sites- collections of 7/29-30 and 8/12/75 combined)	C. p. quinquefasciatus	851	18	3	3 SLE
	C. salinarius	7	2		
	C. erraticus	2	1		
	Culex spp.	873	18	5	5 SLE
	An. quadrimaculatus	7	2		
	others	2	2		
		1742	43		
Total for all sites		5679	276		

Table 3

ILLINOIS MOSQUITO COLLECTIONS

July 29 to August 1, 1975

Location	Species	No. Pools	No. Mosquitoes	No. Pos. Pools
Belleville (8 sites)	C. pipiens	10	204	1
	C. restuans	6	71	
	C. salinarius	7	116	1
	Anopheles quadrimaculatus	6	9	
	An. punctipennis	6	33	
	A. vexans	1	1	
	C. (Mel.) erraticus	4	6	
	C. territans	1	1	
	A. triseriatus	2	2	
	A. sp. nr. trivittatus	1	1	
	Culex spp.	6	180	
McLeansboro (3 sites)	C. pipiens	3	26	
	C. restuans	2	2	
	C. salinarius	3	24	
	C. (Mel.) erraticus	1	7	
	An. quadrimaculatus	2	18	
	An. punctipennis	2	17	
	Culex spp.	3	67	1
Eldorado (2 sites)	C. pipiens	4	96	
	C. restuans	1	2	
	C. salinarius	3	28	
	An. quad. & punctipennis	1 ea.	1 ea.	
	Culex spp.	3	83	2

Structural Proteins of the California Encephalitis Virus Complex

The virus particle structural proteins of some of the viruses in the California subgenus of the Bunyaviruses have been characterized in various laboratories. As a group, the California viruses contain three structural proteins: a major glycoprotein, a minor glycoprotein, and a nucleocapsid protein. Because of the serological complexity and extensive cross reactivities within the California subgenus, we have undertaken a comparative electrophoretic analysis of the structural proteins of nine California viruses as well as Guaroa and Bunyamwera viruses to determine if a meaningful classification scheme could be established on this basis. Similar studies have been reported on alphaviruses, flaviviruses, and rhabdoviruses.

California viruses analyzed in this study were plaque-purified and propagated in roller bottle cultures of BHK-WI2 cell grown in reinforced MEM and labeled with tritiated or carbon 14 amino acids in the same medium containing reduced amino acids. Purified virions were prepared by polyethylene glycol precipitation, discontinuous sucrose gradient centrifugation and equilibrium: viscosity centrifugation in glycerol-potassium tartrate gradients. Molecular weight determinations were made by coelectrophoresis of the CEV virus in question with vesicular stomatitis virus, New Jersey serotype, to provide internal molecular weight marker proteins. When each of the California viruses were examined in this manner, minor variations between serotypes in the molecular weights of the three structural proteins were observed (Table 1). All of the viruses evaluated contained a major glycoprotein with a molecular weight between 120,000 and 130,000 daltons. The molecular weights of the large glycoprotein were so similar between the CEV serotypes that no meaningful distinctions could be made on the basis of this protein subunit alone. However, some difference became apparent when molecular weights of the smaller glycoprotein are examined and division of the viruses into two groups can be made on the basis of differences in molecular weight of this subunit. All of the California complex viruses with the exception of Trivittatus have a minor glycopeptide with a molecular weights of 39,000 daltons. The analagous subunits of Trivittatus, Guaroa, and Bunyamwera were significantly smaller at 28,000, 32,000, and 30,000 daltons, respectively. An analysis of the molecular weights of the nonglycosylated proteins of the Bunyaviruses studied revealed that one-half of the CEV examined (Snowshoe Hare, Jamestown Canyon, and New Jersey) and Guaroa and Bunyamwera viruses have a nucleocapsid protein with a molecular weight of 22,000. LaCrosse, BFS-283 and Melao virus nucleocapsid proteins have a molecular weight of 25,000. Although these differences in molecular weight are significant, no distinct pattern can be discerned which correlates with the more pronounced variations in the small glycopeptide. Typical electrophoretic patterns in 10% polyacrylamide gels which illustrate these differences are shown in Figure 1. Panel (a) presents a coelectrophoresis of LaCrosse and Snowshoe Hare viruses. The major glycoproteins of both viruses which were located in fraction 5 and the minor glycoproteins which are seen in fractions 17 and 18 coelectrophoresed. The nonglycosylated protein of LaCrosse virus in fraction 22 migrated relatively more slowly than the corresponding subunit of Snowshoe Hare virus which is located in fraction 24. Panel (b) of Figure 1 illustrates a coelectrophoresis of Trivittatus

and Snowshoe Hare viruses. The major glycopeptides and the nucleocapsid proteins of both viruses have migrated together. On the other hand, the minor glycopeptide of Trivittatus migrated relatively faster than the corresponding protein of Snowshoe Hare virus.

These data indicate that the polypeptides of the California encephalitis viruses are distinguishable from those of Bunyamwera virus on the basis of differences in the molecular weights of their minor glycopeptides. Guaroa virus is a serological intermediate between the Bunyamwera serogroup and the California complex and contains a Bunyamwera-type polypeptide pattern. It was an unexpected finding that Trivittatus virus, a relatively distinct member of the California complex, also has a Bunyamwera-type electrophoretic pattern. Further investigation of the structural proteins of other Bunyaviruses may shed light on the evolutionary relationships within this large group of viruses.

(A. Vance Vorndam and D. W. Trent)

Table 1

COMPARATIVE ELECTROPHORETIC ANALYSIS OF BUNYAVIRUS STRUCTURAL PROTEINS

Virus	Major Glycoprotein Mol. Wt. ($X10^{-3}$)*	Minor Glycoprotein Mol. Wt. ($X10^{-3}$)	Nucleoprotein Mol. Wt. ($X10^{-3}$)
California BFS-283	125	39	25
Inkoo	125	39	24
Jamestown Canyon	130	39	22
Keystone	130	39	24
LaCrosse	120	39	25
Melao	130	39	25
New Jersey	130	39	22
Snowshoe Hare	125	39	22
Tahyna	125	39	23
Trivittatus	125	28	23
Guaroa	125	32	22
Bunyamwera	125	30	22

*Molecular weights estimated by coelectrophoresis with vesicular stomatitis viral proteins.

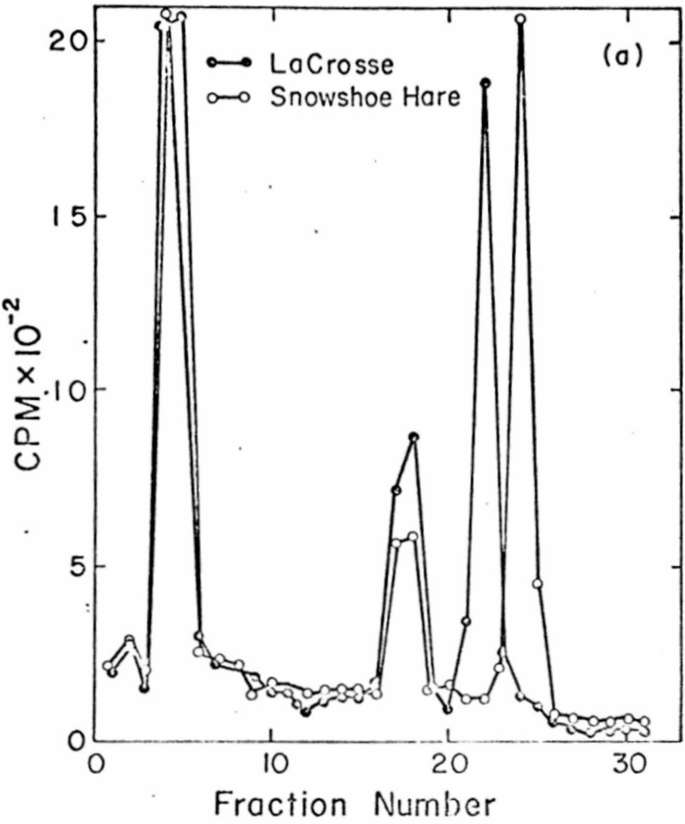
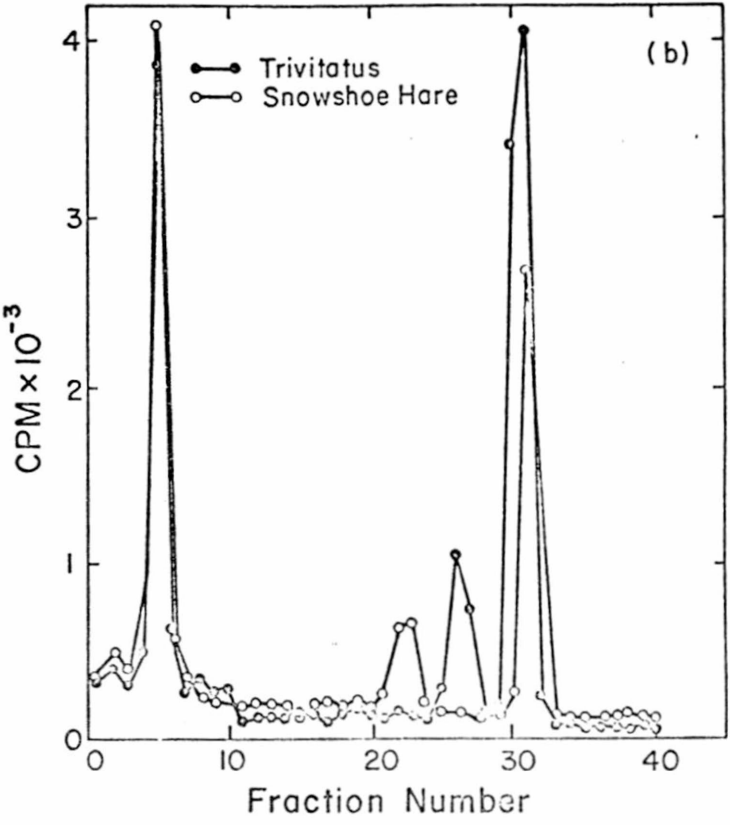

LaCrosse
Snowshoe Hare
(a)
CPM x 10^-2
Fraction Number
0
5
10
15
20
0
10
20
30

Figure 1


Trivitatus
Snowshoe Hare
(b)
CPM × 10⁻³
0
1
2
3
4
0
10
20
30
40
Fraction Number

Homologous and Heterologous Interference Mediated by Defective Particles of California Encephalitis Virus

Infection of baby hamster kidney (BHK) cells with California encephalitis viruses (CEV) under conditions of high multiplicity and serial undiluted passage results in the generation and accumulation of defective-interfering (DI) particles. Although these noninfectious particles cannot be isolated free of standard infectious virions, their presence has been clearly established by a number of criteria: (1) late-passage virus populations inhibit the formation of homologous cloned standard virus and are much less effective in inhibiting the replication of other serologically distinct CEV and do not inhibit the replication of heterologous Sindbis, vesicular stomatitis, and St. Louis encephalitis viruses; (2) the interfering activity migrates with viral infectivity in sucrose and Hypagne gradients and bands at the same density as cloned virus in CsCl and potassium tartrate gradients; (3) purified virions from cloned stocks and undiluted passages contain the same three viral proteins without significant changes in molar ratios of individual polypeptides; (4) virion RNA from late undiluted passage is predominantly of one species, 1.2×10^6 in contrast to cloned virus which contains RNA of 2.9×10^6 2.0×10^6 and 0.52×10^6 daltons; (5) cells coinfected with early and late passage virus synthesize as much intracellular viral-specified protein as is made after infection with cloned virus alone, although viral-RNA and infectious virus production is inhibited by 90% or more; and (6) the restricted interfering activity of CEV DI particles is not interferon mediated nor does it depend upon inhibition of attachment, penetration, maturation, or release of infectious virus. The data strongly suggest that CEV defective particles contain multiple copies of an altered RNA genome whose transcription competes with that of parental type RNA. The specificity of CEV mediated DI competition correlates with serologic relatedness and structural polypeptide patterns of immunologically distinct CEV, thus suggesting a genetic basis for individual serotypes within the CEV complex of the Bunyaviridae.

(D. W. Trent and J. F. Obijeski)

Because of our continuing interest in the Bunyamwera Supergroup, we are cross-comparing all catalogued (and some, as yet, uncatalogued) Bunyamwera group viruses by plaque reduction-neutralization tests in Vero cells.

In general, African members of the group (Table 1) and North American members (Table 2) appear to be related on a geographic basis. Cross reactions were extensive, if often one-way. South American members (Table 3) are less closely related.

A number of interesting conclusions may be drawn, if certain liberties are allowed. For one, the one-way relationship between Batai virus (Europe and Asia) and Northway virus (Alaska) may indicate an evolutionary ascent. That is, if the closely related African and North American Bunyamwera viruses have common ancestors, they may be Batai (called Calovo in eastern Europe, Chittoor in India, Batai in Malaya and Olyka in the Ukraine) and Northway viruses; ecologically distinct viruses could be closely related serologically without interfering with the prevalence of one another. Alternately, the general paucity of cross-reactivity of the South American members (Anhembi and Sororoca may be subtypes of one virus) may reflect the geographic closeness of these viruses; ecologically similar viruses could not be cross-protective and remain commonly occurring entities. Certain of the Bunyamwera group replicate in rabbits but not hares (Francy, personal communication), Batai virus has been isolated from birds (Gaidamovich et al., personal communication), and at least two, Main Drain and Lokern, have been isolated from Culicoides sp. We are studying the entire Bunyamwera group based on not only serologic cross reactivity but host spectrum characteristics in cell cultures and pathogenicity, with the view that an ecological-evolutionary hypothesis may be possible for not only this group but others of the Supergroup as well.

(C.H. Calisher)

TABLE 1. Cross Reactivities of African Bunyamwera Group Viruses (SDPRNT)

Virus	ANTIBODY TO: BAT	CVO	BEL	BUN	ILE	GER	SHO	TAH
Batai	≥640	≥640	160	160	160	-[a]	-	-
Calovo	≥640	≥640	320	80	≥640	-	-	-
Beliefe	≥640	≥640	≥640	160	≥640	-	20	-
Bunyamwera	40	160	-	≥640	-	80	-	-
Ilesha	-	-	40	-	320	-	-	-
Germiston	-	-	-	320	160	≥640	-	-
Shokwe	-	-	40	-	-	-	320	-
Tahyna	-	20	-	-	-	-	-	≥640

a) - = <20

TABLE 2. Cross Reactivities of North American Bunyamwera Group Viruses (SDPRNT)

Virus	ANTIBODY TO: BAT	NOR	CV	TEN	DUR	STR	LOK	MD	TLN	MAG
Batai	≥640	40	-[a]	-	80	20	20	-	-	-
Northway	320	320	160	40	40	20	-	-	-	20
Cache Valley	-	80	320	320	-	20	-	-	-	80
Tensaw	80	80	40	≥640	80	80	40	20	-	160
Durango	40	20	≥640	160	≥640	80	-	-	160	160
Santa Rosa	40	20	160	40	80	320	320	-	160	20
Lokern	-	20	160	80	80	40	≥640	-	40	40
Main Drain	-	-	-	-	-	-	40	160	-	-
Tlacotalpan	40	40	80	40	≥640	160	40	20	320	80
Maguari	80	20	40	80	40	80	40	-	40	320

a) - = <20

TABLE 3. Cross Reactivities of South American Bunyamwera Group Viruses (SDPRNT)

Virus	ANTIBODY TO: ANH	SOR	WYEO	TAIA	KRI	MAG	GRA	TUC	TLN	DUR	STR
ANHEMBI	≥640	320	-	40	-	-	20	-	-	-	-
SORONOCA	160	≥640	-	80	-	20	-	-	20	-	-
WYEOMYIA	-	-	320	-	-	40	-	40	-	20	-
TAIASSUI	-	20	-	160	-	-	-	40	-	-	-
KAIRI	-	20	20	-	≥640	20	20	-	20	-	-
MAGUARI	-	20	-	-	20	640	-	-	40	40	80
GUAROA	-	-	-	-	-	-	320	-	-	-	-
TUCUNDUBA	-	-	40	-	-	-	-	160	-	-	-
TLACOTALPAN	-	-	-	-	-	80	40	-	320	320	160
DURANGO	-	40	160	160	20	160	-	-	320	≥640	80
SANTA ROSA	-	-	-	-	20	20	-	-	80	320	320

REPORT FROM THE ROCKY MOUNTAIN LABORATORY,

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, NIH,

HAMILTON, MONTANA

A new Bunyamwera-like virus from argasid ticks

A single isolate obtained in 1969 from Argas cooleyi ticks of a Cliff Swallow colony in Randall Co., Texas, appears to be unrelated to other known arboviruses tested in screen CF tests by us and by Dr. Jordi Casals at YARU. Previously, we reported isolation of numerous strains of Sixgun City [Kemerovo group] and Sapphire II [Hughes group] viruses from these ticks in the same colony. The new virus (RML 52301-11) is lethal for both newborn and adult mice by the i.c. route of inoculation, but kills only suckling mice by the i.p. route. It causes CPE and plaques in Vero cells. It is an RNA virus, as judged by its resistance to exposure to 5-bromodeoxyuridine, susceptible to action of lipid-solvents and relatively resistant to acid pH. Electron microscopic studies made by Dr. Fred Murphy and Mrs. Alyne Harrison at CDC have indicated that this virus is of about 100 nm in size, enveloped, and Bunyamwera-like in appearance. Therefore, we performed additional screen CF tests with RML 52301-11 virus using antisera prepared against certain tick-borne viruses classified as Bunyavirus-like. In all, one or more representatives of the following serological groups were tested: CHF-Congo, Bakau, Ganjam, Kaisodi, Thogoto, and Uukuniemi. Results were uniformly negative, and the virus will be registered in the International Catalogue of Arboviruses and named Sunday Canyon for the locality from which it was recovered (C. E. Yunker).

Isolation of a Soldado-like virus from Ornithodoros maritimus ticks in Ireland

Incidental to a search for seabird-infesting ticks in southern Ireland by Mr. Thomas C. Kelly of the University of Cork, a virus was isolated and identified as a member of the Hughes group. Three pools of Ornithodoros maritimus (capensis group) collected from nesting sites of marine birds on Saltee Island (off the Alexford Coast) yielded the virus. Complement-fixation and neutralization tests demonstrated that this virus is most closely related to Soldado virus. The latter was described from Ornithodoros ticks (capensis group) and terns in the Caribbean Sea, and is also known from related ticks in Ethiopia. A virus from Puffin Island, Wales, which is similar to ours, is currently under study at the U.S. Naval Medical Research Unit #3 in Cairo, A.R.E. Reported association of Hughes group viruses with human febrile illnesses indicates a need for further investigation of the Saltee I. virus (J. E. Keirans).

Isolation of arboviruses from ticks of the Ornithodoros capensis complex in Hawaii

Four isolations of viral agents were recovered from pools of argasid ticks (Ornithodoros capensis complex) collected on Manana I., a bird nesting island off Oahu, Hawaii. Viruses identified from these isolations were Johnston Atoll, Midway, and a member of the Hughes group near Soldado virus. Viruses of the Hughes group are associated mainly with seabirds of the family Laridae (gulls and terns) and their tick parasites, Ornithodoros spp. of the capensis complex. In the Western Hemisphere these seabird viruses are known to be present in

Caribbean Islands (Hughes and Soldado viruses), Gulf of California (Raza virus), the Pacific Coast of the U.S. (Farallon virus) and Peru (Punta Salinas). Johnston Atoll virus, of the Quaranfil group, and Midway virus (Nyamanini group) were previously recovered from Ornithodoros ticks on Central Pacific Islands. However, this is the first report of any of these agents from the main Hawaiian Islands, where their presence represents a potential human health problem (C. M. Clifford).

Serological tests for viruses associated with seabird-parasitizing ticks (Ixodes uriae and Ornithodoros capensis complex)

In June of 1973 and 1974 we collected a limited number of sera from Common Murres (Uria aalge) and Western Gulls (Larus occidentalis) on a rock off Yaquina Head, Oregon. A high percentage of murres were positive for both Tyuleniy (B serogroup) and Yaquina Head (Kemerovo serogroup) antibodies (Table 1). Gull chicks, which failed to react to either virus in 1973, had HI antibodies to Tyuleniy virus in 1974. Because the seropositive birds sampled in 1973 included all newly hatched murre chicks, passively acquired antibodies were suspected. Therefore, in 1974 we collected blood directly from developing embryos within murre eggs. Some of these reacted to both viruses, hence it appears likely that maternal antibodies to these viruses are transferred in the egg, in a manner similar to that known for RSSE virus. No evidence of alphavirus or mosquito-borne flavivirus activity among any of the seabirds was noted.

We tested by plaque NT test 445 human sera for evidence of exposure to certain Kemerovo-group viruses. These sera had been collected from

adult male Alaskans by Dr. Robert Philip, of this laboratory. Approximately 5% possessed neutralizing activity against an undescribed virus from Ixodes uriae in St. Lawrence Island. Also, a large sample of these viruses were tested, with negative results, for HI antibodies to selected Togaviruses, including Tyuleniy (C. E. Yunker, L. A. Thomas, J. Cory).

Table 1. Antibodies to two tickborne viruses in nesting seabirds of Yaquina Head, Oregon*

	1973			1974		
	Murres		Gulls	Murres		Gulls
	Adults	Chicks	Chicks	Eggs	Chicks	Chicks
Tyuleniy virus (HI antibodies)	13/17 [76.5%]	4/4 [100.0%]	0/5 [0.0%]	2/16 [12.5%]	6/19 [31.6%]	6/9 [66.6%]
Yaquina Head virus (NT antibodies)	11/15 [73.3%]	1/3 [33.3%]	0/5 [0.0%]	4/14 [28.5%]	7/15 [46.6%]	0/9 [0.0%]

*No. positive/no. tested; percent positive in brackets. Neutralization tests done in Vero cells by plaque-reduction method.

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY
UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, CANADA

California encephalitis (CE) virus, snowshoe hare subtype, was isolated from 2 of 140 pools of Aedes sp. mosquito larvae containing 100 to 200 larvae per pool, which were collected in the Yukon Territory, Canada at latitude 61°N during April and May 1975, before adult mosquitoes emerged. To date no virus has been isolated from 200 pools of adult mosquitoes, containing an average of 40 Aedes communis or A. hexodontus mosquitoes per pool, which were collected at the same latitude during June and early July 1975.

Employing both the demonstration of viral antigen in cytoplasm of acinar cells of salivary glands of wild-caught A. communis mosquitoes by the direct immunoperoxidase technique, and infectivity assays of mosquito salivary glands in mice, preliminary results have shown that the minimum infectivity dose of a Yukon mosquito CE isolate, 74-Y-234, for A. communis was 0.1 mouse LD_{50} following incubation at 13°C and at 23°C for 14 days after intrathoracic injection. Comparable infectivity doses were demonstrated for a 1975 larval CE isolate, 75-L-10 and a 1973 mosquito isolate 73-Y-347. Each of the above isolates were in their first suckling mouse brain passage. Unpassaged 74-Y-234 virus also showed an infectivity dose of 0.1 mouse LD_{50} for A. communis. In electron micrographs of preparations of A. communis salivary glands 13 days after intrathoracic injection of 74-Y-234 virus following incubation at 13°C, CE virions coated by peroxidase particles were observed following treatment with CE antiserum labelled with horseradish peroxidase.

The infectivity dose of Northway virus for A. communis mosquitoes was at least 1 mouse LD_{50} and of Murray Valley encephalitis 0.1 mouse LD_{50} following incubation for 14 days at 13°C and 23°C after intrathoracic injection.

(D. M. MCLEAN)

REPORT FROM THE DEPARTMENT OF
TROPICAL MEDICINE AND MEDICAL MICROBIOLOGY
UNIVERSITY OF HAWAII SCHOOL OF MEDICINE
HONOLULU, HAWAII

Previous studies in our laboratory demonstrated that dengue virus replicated in cultures of peripheral blood leukocytes (PBL) from dengue immune rhesus monkeys but not in PBL from non-immune animals. Permissiveness of PBL to dengue virus developed 2-3 weeks after experimental infection of susceptible animals and persisted for at least two years. A preliminary study of a small number of patients at Ramathibodhi Hospital, Bangkok, Thailand in 1972 suggested that permissiveness of human PBL to in vitro dengue infection essentially paralleled that described for monkeys.

In the summer of 1974 a more extensive study was undertaken at Children's Hospital and SEATO Medical Research Laboratory Bangkok in collaboration with Drs. Suchitra Nimmannitya, William Bancroft and Robert Scott. Blood was collected at intervals during the course of illness from 36 patients with clinical (subsequently laboratory confirmed) dengue hemorrhagic fever (DHF), 5 patients with serologically confirmed primary dengue infection, 13 dengue immune patients with non-dengue viral illness and 12 dengue immune healthy individuals. Leukocytes were separated from heparinized whole blood by dextran sedimentation, washed two times with Hanks balanced salt solution (HBSS) containing EDTA and once with HBSS without EDTA. The cells were counted in a hemacytometer and suspended to a concentration of 1.5×10^6 live mononuclear cells/ml in RPMI 1640 medium suplemented with 10% heat inactivated fetal calf serum, glutamine and antibiotics. The cells were dispensed in 1.0 ml amounts into one dram screw-cap vials and 0.1 ml of Dengue 2 (16681) virus tissue culture seed containing approximately 5×10^6 pfu/ml was added to each. The cultures were gassed with 5% CO_2 and incubated at 37°C. After 1-6 days of culture, replicate vials were frozen at -70°C and subsequently assayed for virus by plaque assay in LLC-MK_2 cells and for presence of dengue 1-4 HAI antibody by the microtiter technique. The results are summarized here.

Little or no virus replication occurred in PBL cultures obtained during the first seven days of secondary DHF. Significant virus replication occurred on the 8th and all subsequent days after onset of the disease (fig. 1). Assuming a 5-7 day incubation period for dengue in man, these results are essentially the same as those observed in rhesus monkeys with experimental primary dengue infections.

Similar results were obtained for the five primary dengue cases (fig. 2)

Cultures of PBL from 13 dengue immune patients with non-dengue viral illnesses supported dengue virus replication throughout the course of the illness (fig. 3).

Cultures of PBL from 12 healthy dengue immune persons all supported dengue virus growth (Table 1). It should be noted that these persons all had broadly crossreacting group B arbovirus antibody suggesting previous infection with multiple viruses.

The 36 DHF patients, ranging in age from 2 to 14 years (mean = 8 years) all had secondary type antibody responses. Since this was not a prospective study it is not known if their PBL were capable of supporting dengue virus growth in vitro before the second dengue infection. All the primary dengue cases studied developed dengue virus permissive PBL during the course of the disease, but it is not known how long such permissiveness persists in man. The observation that permissiveness developed at approximately the same time during the course of both primary and secondary dengue illness suggests that the initial PBL permissiveness had disappeared before the second dengue infection. However, these data do not rule out the possibility that the initial permissive PBL population (induced by the first dengue infection) actually persisted and was destroyed or was in some way rendered non-permissive during the acute phase of the second dengue infection. This phenomenon is currently being studied experimentally in rhesus monkeys.

Cultures of washed PBL were also tested for production of dengue HAI antibody at varying times during culture. Antibody appeared only in cultures of PBL from patients in the acute and early convalescent phase of secondary dengue infection, days 4 through 10 of disease (Table 2). On the seventh day of disease PBL cultures from 86% of the patients tested contained antibody. Titers as high as 1/160 were recorded but most were between 1/10 and 1/40.

The presence of antibody in cultures of washed acute phase PBL could be due to production by active plasma cells or to the elution of antibody-antigen complexes from the surface of leukocytes and their subsequent dissociation. Attempts to reproduce these observations in dengue immune monkeys experimentally reinfected with a second dengue type have not been successful.

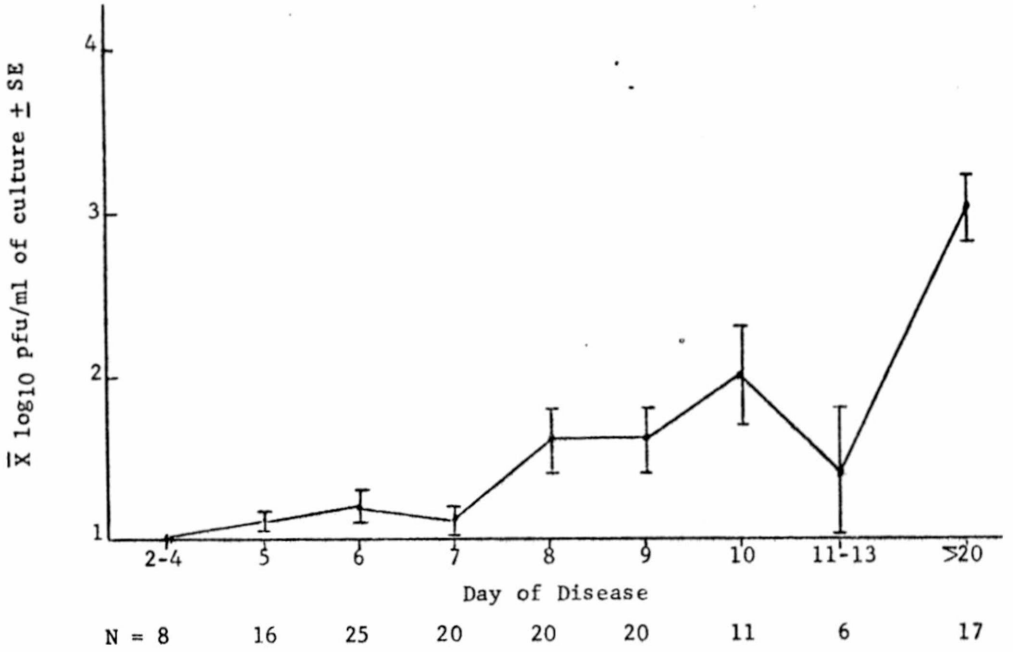


Fig. 1. Dengue virus replication in cultures of PBL from DHF cases obtained at various times during the course of illness. Mean and standard error of the Log_{10} pfu/ml of 3-5 day cultures. N = number of PBL cultures obtained and tested for each period of illness.

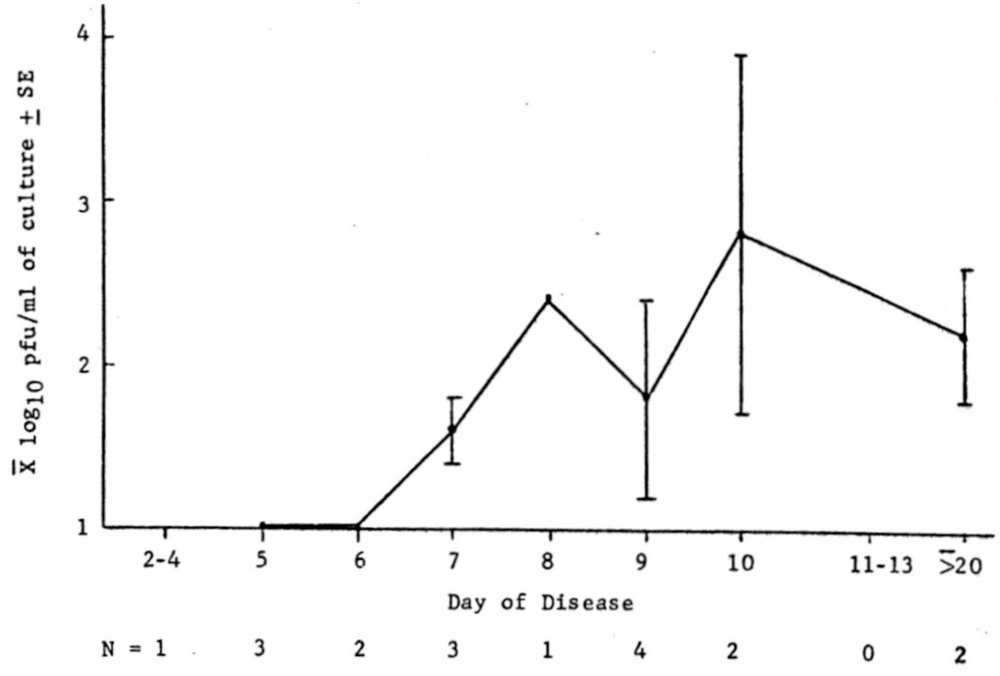


Fig. 2. Dengue 2 virus replication in cultures of PBL from cases of primary dengue infection. Mean and standard error of the $\log_{10}$ pfu/ml of 3-5 day cultures. N = number of PBL cultures obtained and tested for each period of illness.

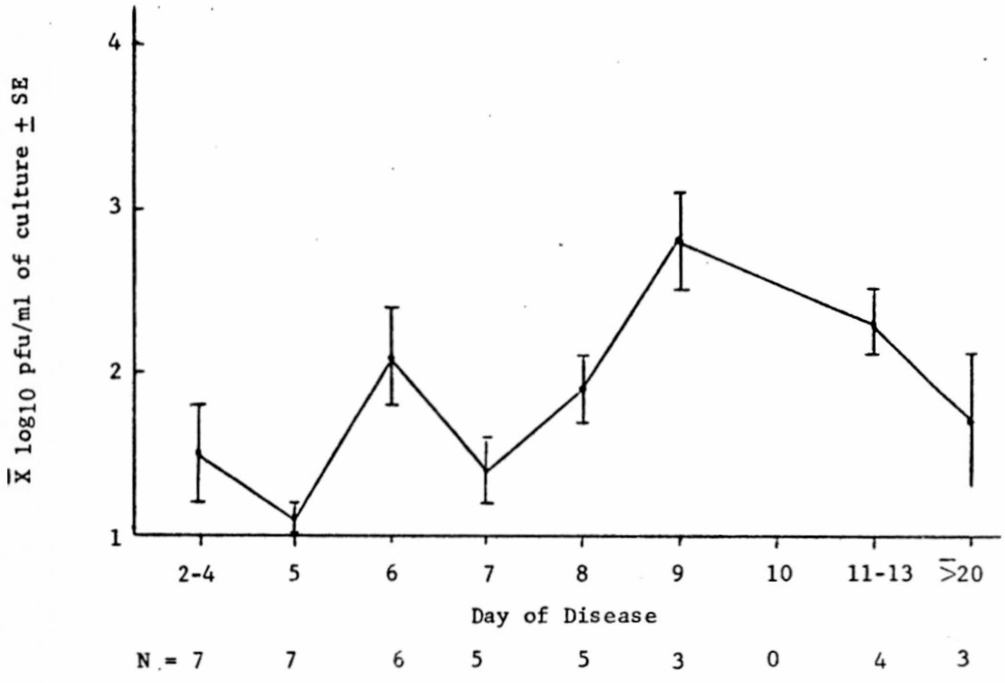


Fig. 3. Dengue 2 virus replication in cultures of PBL from dengue immune patients with non-dengue viral illnesses. Mean and standard error of the $\log_{10}$ pfu/ml of 3-5 day cultures. N = number of PBL cultures obtained and tested for each period of illness.

Table 1. Dengue 2 virus replication in cultures of peripheral blood leukocytes from 12 healthy dengue immune individuals.

Patient No.	Days in culture*			$\bar{X}$
	3	4	5	
12	3.0**	1.7	1.0	1.6
16	1.4	1.0	ND	1.2
18	3.1	3.3	3.6	3.3
21	3.3	3.7	3.7	3.6
59	2.1	2.8	3.7	2.9
61	ND	ND	2.5	2.5
63	1.7	1.4	1.0	1.3
66	2.4	2.9	2.5	2.6
73	2.5	2.5	1.0	2.0
80	3.1	3.7	ND	3.4
85	2.4	2.9	2.9	2.7
94	2.8	2.9	2.2	2.6

*PBL cultures of 1.5×10^6 live mononuclear cells/ml of RPMI 1640 medium were inoculated with approximately 5×10^6 pfu of D_2 virus and incubated for 5 days at 37°C. On culture days 3, 4, and 5 replicate cultures were harvested for virus assay.

**Log_{10} pfu/ml of PBL culture. ND = Not Done.

Table 2. Cultures of PBL from DHF patients containing dengue HAI antibody at 1/5 or higher titer.

D 0 D*	No. of Patients with PBL cultures with HAI Ab at 1/5 or greater				No. Tested	% with Ab
	D_1	D_2	D_3	D_4		
2	0	0	0	0	1	0
3	0	0	0	0	1	0
4	3	3	4	4	6	67
5	9	11	13	12	29	68
6	10	12	13	14	23	61
7	15	13	17	18	21	86
8	4	8	11	10	19	58
9	4	2	7	7	18	39
10	0	0	1	1	8	13

*Day of disease on which blood was collected and PBL cultures established.

REPORT FROM THE VIRUS RESEARCH UNIT, MEDICAL RESEARCH COUNCIL OF NEW ZEALAND, UNIVERSITY OF OTAGO, DUNEDIN, NEW ZEALAND.

TYPE 1 DENGUE EPIDEMIC IN FIJI:

Fiji has experienced major epidemics of dengue type 1 (1943-45) and type 2 (1971-72). In January,1975, cases of dengue-like illness were reported in Suva, the capital city of Fiji. Studies in this Unit and in the laboratory of Dr Leon Rosen in Honolulu showed that these were cases of dengue type 1. Since a very large percentage of the population had no antibody to type 1 and since type 2 had been through the region very recently, a major outbreak with possible occurrence of dengue haemorrhagic fever (DHF) was anticipated.

Cases of dengue continued throughout February in Suva, but it was not until mid-March that a dramatic increase in the number of cases occurred, accompanied by the appearance of haemorrhagic manifestations. Cases were reported from all regions of the Fiji group by the end of March and DHF cases occurred in several districts. By the end of May nearly 11,000 cases of dengue and over 100 cases of DHF with 6 deaths, had been reported. Serological studies indicated that at least 50,000 cases had occurred by this time.

Control measures including back-pack spraying and ULV spraying with a truck-mounted ULV unit were begun in Suva in March and the epidemic began to subside by the end of April. The main urban areas of the main island, Viti Levu, were also sprayed in April. Tests in Suva indicated that malathion was very effective in controlling adult mosquitoes during the epidemic.

Infections were confirmed by serological testing in 163 patients and 55 strains of virus were isolated using a combined mouse or mosquito inoculation followed by fluorescent antibody staining. Transmission experiments were attempted, but were badly affected by the control spraying programme.

Fluorescent antibody staining of house-caught and man baited outdoor landing catches of mosquitoes during and after the epidemic peak showed that only Aedes aegypti was acting as a major vector in Suva, but the infectivity rate was very high (5.4% for house captured, and 6.1% for outdoor caught mosquitoes) during the peak of the epidemic. However, the isolation of virus and serological evidence from the isolated island of Rotuma where Aedes aegypti does not occur, suggests that other Stegomyia

mosquitoes were acting as dengue virus vectors in parts of the Fiji group.

Two aspects of this dengue epidemic are of particular interest. Firstly, most of the DHF cases occurred not in children, but in young adults. This contrasts markedly with experience in the cases of DHF occurring in South East Asia. Secondly, the patterns of antibody response showed that approximately half the cases of DHF occurred in persons with no prior exposure to dengue virus. Thus the suggestion that sensitisation might be an explanation for the initiation of haemorrhagic manifestations in DHF was not supported by the observations made here.

It became obvious that the main cause of the explosive nature of this dengue epidemic was complete lack of a mosquito control programme in the urban areas of Fiji. The severity of,and massive publicity arising from this epidemic,have helped to overcome this problem and control measures have been established on a long term basis.

At about the same time as dengue cases peaked in Fiji, a massive outbreak of type 1 dengue was reported from the Kingdom of Tonga where, by the end of April, many thousands of cases had occurred and 12 deaths were reported. Dengue was also reported from other island groups, Australia and New Zealand, but nearly all of these cases were secondary cases among those who had recently travelled to Fiji or Tonga.

(T.Maguire, F.J.Austin, J.A.R.Miles.)

QUEENSLAND INSTITUTE OF MEDICAL RESEARCH
BRISBANE, AUSTRALIA

The following summary of work in 1974-75 is extracted from the Institute's 30th Annual Report. A limited number of copies of the full report will be available after November 1975 from the Director, Dr R.L. Doherty, QIMR, Herston Road, Brisbane, 4006, Australia.

Epidemiological studies of arboviruses

Much material collected during the 1974 epidemic of Murray Valley encephalitis (MVE) remained for examination in the year under review. The results obtained, along with parallel findings by other laboratories, added to knowledge in several ways. The standard serological tests provided diagnostic results in most patients, but a small proportion, perhaps especially among old people, had only low titre or late responses which were significant only when serial samples over many weeks were tested. In particular the complement-fixation test, a major basis of diagnosis in previous epidemics, would not have diagnosed 10 of 53 patients on the samples available. The geographical distribution of the 1974 epidemic was unique in the occurrence of cases in east central Queensland and in the Northern Territory. Serological tests confirmed that infection without disease had occurred in both those areas. In particular antibody after the epidemic gave evidence that infection was widespread in Australian Aborigines in central

Australia.

The summer and autumn of 1975, in contrast to 1974, provided no proven cases of MVE in south-east Australia. Sentinel chickens at Charleville, which showed high infection rates in 1974, showed only equivocal evidence of MVE virus infection in 1975 although another virus, Sindbis, was active. Similar sentinel studies, in collaboration with CSIRO Division of Animal Health and the Queensland State Department of Primary Industries, showed infection with arboviruses of several groups in late summer at centres in the Flinders River basin in north Queensland. Further north still, at the Institute's long term study area at Kowanyama, high isolation rates of several viruses, including MVE, were obtained from mosquitoes collected in the wet season (February 1975). Strains of Ross River virus isolated in July and November 1974, and of Kokobera virus isolated in November, are of significance as the first isolations of togaviruses at Kowanyama in the dry season. They are important indications that transmission by insects occurs throughout the year at that centre, and therefore that other hypothetical bases for virus survival need not be invoked.

The Institute has for some years acted as a Regional Centre for Arbovirus Reference and Research for the World

Health Organization. Most of the reference work has been concerned with virus isolates from our own field programme, but an increasing number of new strains are being received from other laboratories. In this year scientists of CSIRO Division of Animal Health submitted 16 strains from mosquitoes, midges and ticks from Northern Territory, Queensland and Tasmania. Identification studies, although not complete indicate that the collection includes at least one "new" virus and provides new host and geographical records for several known viruses.

(R.L. Doherty, J.G. Carley,
Cheryl Filippich, G.J. Barrow,
Robyn Wilson, Karen Brown.)

Entomology: mosquito biology and systematics

Forty-one requests for advice or identification of mosquito specimens were processed. Besides giving a service to Local Authorities, scientists and others, these have provided important research material and new distribution records, with four new records from Northern Territory (including one new undescribed species of Culex and one of Uranotaenia) and four from Western Australia. Study of these and other collections led to recognition of characters to separate species pairs currently identified as Coquillettidia linealis, Hodgesia

quasisanguinae and Aedes eidsvoldensis.

Taxonomic studies have been concerned principally with the genus Coquillettidia and the subgenus Geoskusea of Aedes and keys to species of both taxa were prepared and are being tested. A new species of Tripteroides was collected in central Queensland. A note on an unrecorded scale character in Aedeomyia catasticta is in press.

Organisations concerned with assessment of mosquito problems in the Murray Valley were assisted with identifications, advice and seminars.

(Elizabeth N. Marks.)

Entomology: mosquito ecology and mosquito-virus relationships

Significant progress was made on a study of factors that contribute to the importance of the mosquito, Culex annulirostris as a vector of arboviruses. Four visits to Kowanyama, Cape York Peninsula, returned 65,951 mosquitoes in investigation of seasonal patterns of abundance, longevity, natural feeding patterns, host preference, biting activity and contact with man in indoors and outdoors situations. Experiments on host preference of C. annulirostris at Kowanyama showed that domestic fowls were very poor bait in relation to pig, dog, man, grey kangaroo and calf. This casts doubt on their relative usefulness as sentinels of arbovirus activity,

especially of MVE virus, in Australia.

Culex annulirostris, probably the main vector of MVE in Australia, was the predominant mosquito taken in regular collections at Charleville and June and October 1974, and December 1974 to April 1975.

Records of C. annulirostris and other mosquito species breeding in winter at Charleville and at Kowanyama contribute to the understanding of mosquito-virus cycles in nature. Further experimental feeding of viruses to mosquitoes continued in an attempt to understand the interaction of virus and host and to incriminate potential natural vectors of arboviruses.

(B.H. Kay, I.D. Fanning, Robyn Wilson.)

Biochemistry of orbiviruses

Orbiviruses constitute a newly recognised group of viruses, the best known members of which are the agents of bluetongue in sheep and African horse-sickness. The study of the ribonucleic acid composition of four orbiviruses isolated in Australia has shown that they are similar to each other and to bluetongue virus but that there are significant differences between the viruses. Two of the viruses (Wallal and NT14952) can be shown related but distinguishable from each other by

use of serological tests. Like bluetongue virus they consist of 10 segments of ribonucleic acid and seven proteins. Two of these proteins lie on the outside of the virus and it is likely that they are primarily involved in the serological reactions which distinguish the viruses. The analysis of the ribonucleic acid segments of the two viruses suggest that they differ in two segments which probably contain the genetic information for production of the two exterior proteins of the viruses.

Genetic analysis seeks to describe the properties of an organism in terms of the structure of its genetic material. This is usually achieved by exploitation of mutations of the genes, to obtain mutants with differences distinguishable from the original organism. Temperature-sensitive mutants of Wallal and NT14952 viruses have been isolated by growing them in the presence of chemicals which induce mutations. These mutants have lost the ability of the original virus to grow at 37^{o}. It is possible that, in cells infected with two temperature-sensitive mutants which are unable to grow at 37^{o} because of different mutations, there could be an interchange of genetic information providing a new individual (a recombinant) capable of growing at 37^{o}.

Recombination of this type has been demonstrated in one experiment using two mutants of Wallal virus. The interchange of genetic information in natural infections with two viruses may be a mechanism for development of new virus strains.

Preliminary results are reported of attempts to introduce radioactive labels into Wallal virus ribonucleic acid. Some difficulty has been found in the use of drugs to inhibit cell synthesis while incorporating radioactive label into replicating virus since the drug used apparently inhibits virus synthesis as well. The work is aimed ultimately at the hybridization of the ribonucleic acids of Wallal and NT14952 viruses in a study of the molecular basis of the relationships between the viruses.

(B.M. Gorman, Jill Sorensen, P.J. Walker, A.J. Melzer, Barbara Waters)

Biochemistry of Ross River virus

The work this year has been devoted to searching for proteins specified by Ross River virus in its in vitro host cell. This has been done by comparing in polyacrylamide gels the profile of protein synthesis in an uninfected cell with that of an infected cell. The uninfected cell is forced to incorporate a specific amino acid labelled with carbon-14 while the infected cell is fed the same amino acid labelled

with tritium. In this way, all protein synthesized in the uninfected cell is labelled with carbon-14 and that in the infected cell is labelled with tritium; thus corresponding peaks in polyacrylamide gel profiles can be "cancelled out" to leave a qualitative picture of proteins which the virus specifies in the course of its infection.

Application of this technique at intervals in the infection cycle initially yielded encouraging results since a multitude of peaks apparently representing virus-specific synthesis were seen even as early as four hours after infection. However, a quantity of "background noise" is generated during the experiment which is impossible to eliminate entirely. Accordingly statistical tests were applied to the results to assess their reliability. These tests have been done on the Treasury computer and show that although there may be evidence of virus-specific protein synthesis occurring early in the infection cycle, prominent proteins are not apparent until between eight and twelve hours after infection. At this time the envelope and core proteins were clearly present as well as two high molecular weight species with weights 10.5×10^4 daltons and 7.7×10^4 daltons. There was also evidence of a small peptide of weight 1.4×10^4 daltons. Sixteen hours

after infection, when the cell was producing virus at its maximum rate, virus-specific proteins with weights 12.5×10^4, 10.5×10^5, 7.7×10^4 and 1.4×10^4 could be seen.

These results are in close agreement with work reported from other laboratories using other alphaviruses. Thus it is possible that, like other alphaviruses, the structural proteins of Ross River virus arise by post-translational cleavage of high molecular weight precursors.

(M.H. Symons.)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF WESTERN AUSTRALIA, PERTH, W.A., AUSTRALIA

SEROLOGICAL EPIDEMIOLOGY OF THE ORD RIVER AREA

Preliminary results from haemagglutination-inhibition tests on human sera are presented in Table I. All sera were collected in Kununurra from people resident in the townsite and nearby cattle stations. These results indicate a high level of group B activity in the area. The few aboriginal infant sera tested have been positive for MVE. If this observation is confirmed and extended, this would favour a very early infection in life.

Table II gives results from H-I tests on avian sera. Collections were made primarily in the swamps around the diversion dam and on the irrigation area near Kununurra. Group B antibody appears to be ubiquitous in bird species associated with these swamps.

(N.F. Stanley, M.P. Alpers, P.F. Liehne, C.G. Liehne, N.P. Hamilton, K.H. Chan).

TABLE I : Results of H-I Tests for Arbovirus Antibody on Human Kimberly Sera

SAMPLE SIZE		*PERCENTAGE POSITIVE BY H-I TEST AGENTS:*		
		MVE	*SINDBIS*	*ROSS RIVER*
ADULT				
Caucasian	114	40	15	20
Aboriginal	43	86	12	25
CHILDREN				
Caucasian	89	12	2.3	15
Aboriginal	47	62	6.3	63

TABLE II : Results of H-I Tests for Arbovirus Antibody on Avian Sera

SPECIES	*SAMPLE SIZE*	*NUMBER POSITIVE BY H-I TEST: AGENTS:*	
		MVE	*SINDBIS*
Sentinel Chicken	30	22	9
Magpie Goose	26	8	2
Black Duck	5	2	1
Tree Whistling Duck	8	7	3
Yellow Honeyeater	13	12	2
Red-tailed Black Cockatoo	7	5	0
Pink and Grey Galah	8	8	0
Little Corella	38	34	3

REPORT FROM THE ARBOVIRUS RESEARCH UNIT
UNIVERSITY OF CALIFORNIA INTERNATIONAL CENTER
FOR MEDICAL RESEARCH
HOOPER FOUNDATION, SAN FRANCISCO
AND
THE UNIVERSITY OF MALAYA, KUALA LUMPUR

We have previously described the earlier phases of our investigations of the ecology of dengue in Malaysia, emphasizing the jungle cycles involving wild monkeys and forest mosquitoes.

Since the initiation of these studies in the Gunong Besout Forest Reserve, a total of almost 300,000 biting arthropods were collected alive. These included 110 species of mosquitoes representing 17 genera. Half of the world's known mosquito genera are present in the study area. All of the female mosquitoes have been, or are being, processed for presence of arboviruses.

During the first months of the study in Gunong Besout, three sentinel leaf monkeys X-2,X-5, and X-7 (Presbytis obscura) in the high canopy trap at 75 feet above the forest floor developed dengue antibodies. From blood specimens taken prior to these serological conversions, strains of dengue virus were recovered, type 1 from one monkey and type 2 from the other two.

It is of particular significance that, although the leaf monkeys in the high canopy trap were infected with dengue of two different types, the macaques in the nearby ground trap were not infected during the same period of time. This, then, appeared to be convincing evidence of enzootic dengue activity in the high canopy of the forest. It also suggested that the forest vector involved in this case is more common in the canopy or that it prefers leaf monkeys to macaques. The latter seems less likely, since we previously recorded several dengue conversions in wild macaques and since our serological surveys of wild monkeys revealed a high incidence of dengue antibody in all species of monkeys captured.

In July 1973, Presbytis cristata monkey #139 developed dengue antibody as demonstrated by hemagglutination inhibition (HI) and plaque-reduction neutralization (PRNT) tests, but the type of dengue involved could not be ascertained since PRNT antibody developed for three types (1,2, and 4) indicating a probable secondary infection although no antibody was detectable in the preconversion serum. This monkey was also in the high canopy trap.

In February 1974, Presbytis cristata monkey #138 in the same canopy trap at 75 feet developed HI antibody for dengue type 4 only. This was confirmed by PRNT.

In May 1975, Presbytis cristata monkey #195, again in the same trap, developed HI antibody at a low titer for all four types of dengue with the highest titer for type 3. Study of this conversion is yet to be completed.

Unfortunately, we have not yet succeeded in isolating virus from mosquitoes collected in the traps during the periods of time when the sentinels were infected. However, through analysis of the mosquito collections from that trap at those times, we can infer which species is most likely a vector in the high canopy.

During the first three periods of the sentinel monkey conversions, over 20 species of mosquitoes were collected alive in the high canopy trap. However, only eight of the 20+ species were collected in all three periods: Anopheles (Cellia) balabacensis introlatus, Culex (Culex) pseudovishnui, Culex (Lophoceraomyia) cinctellus, Mansonia (Mansonioides) bonneae/dives, Coquillettidia crassipes, Heizmannia spp., Aedes (Finlaya) 'niveus', and Tripteroides spp.

In addition, only some of the captured mosquitoes were freshly engorged. Of the eight species present in all three collections, none of the Tripteroides spp. were engorged; some of the Heizmannia spp., Coquillettidia crassipes, and Culex pseudovishnui were engorged in two of the three periods; and some of the Anopheles b. introlatus, Culex cinctellus, Mansonia bonneae/dives, and Aedes 'niveus' were engorged in all three periods.

For the reasons about to be given, we believe that Aedes (Finlaya) 'niveus' and Anopheles (Cellia) balabacensis introlatus are the most likely jungle canopy vectors of dengue in the Gunong Besout Forest. Aedes (Stegomyia) albopictus, which can be found in greater numbers at the forest fringe and in agricultural and village areas, is undoubtedly the important link between the jungle and urban environments. These three species, and Coquillettidia crassipes, Heizmannia spp., Armigeres subalbatus, and Aedes (Stegomyia) albolineatus, are the seven that we have given priority consideration as suspect vectors.

The two Culex species are the mosquito species most abundant in the forest collections. Although both have been taken in large numbers at both ground and canopy levels, 77.4% of Culex cinctellus and 72.6% of Culex pseudovishnui attracted to monkeys were found at ground level in 1974. Furthermore, of 38,586 Culex cinctellus females attracted to monkeys, only 0.7% were engorged. Of 22,463 Culex pseudovishnui attracted to monkeys, only 2.3% were engorged.

Mansonia bonneae and M. dives are also very common in the forest and are collected at both ground and canopy levels. (We usually pool these two species because they are difficult to separate in rapid identifications.) Although many (42.3% of 1,202) Mansonia bonneae/dives females attracted to monkeys in 1974 were engorged, 65.9% were taken at ground level.

Of all of the 1974 collections of mosquitoes (as an example) attracted to monkeys at ground and canopy levels, Heizmannia were found mostly in the low canopy, whereas Aedes 'niveus', Coquillettidia crassipes, and Anopheles b. introlatus proved to be more common in the high canopy, where the dengue conversions occurred and in the emergent canopy. Heizmannia represented 2.1% of

the total female mosquitoes attracted to monkeys at 56', 0.6% at ground level, 0.4% at 75' and none at 147' in the emergent canopy. Aedes 'niveus' represented 13.8% of the collections at 147', 2.8% at 75', 0.04% at 56', and 0.03% at ground level. Coquillettidia crassipes represented 18.4% of the total collections at 147', 2.8% at 75', 4.2% at 56', and 1.0% at ground level. Anopheles b. introlatus represented 1.3% of the total collections at 147', 2.6% at 75', 0.06% at 56', and 0.03% at ground level.

Considering engorged specimens, we find only 4.0% of the Heizmannia and 5.7% of Coquillettidia crassipes engorged. In contrast, 57.8% of 422 Aedes 'niveus' and 65.1% of 335 Anopheles b. introlatus were engorged.

In traps employing animals other than monkeys as bait, Coquillettidia crassipes had an engorgement rate of 58.7%. It does not appear to prefer monkeys and is generally considered principally a bird feeder.

Of 335 Anopheles b. introlatus females attracted to monkeys in 1974, 94.6% were taken in the canopy at 75' or higher; of 422 Aedes 'niveus' females attracted to monkeys in 1974, 95.6% were taken in the canopy at 75' or higher. During the first three periods of the sentinel monkey dengue conversions, 78.7% of 47 Anopheles b. introlatus females and 44.3% of 79 Aedes 'niveus' females taken in the 75' trap were engorged.

Aedes albopictus was formerly highest on our list of suspect jungle vectors simply because it is a known vector of dengue and occurs in the jungle. However, despite intensive efforts to collect that species, we found it only in small numbers in the deep forest, and more commonly at ground level than in the canopy.

Aedes 'niveus', as reported in our collections in the Gunong Besout Forest Reserve, represents two or more related species. The majority of the specimens are probably pseudoniveus, but further study will be required to clarify the species composition of this complex in our collections.

Dengue antibody is common in monkeys in all types of forest habitats, except high mountain, in Malaysia, indicating that the jungle vector may be a species common to all those habitats. Since 'niveus' is widespread in all habitats throughout Malaysia and introlatus is found only in central peninsular Malaysia in low hill forest, it is most likely that 'niveus' is the important jungle canopy vector.

A new method designed to detect the jungle canopy vector and to demonstrate transmission was initiated in 1975 and is currently in use. Antibody-free monkeys are placed in mosquito-proof cages in the study site. Each day, unengorged specimens of the suspect vectors, which are captured alive, are allowed to engorge on the susceptible monkeys, one or two monkeys for each mosquito species. Blood samples from the monkeys are then examined twice weekly for detection of dengue viremia and development of antibody. This method will continue to be used during the remaining period of these studies.

Results of a serological survey of isolated jungle-dwelling aborigines for the presence of arbovirus group B antibodies have provided additional conformation of dengue activity in Malaysian jungles. Of eight group B viruses tested,

20% of 175 sera examined to date had specific neutralizing antibody only for dengue of one or more types, one serum was positive for Langat virus only (at a low titer), and none were positive only for Japanese encephalitis, tembusu, or Zika viruses. In addition, 30% of sera of deep jungle residents were positive for dengue only. Analysis of the prevalence of antibody to one virus only and for different combinations of cross-reacting antibody to two or more viruses clearly demonstrated that dengue is the principal virus responsible for group B antibodies in jungle residents.

Attempts to colonize suspect jungle mosquito vectors have not been successful to date, except for Armigeres subalbatus. A collaborative effort to colonize jungle mosquitoes, however, has been set up with Dr. A. Ralph Barr, Professor of Medical Entomology, University of California, Los Angeles. Dr. Barr is in Malaysia for one year under UC ICMR sponsorship. To date, after several months of intensive effort, he has successfully obtained hatching of a high percentage of Aedes 'niveus' eggs and he is optimistic that a laboratory colony can be developed for dengue transmission experiments.

In relation to our goals during the period of this report, the results have established with certainty that enzootic dengue, involving at least three of the four known types, occurs in the high canopy of the Gunong Besout Forest and that Aedes 'niveus' mosquitoes are most likely the important jungle vectors.

In related studies during the period of this report, six new viruses, including three in group B, were characterized, named, and registered in the Catalogue of Arthropod-Borne Viruses. They are Batu Cave, Carey Island, Jugra, Puchong, Seletar and Tanjong Rabok viruses. Epidemics of dengue hemorrhagic fever occurred in Malaysia in 1973, 1974, and 1975. Our staff assisted the Virus Research Division of the Institute for Medical Research in the study of these outbreaks. Results of studies of the epidemiology of tembusu and Umbre viruses demonstrated that domestic chickens are significantly involved in their cycles, that Culex annulus is the principal vector, and that man is infected in Malaysia.

(A. Rudnick, A.B. Knudsen & H.G. Wallace)

REPORT FROM THE DEPARTMENT OF VIROLOGY, SCHOOL OF TROPICAL MEDICINE, CALCUTTA, INDIA

Haemorrhagic manifestation in mice after second heterotypic dengue virus infection

Laboratory-bred white Swiss mice were injected intraperitoneally with a 10 LD_{50} dose of dengue virus type #1 (D_1). After intervals of 1, 2, 3 and 4 weeks, separate groups of these mice were challenged intracerebrally with a 10 LD_{50} dose of adult mouse-adapted dengue type #2 (AD-2) virus. When the mice became sick, their blood was examined for bleeding time (B.T.), coagulation time (C.T.) and platelet count. Subsequently they were sacrificed, autopsy performed and histopathological examinations of liver, brain, kidney and lungs were made. As control, normal un-inoculated mice and Ad-2 infected mice were similarly studied. It was found that the platelet count was 69% of normal in the Ad-2 infected mice, but became as low as 26.6 to 44.5% of normal in the re-infected mice.

The number of mice inoculated and the number showing macroscopic haemorrhage are shown in Table 1.

Table 1
Showing results of four experiments

Mouse Groups	Number Studied	Number Sick	Number Autopsied	Congestion & Patchy diffuse redness	Macroscopic haemorrhage
Control	24	20	16	2	Nil
dengue					
A	30	20	17	7	Nil
B	30	19	16	8	1 (lung)
C	32	25	21	10	Nil
D	32	20	16	3	2* (peritoneal)

* Superficial to peritoneum.

The number of mice in the different groups showing haemorrhage histopathologically are given in Table 2.

Table 2

Number of organs showing haemorrhage histologically

Mouse group	Brain	Lungs	Kidney	Liver
Control dengue	*0/4	0/4	0/4	0/4
A	1/4	1/4	1/4	2/4
B	2/4	2/4	3/4	3/4
C	1/4	1/4	2/4	1/4
D	2/4	0/4	3/4	2/4

* Numerator - number showing haemorrhage.

Denominator - number examined.

(J.K. Sarkar, B.C. Das, K.K. Mukherjee & S.K. Chakravarty)

REPORT FROM THE ARBOVIROLOGY DIVISION, DEPARTMENT OF MICROBIOLOGY,
NAVAL MEDICAL RESEARCH UNIT NO. 2 (NAMRU-2)
BOX 14, APO SAN FRANCISCO 96263

1. Virus Isolations from Mosquitoes Trapped in Indonesia.

Virus isolation studies from mosquitoes trapped in various areas of Indonesia have been conducted by this laboratory in cooperation with the Indonesian Ministry of Health. Mosquitoes are caught by using sucking tubes and by placing CDC battery operated light traps in pre-selected locations. Mosquitoes thus collected are brought alive to the NAMRU-2 Detachment laboratory in Jakarta, where females are separated and held for 48 hours or longer at room temperature in paper cups containing sugar water. Mosquitoes are processed for virus isolations as reported in our paper on isolations of JE virus from Java (1). Aliquots of original suspensions and infected mouse brains are transmitted to the parent laboratory in Taipei for reisolation and identification of the viruses. Isolates which we could not identify were submitted to Yale Arbovirus Research Unit for identification. In this laboratory the plaque reduction neutralization test (PRNT) is used to identify the viruses. Antiserums to the isolates and JE-Nakayama strain are produced in rabbits. Occasionally hyperimmune mouse ascitic fluid (IMAF) is used. Only those viruses reisolated from the original material by this laboratory are subjected to further study. Figure 1 shows a summary of JE isolates from mosquitoes.

Figure 2 shows a summary of isolates obtained from Aedes (sp) collected during the months January - April 1975. Thus far we have identified 5 of the isolates as Dengue 2. Final identification of the other isolates is pending.

2. Serological Survey for JE Antibodies in the Philippines.

Pig sera were collected at abattoirs in two locations in the Philippines - Olongopo and Angeles City. These locations are in close proximity to two large U.S. military facilities. The blood was collected at the time of slaughter, sera were extracted and transported to NAMRU-2, Taipei, in dry ice containers. Sera were stored at -70°C until processed for virus isolation and antibody studies. Virus isolation was attempted by passage in Vero cells liquid culture and by plaquing on Vero cells. For antibody studies the sera were screened at a 1:20 dilution against 75-100 PFU's. The results shown in Figure 3 are for the months April - October. We did not isolate JE virus, however, Inguavuma virus was isolated from sera collected in July. To our knowledge, JE virus has not been isolated in the Philippines. These antibody studies by PRNT strongly suggests the presence of JE or a virus very closely related.

Figure 1. Virus Isolates from Indonesia - JE

Place collected	Source	No. of JE Isolates
Kapuk	C. tritaeniorhynchus	12
Kapuk	Pig serum	3
Bekasi	C. tritaeniorhynchus	4
Bekasi	Pig serum	1
P. Babepan	C. gelidus	1
P. Babepan	C. tritaeniorhynchus	1

Figure 2. Virus Isolations from Mosquitoes in Indonesia - Dengue

nth llected 75	Species	No. of Mosquitoes/ No. of collections	Location	Virus
nuary	A. albopictus	339/3	Ragunan	-
"	A. albopictus	3/1	Pondok, Habu	+
"	A. albopictus	229/1	Raunan	Dengue 2
"	A. aegypti	68/3	Cilandak (JKT)	+
"	A. aegypti	49/2	Manggarai	-
"	A. aegypti	11/1	Cempaka	-
bruary	A. albopictus	463/4	Ragunan	+(2)
"	A. albopictus	2	Tebet	Dengue 2
"	A. aegypti	48/2	Cilandak	-
"	A. aegypti	20/2	Manggarai	-
"	A. aegypti	32/3	Cempaka	-
"	A. aegypti	7/1	Tebet	Dengue 2
rch	A. albopictus	464/4	Ragunan	Dengue 2, + 1
"	A. aegypti	74/2	Cilandak	-
"	A. aegypti	7/1	Manggarai	-
"	A. aegypti	8/1	Cempaka	-
ril	A. albopictus	347/2	Ragunan	-
"	A. albopictus	53/1	Kebayaran	-
"	A. aegypti	38/1	Cilandak	-

= Isolate not yet identified.

Figure 3. Philippine Pig Sera Study

Month collected	JE No. Pos/No. Tested	%	Ingwavuma No. Pos/No. Tested	%
April-May 1974	14/109	17.0	31/109	28.0
June	13/50	26.0	14/50	28.0
July	31/54	57.4	20/54*	37.0
August	15/56	26.7	28/56	50.0
September	34/60	56.6	17/60	28.3
October	22/59	37.2	18/59	30.5

* Ingwavuma virus isolated.

Reference:

1. Van Peenen, P. F. D., et al. 1974. First isolation of Japanese encephalitis from Java. Mil. Med. 139(10):821-823.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY

KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Specificity of anti-dengue-1 IgM and IgG

Three components, separated from dengue type 1 Mochizuki strain virus, RSH, SSH and MSC (previously reported in Arbovirus Info-Exchange, No. 25, 125-126, 1973) were intravenously injected into rabbits three times at weekly intervals; and 8-9 weeks after the first injection, two additional shots were given as booster. At appropriate time the blood was taken and the serum separated.

Immunoglobulins were separated by sucrose gradient centrifugation, and IgM (19S fractions) and IgG (7S fractions) were examined for HI reactivities against the Mochizuki virus antigen as well as antigens of related arboviruses such as dengue type 1 Hawaiian, type 2 Trinidad, type 3 H-87, type 4 H-241, yellow fever 17D and Japanese encephalitis JaGAr#01 viruses.

In the whole serum from rabbits immunized with the RSH, the antibodies against all the antigens employed appeared at two weeks after the first injection. Similar data, though of a little lower titers, were obtained as for the IgG. The IgM contained only the homolougous antibodies, i.e., anti-dengue type 1. Nine weeks after the first injection, the antibody titers were more elevated than those at 2 weeks. In this cases, the IgM showed no reactivity either against the homologous or heterologous antigens.

In the sera from rabbits immunized with the SSH or MSC, the HI titers were significantly lower than those by the RSH, although the general aspects were similar to each other.

The IgG revealed cross reactivity with all the antigens employed, although there were differences in the levels of titer. The reactivity of IgM was practically homologous.

The results indicate (i) that the RSH is stronger in immunogenic capacity than the SSH and MSC, and (ii) that the IgM is more specific in the HI reactivity than is the IgG or the whole serum.

(N. Fujita and S. Hotta)

REPORT FROM THE NATIONAL INSTITUTE
OF HEALTH, TOKYO, JAPAN

Immune response in old men and adults living in endemic and non-endemic areas of Japanese encephalitis following Nakayama and JaGAr-01 vaccination

The formalin-inactivated mouse brain vaccine of Japanese encephalitis (JE) has been known to be protective, to some extent, against JE virus infection in mice. Such a vaccine injected subcutaneously twice 7 days apart into people living in Tokyo resulted in production of neutralizing (Nt) antibody, while similar injection of people living in an almost non-endemic area such as Sapporo showed hardly any Nt antibody. It was apparent that man in the endemic area has been subclinically infected, resulting in an immune state in various grades; the vaccine produced a booster effect, anamnestic reaction (Mitamura et al., 1940).

It is also known that different strains of JE virus may differ in antigenic structure (Hale and Lee, 1954; Ogata, 1959; Okuno et al., 1961; Kitaoka, 1971). The Nakayama strain was isolated from spinal fluid of man in 1935 and was maintained through serial brain passages in adult mice or suckling mice. The JaGAr-01 strain was isolated from a mosquito pool inoculated into the brain of suckling mice and passed in adult mice by the intraperitoneal route. The optimum pH of the Nakayama strain is 6.4 and that of the JaGAr-01 strain is 6.7. In the diagnosis of JE, the HI titer is found to be higher for the JaGAr-01 strain than for Nakayama. Accordingly, it is said the JaGAr-01 strain is a current strain.

The technique and procedure of vaccine preparation has been so much improved that there is no adverse reaction following repeated injections. Both vaccines, Nakayama and JaGAr-01, were prepared and authorized by the national assay.

Man of two groups, one being older than 60 years of age and the other being adults of lesser age, living in Tokyo and Sapporo were selected for study. Each group was divided into two subgroups. One subgroup was vaccinated with Nakayama and the other with JaGAr-01. The first and the second injections were 14 days apart and the second and third injections were 6 months apart. At the third injection each subgroup, old and adult, in Tokyo and Sapporo, was divided into two smaller groups; one was inoculated with Nakayama vaccine and the other with JaGAr-01 vaccine as booster. In Tokyo, preinoculation titers of 1.19 or 2.07 were noted in the old men, and of 1.43 and 1.43 in the adults (geometric means of HI titer); in Sapporo they were 0.38 and 0.61 in the old men and 0.20 and 0.39 in the adults. Other studies in mothers and their new borns in Sapporo indicated that JE has increased in endemicity in that area as compared with 1941. The accompanying Table indicates that the prevaccination HI titers of both the old and adult groups in Tokyo were elevated to as high as 3.62-5.23 by 2 weeks after the 2nd shot (booster effect). In the old group in Sapporo, they remained low, but in the adult group increased to 2.76-3.10.

At the third shot the HI titer (geometric means) of the Tokyo groups was

not significantly increased, probably due to no booster effect; no original antigenic sin phenomenon was seen as in influenza except in the old-man Nakayama vaccine group, which showed a higher Nakayama response. Both Sapporo age groups, old and adult, showed a higher HI titer (geometric means) for the homologous antigen than the heterologous one. In Tokyo each half of Nakayama and JaGAr-01 vaccinated groups was boostered with heterologous vaccine. The grade of initial immune memory and grade of antigenic structural difference might be so reflected on the original antigenic sin phenomenon as seen in influenza viruses. It is noteworthy that the sin phenomenon was not always evident, and not only in Sapporo which has been mentioned by Kanamitsu et al. It might be due to no marked antigenic difference in JE viruses, and the initial immune memory to JE virus might not always be boosted by a heterologous strain.

(Masami Kitaoka and Takayuki Ogata, National Institute of Health, Tokyo; Hassaku Imagawa, The Bokuto Municipal Hospital, Tokyo; Isao Tomizawa and Toru Akiba, The Sapporo Municipal Hospital, Sapporo)

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Geometric means of HI-antibody following JE vaccination in both old men and adults living in Tokyo and Hokkaido

Vaccine used			Nakayama NIH(A)	JaGAr-01(B)
Tokyo	Old men	I	1.19 (26)	2.07 (26)
		II	4.42 (26)	5.23 (26)
		III(A)	4.66 (12)	5.66 (12)
		III(B)	4.75 (12)	5.83 (12)
	Adults	I	1.43 (16)	1.43 (16)
		II	3.81 (16)	3.62 (16)
		III(A)	3.25 (8)	3.75 (8)
		III(B)	4.12 (8)	3.12 (8)
Hokkaido	Old men	I	0.38 (18)	0.61 (18)
		II	1.43 (16)	1.56 (16)
		III(A)	3.00 (8)	1.62 (8)
		III(B)	2.25 (8)	2.33 (8)
	Adults	I	0.20 (43)	0.39 (43)
		II	2.76 (38)	3.10 (38)
		III(A)	5.05 (19)	4.89 (19)
		III(B)	4.05 (19)	5.05 (19)

Geometric means of log of HI titer are in prevaccination and those in 2 weeks after the second and booster shots.

Figure in parenthesis indicates number of persons tested.

I and II with 2 weeks apart, II and III with 6 months apart.

Noninfectious hemagglutinating antigen prepared by B-propiolactone-treatment of Japanese encephalitis virus-infected mouse brains and tissue culture

The hemagglutination (HA) and hemagglutination-inhibition (HI) tests are needed for the identification and classification of arbovirus and for the diagnosis of patients with arboviral infections. To avoid the laboratory infection with Japanese encephalitis (JE) virus of laboratory workers performing serological diagnosis, the antigen used in HA and HI tests should not contain any infectious virus particles. At present, the acetone-ether extract of suckling mouse brains or tissue cultured cells infected with JE-virus is routinely used as HA antigen. However, these solvents act on the surface (projection) and evelope of the virion, but not directly on the inner core; and although the treatment might much reduce the infective activity of JE virus, the active virus can still be detected (see below). On the contrary, B-propiolactone (BPL) is known to be an alkylating agent that renders Sendai virus noninfective while leaving unaffected the capacity of the virus to fuse cells rapidly in culture (Neff and Enders, Pedreia and Tauraso). Roberts and Warwick have shown that BPL reacts with ribonucleic acid (RNA) to yield 7-(2-carboxyethyl) guanine after RNA hydrolysis in 1/N HCl. Extending this observation, alkylation at the N-9 position also occurs to yield 7.9-di-(2-carboxyethyl) guanine. Moreover, inactivation of polymerase in the viral envelope or nucleo-capsid might occur at a concentration of BPL lower than that affecting other envelope functions (Weinberg et al.). At any rate, BPL acts on the infective activity but not on the HA activity of JE virus even though HA activity of influenza virus is considerably reduced by BPL.

BPL was diluted in water 1:10 by method of Neff and Enders. This 1:10-diluted BPL solution was again diluted 1:10 in a concentrated virus suspension (infected tissue cultured cells, and an acetone-ether extract of a 15% emulsion of infected suckling mouse brain) and the mixture shaken every 10 min. The treated virus was used the next day after overnight refigeration at 4°C to insure complete hydrolysis of the BPL. Three JE virus strains were used: Nakayama-NIH, isolated from spinal fluid of a patient in 1935; JaGAr-01, isolated from a mosquito pool near Tokyo in 1959; and Peking 1, isolated from man in 1947, first passed in adult mouse brains and then cultured in primary sheep kidney cells. In Tokyo the strains were cultured in hamster kidney cells in medium 199+2% human albumin. They had been inactivated by 0.1% BPL and concentrated by dialysis against saturated sucrose. 2% of gelatin was added to the antigen before lyophilization. The process of lyophilization was performed during 24 hours at -20°C.

Optimum pH of HA: The optimum pH of BPL-treated antigen (BPL(+)) and BPL-untreated antigen (BPL(-)); for Nakayama-NIH (-) was 6.4 and (+), 6.4; JaGAr-01 (-), 6.6 and (+), 6.4; and Peking-1 (-), 6.2 and (+) 6.2. Antigen dilutions: Antigen dilutions of Nakayama-NIH (+), (-), JaGAr-01 (+), (-) and Peking-1 (+), (-) were 1:800, 1:600, 1:800, 1:300, and 1:240, 1:240, respectively, corresponding to 8 units of HA. In general BPL (+) seemed to move a little to the acid side but almost the same as illustrated in the Fig.

In the Acetone-ether extracted antigens of Nakayama-NIH and Peking-1, virus was detected in titers as high as 1.4 and 2.0, respectively (log of LD_{50} per 0.02 ml intracerebral inoculation into suckling mouse brain). Virus was not detected in the acetone-ether extracted JaGAr-01 antigen. No virus could be detected in any of the three BPL (+) antigens.

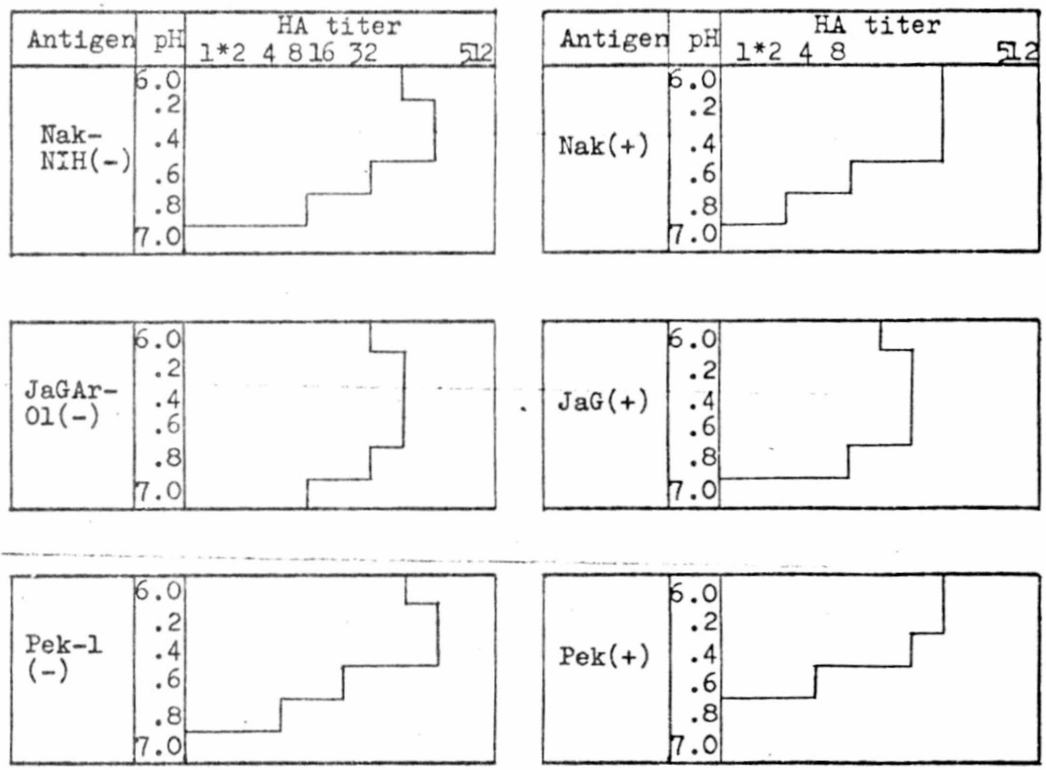
HAI tests on JE patients revealed no marked difference in the sensitivity and specificity of the acetone-ether extracted antigens and the BPL-treated antigens. The BPL -- inactivated antigens were preserved for at least one month in the lyophilized state; they probably are preserved for much longer than this, as seen in acetone-ether extracted antigens.

In conclusion, BPL-treated JE antigens have been found to be non-infectious but to retain HA activity. The sensitiveness and specificity of the BPL-treated antigens are almost the same as acetone-ether extracted antigens. BPL-treated JE antigens are to be recommended to avoid laboratory infections.

The paper will be submitted to Acta Virologica.

(M. Kitaoka, National Institute of Health, Tokyo; and S. Y. Gaidamovich, Ivanosky Institute of Virology, Moscow)

HA-pattern of 3 JE antigens, Nakayama-NIH, JaGAr-01 and Peking-1, BPL-treated: (+) and BPL-untreated: (-)



*HA-positive of two-fold dilution starting from 1:100 antigen dilution which is presented as 1.

Antigen		HA titer	Optimal pH	Infectivity
Nakayama-NIH	(-)	12800	6.2	+ LD_{50} $10^{-1.4}$ 0.02ml
Nakayama-NIH*	(+)	6400	6.2	-
JaGAr-01	(-)	6400	6.6	-
JaGAr-01*	(+)	3200	6.4	-
Peking-1	(-)	12800	6.2	+ LD_{50} $10^{-2.0}$ 0.02ml
Peking-1**	(+)	6400	6.2	-

*Aceton-ether extracted antigen from 15% suckling mouse brains infected with Nakayama-NIH and JaGAr-01 which is treated with 0.01% BPL.

**Acetone-ether extracted antigen from HK cell culture infected with Peking-1, which is treated with 0.01% BPL.

REPORT FROM THE IVANOVSKY INSTITUTE OF VIROLOGY
ACADEMY OF MEDICAL SCIENCES
MOSCOW, USSR

Application of the IHI test to detection of antibody to LCM virus

The question of the presence of antibodies in chronic LCM infection is debatable. However, some scientists (Oldstone and Dixon 1967, 1969) have succeeded in detecting antibodies in mice with chronic LCM infection in the immunofluorescent staining and complement-fixation tests (Traub 1972). The present communication reports the application of the indirect hemagglutination (IHI) test, developed in the laboratory for LCM virus, to the detection of antibodies in mice in acute and chronic infection.

To reproduce chronic infection, newborn mice (16-24 hours old) were inoculated intracerebrally with 0.01 ml of brain suspension containing 3-4 $\log_{10}$ LD_{50}/0.03 ml virus. The blood was examined for virus and antibody, starting with postinfection day 30. The virus in the blood of infected mice was titrated by the intracerebral inoculation in adult mice weighing 10-12 g.

Antibodies in the blood were detected in CF and IHI tests. The IHI was run according to the technique described earlier for Colorado tick fever (4) employing sheep erythrocytes sensitized with 0.05% immunoglobulin and the sucrose-acetone antigen from infected suckling mice brain.

Upon reproduction of chronic infection the survival rate in infected suckling mice averaged 35-40%. Between post-infection days 10 and 30 some animals showed the evidence of infection: retardation in body weight, ruffled hair with alopecia, blepharitis. Most of the mice which survived were virus carriers. The titer of LCM virus in the blood did not exceed 3.0-3.5 $\log_{10}$ LD_{50}/0.03 ml.

The table represents the results of examination of blood for viremia and antibodies between days 35 and 90 of infection. One group of mice demonstrated viremia and the other did not. There were two types of the immunological response in the mice free of virus. In some animals high-titered antibodies were revealed in IHI and CF tests (see mice No. 901-903); these mice apparently recovered from the infection. In other animals the immunological response was very weak; antibodies were absent or were revealed in a low titer. In mice which were virus carriers, with negative or equivocal results in the CF test, we succeeded in detecting antibodies in the IHI test. Unfortunately, antibody titration was not always performed because of the small amount of the material.

Apparently, antibody detecteable in the IHI test corresponds to antibody against soluble CF antigen, so far as mice do not develop neutralizing antibody. But the IHI test is more sensitive than the CF test. Experiments carried out with sera from immunized mice, which had no measurable amounts of NT antibodies, and sera of guinea pigs with both CF and NT antibodies, showed perfect correlation of CF and IHI antibody.

(Sofia Ya. Gaidamovich)

Viremia and antibodies in

chronically infected mice.

Virus carriage	No. of mice	Viremia (lg LD_{50}/0,03ml)	IHI	CF
No Viremia	351	0	80	traces
	521	0	(10)	0
	522	0	(10)	0
	523	0	traces	0
	524	0	(10)	32
	525	0	traces	0
	526	0	(10)	8
	611	0	80	0
	801	0	(10)	traces
	901	0	640	640
	902	0	320	160
	903	0	320	320
Viremia	401	(2)	10	0
	402	(2)	traces	0
	403	(2)	40	10
	404	(2)	20	0
	405	(2)	40	0
	406	(2)	traces	0
	407	(2)	20	traces
	761	2,75	traces	traces
	762	3,75	0	0
	763	2,25	10	0
	801	1,75	(10)	traces
	802	3,0	(10)	0
	803	1,75	(10)	0
	804	3,5	(10)	0

Designation: the first two figures indicate the post inoculation days

The antibody titre is expressed in reciprocals

( ) - quality test

REPORT FROM THE INSTITUTE OF POLIOMYELITIS AND VIRUS ENCEPHALITIDES
ACADEMY OF MEDICAL SCIENCES
MOSCOW, USSR

Three antigenic types of tick-borne encephalitis virus; their dependence upon the main tick vector species and geographical distribution

By the use of a set of new methods developed by S.G. Rubin (1971-1975) for intraspecies antigenic differentiation of numerous groups of tick-borne encephalitis virus strains (TBE) isolated from different sources in several geographic regions of Europe and Asia, the existence of at least three antigenic types of human tick-borne encephalitis virus was established, and their dependence upon the species of the tick reservoir and vector of infection prevalent in the region was demonstrated. Although all TBE virus strains show considerable cross relationships with each other, they may be clearly differentiated in AGDP and HI tests into three antigenic groups using specially prepared type-specific sera or quantitatively dosed adsorption of type-specific antibody. According to the antigenic characteristics, type I of TBE virus includes strains isolated from Ix. persulcatus ticks or from human patients and animal virus carriers in the region where Ix. persulcatus is the predominant vector; TBE type II includes strains isolated from Ix. ricinus ticks or from human patients and animal virus-carriers in the region where Ix. ricinus in the main vector; TBE virus type III has so far one strain isolated by Dr. O. Papadopoulos in 1969 in Greece from the brain of a paralysed goat which contracted the disease in a pasture where Rhipicephalus bursa ticks were prevalent.

It is important to state that TBE virus strains isolated from fleas, gamasoid mites and from different species of Ixodid ticks which are not main vectors of virus to man, usually exhibit the antigenic characteristics of either type I (persulcatus) of type II (ricinus) virus prevalent in one or another part of the endemic region. In the extreme west of the USSR, in the Baltic republics, there are districts where either type I or type II TBE virus is prevalent. Consequently, the conventional differentiation of TBE virus strains into eastern and western variants or into Central-European and Far-Eastern, i.e. based on the geographical principle, requires revision with due consideration to the new evidence.

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(M.P. Chumakov, S.G. Rubin and M.B. Linev)

Abstracts in English of important reports on Crimean hemorrhagic fever and other viruses, published only in Russian (from Medical Virology, Vol. XXII, N2, Moscow, 1974, pp. 277-284). (The following abstracts of interest to arbovirus workers were kindly submitted by Academician M.P. Chumakov, Editor in Chief of the Transactions of the Institute of Poliomyelitis and Virus Encephalitides. R.W. Chamberlain)

ON 30 YEARS OF INVESTIGATION OF CRIMEAN HEMORRHAGIC FEVER

M. P. Chumakov

During 30 years after first description in the USSR of a hitherto unknown human viral disease designated in 1944 Crimean hemorrhagic fever (CHF) considerable scientific materials have been accrued comprehensively characterising this natural-focus tick-borne infection thich has been found to occur not only in the Crimea but also in a number of souwhern areas of many republics of the Soviet Union and in the southern parts of other countries of Europe, Asia and Africa. The filterable virus first isolated in 1945 from Hyalomma marginatum marginatum ticks (new name Hyalomma plumbeum plumbeum) and identified then as the causative agent of CHF later proved antigenically and biologically to be closely related to Congo fever virus isolated in 1956 in Africa and partially related to Hazara virus isolated in 1964 in Pakistan. Diseases of the type of CHF in the USSR, Bulgaria and Yugoslavia and Congo fever in African countries are similar clinically and epidemiologicaly but, evidently, not identical. There are reasons to include both infections in one CHF-Congo group.

The CHF-Congo group viruses have properties typical of RNA-containing, ether-sensitive, moderate size arboviruses occurring in many species of blood-sucking ticks. To-date, CHF virus in the USSR has been isolated from ticks of 15 species, including 5 Hyalomma species, 5 Rhipicephalus species and thus far occasional species of Haemaphysalis, Ixodes, Dermacentor, Boophilus calcaratus and Argas. In Africa and South-East Asia (Pakistan), the closely related Congo virus was isolated from 8 other tick species parasitising on cattle and also was once isolated from a mixture of midges (Culicoides). Evidently, only those species of virus-infected ticks which have sufficiently frequent contacts with man may become vectors of human disease. CHF virus has been isolated from the blood and the liver of hares in the Crimea during the period of tick parasitising on them, and Congo virus from the blood of cows, goats and from the viscera of hedgehog also during the time of ticks parasitising on them. No diseases in domestic and wild animals infected with CHF virus have been detected, but recently cases of human infection with CHF through the contact with the blood of a cow developing "gastritis" after tick bites have been reported. Several nosocomial cases of CHF contracted by contact with the blood of CHF cases are known also.

CHF has quite characteristic clinical pathology with predominance of acute hemorrhagic syndrome. The disease is usually severe, its lethality being from 8—10 to 30—50%, but leaves no residuals in the survivors.

The laboratory diagnosis of CHF is possible on the basis of typical changes in the blood picture (early leukopenia, with a marked shift to the left, thrombocytopenia, prothrombinopenia) and at autopsy by the results of specificimmunofluorescence in impressions of liver and spleen sections. Besides, in specially equipped laboratories attempts at isolation of virus pathogenic for newborn wite mice may be made with the blood from a febrile patient (or from the organs in fatal cases) and the isolate may be identified by the complement fixation test (CFT) or other tests.

By the use of the fluorescent antibody procedure an effective method for detection of CHF virus antigen in preparations of the salivary glands of virus-carrying ticks has been developed, contributing to detection of latent tick foci of this infection.

For detection of antibody to CHF virus in humans and animals in active or potential natural foci of infection, the agar gel diffuse precipitation test (AGDP) and, under certain conditions, the CFT are used successfully as well as virus neutralization test in several modifications. A formalin-inactivated, protamine sulfate-purified mouse brain vaccine has been developed and tested in the USSR for specific prophylaxis of CHF. Vaccination is recommended in foci of infection for protection of some contingents of cattle breeders developing CHF most frequently, medical staff of infectious wards in hospitals and workers of some virological laboratories handling CHF-Congo group viruses.

PROBLEMS OF CRIMEAN HEMORRHAGIC FEVER VIRUS ECOLOGY IN NATURAL FOCI OF THIS INFECTION IN THE CRIMEA

M. P. Chumakov, S. K. Andreeva, T. I. Zavodova, G. N. Zgurskaya, N. V. Kostelsky, L. I. Martianova, A. M. Nikitin, K. M. Sinyak, S. E. Smirnova, L. I. Turta, E. D. Ustinova, S. P. Chunikhin

Crimean hemorrhagic fever (CHF) first described in 1944—1945 on the basis of the materials of an epidemic outbreak (about 200 cases in the Crimea) in subsequent 25 years did not occur annually in the Crimea and was observed as small accumulations of cases or occasional cases. In the past 5 years no CHF cases were reported. Natural foci of this infection have been shown to still exist in the Crimea as indicated by isolation of 33 CHF virus strains from 5 species of blood-sucking ticks collected from cattle in 11 places, practically in all landscape-geographical areas of the Crimea including the Southern Coast, vicinities of Sebastopol, central and eastern (Kerch) areas. CHF virus has been isolated from the blood and livers of three hares at the time of tick parasitising on them. This is the first direct evidence of the important role of hares in the ecology of the causative agent of CHF.

Selective serological surveys of domestic cattle for antibody to CHF in the Crimea confirmed the existence of CHF virus circulation.

FIRST ISOLATION OF CHF VIRUS IN ARMENIA FROM THE BLOOD OF A PATIENT WITH CRIMEAN HEMORRHAGIC FEVER

I. V. Semashko, M. P. Chumakov, R. M. Karapetyan, A. G. Vorobiev, T. I. Zavodova, K. Sh. Matevosyan, M. A. Nersesyan

This is the first report in Armenia of a case of hemorrhagic fever confirmed by isolation of Crimean hemorrhagic fever virus from the blood of the patient.

A CASE OF CRIMEAN HEMORRHAGIC FEVER IN THE ARMENAIN SSR

R. M. Karapetyan, A. G. Vorobiev, I. V. Semashko, K. Sh. Matevosyan

This is the first report of the clinical picture of Crimean hemorrhagic fever (CHF) in the Armenian SSR developing 4 days after detachment of an attached Ixodid tick. The diagnosis was confirmed by isolation of CHF virus from blood specimens collected at 7 and 8 days.

CASES OF TRANSMISSION OF CRIMEAN HEMORRHAGIC FEVER VIRUS IN UZBEKISTAN BY CONTACTS WITH THE BLOOD OF A SICK COW AND A HUMAN PATIENT AS WELL AS BY TICK BITES

M. P. Chumakov, B. Kh. Vafakulov, T. I. Zavodova, V. Ya. Karmysheva, S. S. Maksumov, L. I. Martianova, V. I. Rodin, S. N. Sukharenko

In 1973 in Uzbekistan, the evidence was first obtained of the possibility of human infection with Crimean hemorrhagic fever (CHF) as a result of casual contacts with the blood of a sick animal bitten by ticks in an endemic area. Three cases of CHF in persons who took part in removing the hideand cutting the carcass of a cow sacrificed in the presence of signs of a disease diagnosed as "acute gastritis" are described. These subjects developed the disease in 3 days and died almost simultaneously at 7—8 days after infection, the typical picture of CHF being observed. Five days after the deaths of these patients a nurse of the surgical ward of the hospital who had had contact with the blood of the patient fell ill. This woman survived after a very severe course of the disease with various manifestations of the hemorrhagic syndrome.

The CHF virus was isolated from two patients and the nurse in the convalescent period was found to have antibody to CHF virus.

In the same area in 1973, 2 more strains of CHF virus were isolated from the organs of human patients who had been bitten by ticks, and died of CHF.

DETECTION OF CRIMEAN HEMORRHAGIC FEVER VIRUS IN SOME SPECIES OF BLOOD-SUCKING TICKS COLLECTED IN 1973 IN THE KIRGHIZ AND UZBEK SSRs

M. P. Chumakov, T. I. Zavodova, L. I. Martianova, A. G. Mukhitdinov, T. P. Povalishina, V. I. Rodin, V. F. Rozina, R. O. Safarova, S. I. Sukharenko, A. G. Tatarov, S. S. Khachaturova, S. P. Chunikhin

Virological examinations in newborn white mice of blood-sucking ticks collected in 1973 from cattle in Osh region of the Kirghiz SSR and in Andizhan, Kashkadarya and Samarkand regions of Uzbekistan resulted in isolation of a large number of strains of Crimean hemorrhagic fever virus from ticks belonging to 5 genera, including four Hyalomma species, two Rhipicephalus species, and one species each of other genera: Dermacentor marginatus, Boophilus calcaratus and Argas persicus.

Consequently, many species of blood-sucking ticks in foci of hemorrhagic fever in Uzbekistan and Kirghizia are capable of maintaining circulation of CHF virus in nature. The epidemiological importance of spontaneously infected ticks as vectors of CHF infection to man appears to be determined by their unequal opportunities and capacities to attack man.

EXAMINATION OF HUMAN AND ANIMAL SERA FROM INDIA FOR ANTIBODY TO BHANJA AND DHORI VIRUSES

J. Shanmugam, S. E. Smirnova, V. N. Bashkirtsev, M. P. Chumakov

Antibody to Bhanja and Dhori viruses has been found in hemagglutination-inhibition tests in the blood sera of people and domestic animals from 5 states of India: Tamil Nadu, Mysore, Kerala, Pondicheri and Maharashtra.

ISOLATION AND IDENTIFICATION OF CRIMEAN HEMORRHAGIC FEVER AND WEST NILE FEVER VIRUSES FROM TICKS COLLECTED IN MOLDAVIA

M. P. Chumakov, V.N. Bashkirtsev, E. I. Golger, T. K. Dzagurova, T. I. Zavodova, Yu. N. Konovalov, L. I. Martianova, I. G. Uspenskaya, A. N. Filippsky

In 1973—1974 in several areas of the Moldavian SSR, 8 strains of CHF virus were isolated from ticks of 3 species: Ix. ricinus, Dermacentor marginatus and Haemaphysalis punctata, and 3 strains belonging to the antigenic subgroup of West Nile virus from Ix. ricinus ticks. Besides, one isolate containing a mixture of CHF and WN viruses was isolated from Dermacentor marginatus ticks. The Moldavian strains of the WN virus subgroup had high plaque titers in tissue cultures under the agar overlay corresponding to the infectious virus titers in newborn mice; the diameter of their plaques in pig embryo kidney cell cultures was about 6—7 mm and in a continuous line of green monkey embryo kidney cultures about 1.5—2 mm. No human cases have been reported in the areas of CHF and WN virus isolations.

THE EFFECT OF SOME PHYSICAL AND CHEMICAL TREATMENTS ON THE CHF-CONGO GROUP VIRUSES

M. A. Donets

The effect of some physical and chemical treatments on the CHF-Congo group viruses (the Khodzha and K67/67 strains) in 10% brain suspensions from sick newborn white mice was studied. Both strains showed similar characteristics in these experiments. They were markedly thermolavile (lost their titers considerably at 22°—37°C for 12—18 hours), biologically active in a narrow pH range (optimal pH 7.4), readily inactivated by formalin, beta-propiolactone, ultraviolet irradiation but retained their complement-fixing activity. Both viruses were rapidly destroyed in hypertonic solutions of 2M NaCl, 2M $MgCl_2$, 2M CsCl but resisted osmotic shock. The CHF-Congo viruses were found to be sensitive to trypsin and RNA-ase. The effect of these enzymes probably resulted in virion destruction, and a portion of CF-antigens was separated on Sephadex G-200 from the macromolecular virus fraction. Ultrasonic treatment of brain virus-containing suspensions produced highly active complement-fixing antigens. In all the experiments the stability of the complement-fixing activity was higher than that of the infectious titers.

ISOLATION AND IDENTIFICATION OF CRIMEAN HEMORRHAGIC FEVER AND DHORI-ASTRA VIRUS STRAINS FROM HYALOMMA PLUMBEUM PLUMBEUM TICKS COLLECTED IN THE AZERBAIJAN SSR

I. V. Semashko, M. P. Chumakov, R. I. Safarov, E. A. Tkachenko, V. N. Bashkirtsev, S. P. Chunikhin

Virological examinations of ticks collected from cattle in Dzhalalabad and Masslin districts of the Azerbaijan SSR yielded 9 virus strains pathogenic for newborn white mice. Eight of the isolates were identified as CHF virus, one as Dhori-Astra virus.

ISOLATION OF DHORI VIRUS FROM DERMACENTOR MARGINATUS TICKS IN THE ARMENIAN SSR, 1973

I. V. Semashko, K. Sh. Matevosyan, M. P. Chumakov, V. N. Bashkirtsev, E. A. Tkachenko, S. P. Chunikhin

A strain related to Dhori virus was isolated from Dermacentor marginatus ticks collected from sheep in Razdan district of the Armenian SSR and identified in 1973.

ISOLATION OF FIVE GETAH UIRUS STRAINS FROM MOSQUITOES IN THE SOUTHERN AMUR REGION, USSR

M. P. Chumakov, A. V. Moshkin, E. B. Andreeva, O. E. Rzhakhova, T. I. Zavodova, G. P. Fleer, G. V. Koreshkova, I. V. Uspensky, E. V. Krasovskaya, V. V. Merekin, V. I. Novitskaya

In 1972—1973 in three places of Blagoveshchensk and Tambov districts of southern Amur region (49°50′—50°30′ N and 127°40′ E) five Getah virus strains were isolated, including 1 strain (AE 17) from a pool of 400 female Aedes vexans nipponii and 4 strains from mixtures (500—1000 females) of several Aedes and Anopheles hyrcanus species; in the mixtures, Aedes vexans nipponii constituted up to 87% of the pool, An. hyrcanus 1—35%. Biologically and antigenically, the isolates were identical to each other and in cross CFT reacted only with the Malayan MM 2021 strain of Getah virus.

This report on the isolation from mosquitoes of 5 strains closely related or identical to Getah virus is the first virological evidence of the existence of natural foci of this infection in the USSR.

THE RESULTS OF THREE-YEAR STUDIES OF GETAH VIRUS CIRCULATION IN THE USSR

N. G. Bochkova, V. V. Pogodina

The paper presents serological evidence of occurrence of mosquito-borne arbovirus Getah belonging to the antigenic Group A (Alfavirus) in Primorsk region on the island of Sakhalin. This virus is new for the USSR. Examinations were carried out on 4652 blood sera collected from humans and domestic animals in 1969—1971 in 17 administrative districts of Primorsk region.

Swine were used as index animals for characterization of the seasonal (summer-autumn) and territorial distribution of Getah virus. Similarities in circulation of Getah and Japanese encephalitis viruses were observed.

Detection of virus-neutralizing antibody to Getah virus in titers of 1 : 2, 1 : 64 in the blood sera from local residents who in the summer-autumn of 1971 had suffered from febrile diseases of unknown etiology suggests the role of this virus in human infectious pathology.

FURTHER STUDY OF THE POSSIBLE ASSOCIATION OF THE DS-MARKER OF THE TICK-BORNE ENCEPHALITIS COMPLEX VIRUSES WITH VECTOR SPECIES

T. I. Dzhivanyan, V. A. Lashkevich, M. V. Chuprinskaya, G. G. Bannova, E. S. Sarmanova

The capacity of dextran sulphate (DS) added to the agar overlay to reduce the size of plaques produced in continuous SPEV-44 cell culture by viruses of the tick-borne encephalitis subgroup (DS marker) was studied in 32 strains of tick-borne encephalitis virus (TBE) isolated in different endemic areas from different species of ticks or from patients as well as in louping-ill, Langat and Negishi viruses. The DS marker was found to be the strain and not the species characteristic of the TBE complex viruses. The data permitting the division of all large-plaque TBE viruses by the DS marker into two groups: persulcatus (DS^-) and ricinus (DS^+) have been obtained. The division of the strains into two groups by the DS marker does not coincide with their division by the geographical origin from the west or the east of the Euro-Asian continent and is associated with the species of their tick vectors. The strains isolated in close geographical areas but from different tick species had different DS markers whereas the strains isolated in remote areas but from the same tick species had the same DS marker. The strains from patients had the DS marker similar to that of the strains isolated from ticks occurring in a given area.

ISOLATION OF TICK-BORNE ENCEPHALITIS VIRUS FROM GAMASOID MITES IN THE EXTREME NORTH (TAIMYR PENINSULA) IN BIOASSAYS IN MATURE GUINEA PIGS

M. S. Shaiman, L. N. Tarasevich

Materials are presented reporting isolation of two infectious agents identified as tick-borne encephalitis virus ("Taimyr" strain) from gamasoid mites collected in burrows of Siberian lemmings in Taimyr peninsula. Different laboratory models were used for virus isolations, including mature guinea pigs inoculated intraperitoneally.

THE INFLUENCE OF ERADICATION OF THE MAIN TICK VECTOR, IXODES PERSULCATUS, ON THE POPULATION OF TICK-BORNE ENCEPHALITIS VIRUS IN A FOCUS

N. N. Gorchakovskaya, M. P. Chumakov, I. M. Rodin, Yu. S. Korotkov, E. V. Finogenova

The study was carried out in tick-borne encephalitis (TBE) foci in taiga forests of Western Siberia. Changes in the rate of occurrence of small rodents possessing antibody to TBE virus indicating virus circulation in nature were studied. Ticks had been eradicated by a single treatment of forests with DDT. The changes were followed for 9, 12 and 16 years after elimination of Ixodes persulcatus population from large forests.

Initially, eradication of Ixodes persulcatus population disturbed the conditions of virus circulation in nature considerably as shown by a significant decrease in the number of seropositive rodents. But later virus circulation was restored. Restoration of the number of seropositive rodents occurred more rapidly (in 4—9 seasons) than that of the abundance of the main vector.

This evidence indicates the dominant significance of I. persulcatus in circulation of TBE virus in Western Siberia foci. L. trianguliceps and other nest-burrow blood-sucking Arthropodae are of secondary importance in Siberian foci but their role increases when I. persulcatus populations are eradicated.

ELECTRON MICROSCOPE EXAMINATION OF THE CENTRAL NERVOUS SYSTEM OF MICE INFECTED WITH VIRULENT AND ATTENUATED VARIANTS OF THE TICK-BORNE ENCEPHALITIS COMPLEX VIRUSES

T. I. Tikhomirova, L. G. Karpovich, M. P. Frolova, N. M. Shestopalova

The hemisphere cortex and the cerebellum of mice inoculated intracerebrally and subcutaneously with virulent and attenuated variants of tick-borne encephalitis and Langat viruses were examined in the electron microscope. All the strains under study were shown to be neurotropic regardless of the route of inoculation. Neurotropism of Langat virus, the Tp-21 strain, was manifested even in the absence of the clinical symptoms after subcutaneous inoculation. In addition to neurons, involvement in the infectious process in the nervous tissue of glial cells was demonstrated, and in them virus particles were observed most frequently in astrocytes and their processes.

The intracellular changes accompanying virus replication were similar for the strains under study and did not depend upon the route of inoculation.

INOCULATION OF CHICK EMBRYO CELL SUSPENSION WITH TICK-BORNE ENCEPHALITIS VIRUS AND FORMATION OF TISSUE CULTURES FROM THESE CELLS. THE CELL MORPHOLOGY AND YIELDS OF IMMUNOGENIC VIRUS IN THESE CULTURES

M. K. Khanina, A. V. Gagarina, N. N. Ginzburg

Formation of chick embryo tissue culture grown on glass from tick-borne encephalitis virus-infected cells in suspension was studied. The morphological condition of the infected cells was compared with antigen accumulation and virus immunogenicity.

SURVEY OF JAPANESE ENCEPHALITIS FOCI AND CHARACTERISTICS OF THE CURRENT SITUATION IN PRIMORSKIY REGION

V.V. Pogodina, N. G. Bochkova, I. N. Polenova

Expedition teams of the USSR AMS Institute of Poliomyelitis and Virus Encephalitides in 1968—1972 examined over 7500 blood serum specimens from humans and domestic animals in 17 administrative districts of Primorskiy region, and obtained serological evidence of annual seasonal

circulation of Japanese encephalitis virus in foci of the Middle and Southern Primorie. Currently the greatest potential danger rests with the areas of developing swine breeding and rice growing in Prikhankaiskaya zone (forest-steppe area) where the highest rates of natural infection of swine with Japanese encephalitis virus were observed by the end of the season (up to 35—70% of swine examined). It was established that in recent years strains close to the Jagar-01 immune type circulated both in Primoskiy region and in Japan.

A method for annual surveys of Japanese encephalitis foci is suggested including 4 main procedures: (1) serological examination of the sensitive index animals, swine; (2) analysis of the seasonal dynamics of antihemagglutinins in sera of 3—6-month-old piglets and detection of 19S antibody in blood specimens collected early in the season; (3) employment of the diagnostically valuable Jagar-01 strain (4) confirmation of the results of HI tests by the agar gel diffusion precipitation test (AGDP).

A STABLE COMBINATION OF THE ANTIGENIC PROPERTIES OF JAPANESE ENCEPHALITIS AND WEST NILE VIRUSES IN AN ATTENUATED ARBOVIRUS VARIANT

V. V. Pogodina, G. V. Koreshkova, L. L. Kiseleva

In the process of attenuation of the Egypt-101 strain of West Nile (WN) virus a peculiar clone 41/WN+JE+ was obtained possessing a stable combination of the antigenic properties of Japanese encephalitis (JE) and WN viruses. The two-way antigenic relationships can be demonstrated using hyperimmune sera in mouse neutralization tests and by the plaque method in tissue culture. The two-way antigenic relationships of the 41/WN+JE+ clone have been shown to be beyond common cross serological reactions of JE and WN viruses. The duplex characteristics persisted after serial passages of the 41/WN+JE+ clone and its recloning. The non-neutralizable fractions obtained in the presence of immune sera to JE and WN viruses also possess the duplex antigenic properties. Clones formed by the infectious RNA manifest duplex antigenic properties.

THE EFFECT OF SYNTHETIC AND OTHER INTERFERON INDUCERS ON FORMATION OF THE VACCINATION PROCESS TO TICK-BORNE ENCEPHALITIS

L. M. Vilner, E. V. Finogenova, I. M. Rodin

The stimulating effect on the formation of the vaccination process to tick-borne encephalitis virus was found with three polyribonucleotides (poly I : poly C, poly G : poly C, poly A : poly C), copolymers of vinylpirrolidone with crotonic acid or maleic anhydride, statolone and endotoxin.

The polyribonucleotides enhanced both the level of specific resistance of mice due to tissue culture vaccine against tick-borne encephalitis and the level of virus-neutralizing antibody to the causative agent of this infection. The other interferon inducers stimulated markedly an increase of specific resistance to virus infection only.

No complete parallelism was found between the effect of the preparations under study on the specific immunogenesis and the intensity of their interferon-inducing and antiviral activity.

REPORT FROM THE DEPARTMENT OF VIROLOGY,
UNIVERSITY OF HELSINKI
HELSINKI, FINLAND

Uukuniemi virus, a proposed member of the Bunyaviridae.

1. Uukuniemi virus surface structure

The surface structure of virus particles negatively stained with different stains reveals a clustering of the two glycoproteins G_1 and G_2 into distinct capsomere-like subunits, resembling hollow cylinders. These are seen in freeze-etched particles as knob-like structures. The subunits are arranged in a T=12 (P=3) icosahedral lattice. Thus there are 122 subunits and 720 structural units per virion. The icosahedral symmetry of the virus surface structure is especially interesting since the internal circular nucleoprotein strands seem to have a helical symmetry.

(Carl-Henrik von Bonsdorff and Ralf Filip Pettersson, J.Virol., 1975, in press).

2. An RNA polymerase associated with Uukuniemi virions.

Purified virus particles catalyzes an RNA dependent RNA transcription. Mn^{++}, but not Mg^{++} ions, are required for expression of the enzyme activity. The enzyme functions most efficiently and rapidly at temperatures around 37-40^{o}. The product consists of small RNA fragments which are complementary to template virion RNA. The finding of an RNA polymerase in Uukuniemi virions suggests that this virus, and probably the whole Bunyavirus group, belongs to the negative-strand viruses.

(Ralf Filip Pettersson and Marjut Ranki, 1975)

Bird studies in East Slovakia

During period of three years (1971-73) a total of 169 birds belonging to 35 species were obtained from the Zahorska lowland, the basin of Ipel river and East Slovakia.

Birds were captured mostly by Japanese mist nets; however, some birds were shot. Each bird was inspected for infestation with ticks. Collected ticks were sorted according to species, sex and stage. For virus isolation attempts suspensions of ticks or bird viscera (brain, liver, spleen, kidney) were prepared in 1 or 2 ml, respectively, of Earle's solution containing 10% inactivated calf serum. Blood was also taken from each bird for virus isolation and serological examination. The blood for virus isolation was collected into a 1% heparin solution and frozen on a dry ice in a Dewar's container until examined further in the laboratory.

For isolation, 2 to 3 day old suckling mice were inoculated intracerebrally (ic.) with 0.01 ml of test material. Bird sera were tested for the presence of VN antibodies against 100 CPD_{50} of Sindbis virus on 1 day old tube cultures of chick embryo cells and against 100 CPD_{50} of TBE, WN, Calovo and Tahyna viruses on a cloned line of porcine epithelial (PS) cells, in which they exert a cytopathic effect.

From the blood specimens of the 169 birds one strain of TBE virus was isolated from *Vanellus vanellus*, two strains of WN virus from *Tringa ochropus* and *Larus ridibundus* and two strains of Sindbis virus from *Vanellus vanellus* and *Sturnus vulgaris*. One strain of Sindbis virus was isolated from the brain and liver of *Acrocephalus scirpaceus* and two strains of WN virus from the brain of *Vanellus vanellus* and *Streptopelia turtur*. The titre of WN virus in the blood of *Tringa ochropus* was 10^1/0.01 ml of mouse ic. LD_{50}, while remaining titres reached only threshold levels. No virus was isolated from tick suspensions. The isolated strains of TBE, Sindbis and WN virus were identified by VN test in mice inoculated ic., and by haemagglutination-inhibition test.

The VN antibodies against Sindbis virus were found in the bird species *Perdix perdix, Vanellus vanellus, Streptopelia decaocto, Dendrocopos major, Sylvia atricapilla, Acrocephalus arundinaceus* and *Sturnus vulgaris* with titres of 8-32; against TBE virus in *Vanellus vanellus, Cuculus canorus, Turdus philomelos, Turdus pilaris, Sitta europaea, Fringilla coelebs* and *Corvus frugilegus* with titres of 4-32; against WN virus in *Perdix perdix, Vanellus vanellus, Tringa glareolus, Streptopelia turtur, Streptopelia decaocto, Locustella luscinioides, Acrocephalus schoenobaneus, Sitta europaea, Fringilla coelebs, Emberiza citrinella, Sturnus vulgaris* and *Corvus frugilegus* in titres 4-32: against Tahyna virus in *Vanellus vanellus, Columba oenas, Streptopelia turtur, Streptopelia decaocto, Cuculus canorus, Turdus philomelos, Merula merula, Sitta europaea, Fringilla coelebs, Sturnus vulgaris* and *Corvus frugilegus* in titres of 4-32; against calovo virus in *Vanellus vanellus, Tringa glareolus* and *Streptopelia decaocto* in titres of 4-128.

Infestation with ticks was found only in birds captured on the Zahorska lowland, which is connected obviously with their habitat in the riverside and forest vegetation. Ticks were mostly larvae and nymphs of *Ixodes ricinus*, less larvae and nymphs of *Haemaphysalis concinna*. The highest tick infestation noted was 56 nymphs and 41 larvae of *I. ricinus* and 2 nymphs and 2 larvae of *H. concinna* which occurred on the bird species *Merula merula*.

In some bird species, mostly of migrating birds (*Vanellus vanellus, Tringa ochropus, Tringa glareolus, Streptopelia turtur, Streptopelia decaocto, Cuculus canorus, Turdus philomelos, Sitta europaea, Fringilla coelebs, Corvus frugilegus*), VN antibodies against several arboviruses were found. Of importance also is the finding of VN antibodies against tick-borne and mosquito-borne viruses in the same bird species (*Vanellus vanellus, Cuculus canorus, Turdus philomelos, Sitta Europaea, Fringilla Coelebs, Corvus frugilegus*), indicating that these bird species come into contact with both tick and mosquito vectors. We suppose that birds as the main hosts of tick nymphs take part in the ecology of tick-borne as well as mosquito-borne viruses. Birds living in the shore vegetation of ponds and marshes are attacked mostly by mosquitoes, whereas bird species living in forests are attacked also by ticks of the *Ixodes* and *Haemaphysalis* genera.

(E. Ernek., O. Kozuch, J. Nosek, J. Teplan, C. Folk)

Sheep milk-borne epidemic of tick-borne encephalitis in Slovakia

During a 9-day period, April 24 to Mayl, 1974, 10 patients from Zavada village were hospitalized at the infectious department in Topolcany hospital (Western Slovakia). All patients, whether employed or not employed in the local cooperative sheep-breeding farm had consumed fresh sheep milk products (cheese) prior to onset of illness. Q fever was originally suspected, but was not confirmed in the laboratory. Brucellosis, leptospirosis, ornithosis, tularemia and typhoid fever were also excluded. Later on, TBE was confirmed serologically by haemagglutination-inhibition (HI) and virus-neutralization (VN) tests. The epidemiological status was also compatible.

Virus isolations were attempted from sheep milk and cheese 6 weeks after the epidemic but results were negative.

The sera of patients reacted with TBE antigen in HI tests in titres of 80-5128 (Table 1). It was demonstrated by mercaptoethanol treatment that the infections were primary, because HI titres of the first samples were lower after mercaptoethanol treatment than without the treatment.

Epidemiological investigation revealed that earlier in April 1974 a group of 13 inhabitants of Zavada village had developed similar symptoms of disease. All excluded the possibility of tick-bite prior the onset of disease. However, they recalled the consumption of sheep milk products, suggesting that cheese was the source of man's infection by the alimentary route.

Having proved the diagnosis of TBE in man and suspecting sheep as a source of infection, 548 samples of sheep sera and 182 samples of sheep milk were examined serologically in collaboration with the veterinary service of Topolcany district. 82 sera out of 548 (15%) gave positive reactions with TBE antigen in titres from 10 to 1280. None of milk specimens reacted in HI tests with TBE antigen.

In the period of June 5 to July 16, 1974, further investigation was carried out aimed at finding other positive cases in inhabitants of Zavada village and serologically examining the most highly exposed gorups of the population. The serological survey included not only all members of the local cooperative farm working at the sheepyard, but also forest workers moving around Zavada village and relatives of patients who might also consume sheep milk products, and schoolchildren from Zavada village.

Out of 102 human sera divided into 4 groups and examined by HI test, 18 sera (17.6%) reacted positively with TBE antigen in titres of 40-1280 (Table 2). Most of the persons with positive reactions had been bitten by ticks in the vicinity of Zavada village in previous years.

It has been experimentally proved that milk-giving domestic animals (goats and sheep) can be a source of infection for man. In sheep an asymptomatic viral infection occurs, both after injection of an infective suspension and following the bite of ticks carrying the virus. Presence of the virus can be demonstrated in both the blood and the milk.

In natural conditions, sheep are infested by ticks. Attention should be paid to the possibility of transmission of the virus to man in sheep's milk products (cheese, cream cheese). Since the virus is destroyed in milk only after heating for 30 minutes at 65° C, it can be assumed that the virus is not destroyed in products made from sheep's milk as the timperature used in preparation of cheeses are generally below that level. The cheese in the Zavada cooperative farm was manufactured from unheated sheep's milk using 5% lactochyn. It was already found that tick-borne encephalitis virus was active in buffer solutions at pH 2.75 on the acid side, and at pH 11.55 on the alkaline side. It was demonstrated that the virus was maintained in sour milk, so the possibility of survival of tick-borne encephalitis virus in dairy products prepared from sour milk was proved.

It was not possible to establish the exact day of infection of the people by tick-borne encephalitis virus; the cheese was prepared and eaten from 1st April up to the time of the epidemic (April 24).

Thus, for the first time the role of sheep in transmission of TBE in the natural focus in Slovak territory was proved, although the possibility of transmission of this infection by sheep milk or its products to man was anticipated.

After the epidemic, no further human cases were recorded. In 6 persons with an influenza-like disease (including headache) who recalled consumption of sheep milk products at the beginning of April 1974, results of serological examination were negative.

After evaluating the results of all examinations connected with the outbreak of tick-borne encephalitis (TBE) in the natural focus of this infection of Zavada village, Slovakia, it was concluded that the epidemic was caused by consumption of fresh sheep cheese and that infection of persons was by the alimentary route. This source of infection of man is being recorded, according to our knowledge, for the first time.

(M. Gresikova, M. Sekeyova, S. Stupalova, and S. Necas)

Table I. Haemagglutination-inhibition reaction with human sera from Závada tick-borne encephalitis microepidemic.

Name of patient	Disease observed on	Hospitalized	Data of sample collection	HI titre	HI titre after merkaptoethanol treatment
B. A.	8.4.74	1.5.74	13.5.74	1280	160
			29.5.74	640	640
			14.6.74	320	320
B. R.	13.4.74	29.4.74	30.4.74	640	10
			13.5.74	320	80
			27.5.74	320	80
K. L.	12.4.74	29.4.74	26.4.74	320	80
			13.5.74	160	160
			27.5.74	640	160
			14.6.74	320	160
Š. J.	12.4.74	29.4.74	30.4.74	1280	40
			13.5.74	1280	80
			27.5.74	1280	160
			14.6.74	320	160
Ž. J.	17.4.74	26.4.74	13.5.74	1280	80
			27.5.74	1280	80
			14.6.74	320	160
M. Š.	-	-	7.5.74	1280	160
M. M.	14.4.74	26.4.74	24.4.74	2560	160
			13.5.74	640	320
			27.5.74	640	320
M. O.	20.4.74	26.4.74	26.4.74	1280	640
			13.5.74	1280	320
			27.5.74	640	320
			14.6.74	320	320
M. J.	-	26.4.74	26.4.74	5128	320
			13.5.74	2560	1280
			27.5.74	5128	2560
			14.6.74	2560	1280
M. M.	not hospitalized		7.5.74	1280	80
			13.5.74	2560	160
			27.5.74	5128	160
			14.6.74	640	160
B. P.	8.4.74	27.4.74	29.4.74	640	80
			13.5.74	640	160
			27.5.74	640	160
B. J.	14.4.74	24.4.74	13.5.74	1280	320
			27.5.74	640	320
			14.6.74	640	320

Table II. Distribution by occupation of seropositive human sera

Occupation	No of examined	No of positive	Per cent of positive
Agriculture workers	69	15	21.70
Forest workers	7	2	28.60
Children	17	1	5.90
Others	9	0	0
Total	102	18	17.60

REPORT FROM THE ARBOVIRUS UNIT, INSTITUTE OF PARASITOLOGY,
CZECHOSLOVAK ACADEMY OF SCIENCES, PRAGUE, CZECHOSLOVAKIA

Study of Lednice Virus

Lednice virus, serologically identical with Yaba 1 virus, was isolated for the first time on the European continent in Czechoslovakia in 1963. Its isolation was confirmed in this country in 1972. Altogether, 7 strains were isolated in Czechoslovakia, all of them from the mosquito, Culex modestus.

Study of experimental infection of animals as well as serological investigation of selected human populations and domestic animals have thus far shown only a limited relation of the virus to mammals. Of suckling and adult mice, rats, guinea-pigs, hamsters (<100 g of weight), rabbits (<1800 g of weight) and 48-hour-old chickens inoculated by various routes, only the mice died after intracerebral and intranasal infection, and their sensitivity decreased with increasing age.

The serological studies of humans and domestic animals also brought mostly negative results (see Table 1). The persons tested were employees of fisheries and agricultural establishments, and school children working or living in close vicinity to ponds where the virus was isolated. The assayed domestic animals originated from the mentioned area as well. The only positive case, namely from the cattle, is left open as yet.

On the other hand, the serological investigation and experimental study of birds suggest that birds could be the hosts of virus. Results of serological examination of 298 samples of sera from wild birds representing 10 families and 29 species were positive in the case of those species bound to water environment, and above all from the family of Anatidae (see Table 2). Among domestic birds, it was again duck and goose which were positive, primarily those from the farms located at ponds (see Table 1). The finding of antibodies in water birds can be explained by the bionomy of the mosquito, Culex modestus, which is strictly bound to the littoral zone of ponds.

Experimental results with 1-day-old ducks and geese, which were assayed for 3 months post inoculation, showed that these animals produced antibodies and could serve as sensitive indicators; however, it is not yet quite clear whether they play a significant role in virus circulation, in view of their low and short viraemia (see Table 3).

Both serological investigations and experimental studies are being continued with the aim to elucidate the epidemiological and epizootological significance of Lednice virus.

(Doubravka Malkova, Vlasta Danielova, Jan Kolman)

Table 1

HI antibodies to Lednice virus in human beings and domestic animals

Source of sera	Number of tested sera	Number of positive sera
Man	180	0
Horse	105	0
Cow	603	1
Pig	139	0
Sheep	100	0
Goat	9	0
Hen	100	0
Goose	374	11
Duck	289	29

Table 2

HI antibodies to Lednice virus in wild birds

Family	Species	Number of tested sera	Number of positive sera
ANATIDAE	Anas platyrhynchos	69	22
	Anas querquedula	1	0
	Anas strepera	1	0
	Anser anser	64	12
	Aythya ferina	16	0
	Cygnus olor	3	1
	Netta rufina	14	0
SYLVIIDAE	Acrocephalus arundinaceus	8	0
	Acrocephalus palustris	7	0
	Acrocephalus scirpaceus	27	0
	Acrocephalus schoenobaenus	16	0
	Carduelis carduelis	12	0
	Carduelis chloris	1	0
	Fringilla coelebs	4	0
	Locustella fluviatilis	1	0
	Locustella luscinioides	1	0
	Phylloscopus collybita	2	0
	Serinus serinus	2	0
	Sylvia atricapilla	4	0
RALLIDAE	Fulica atra	9	0
TURDIDAE	Erithacus rubecula	1	0
	Turdus merula	1	0
PARIDAE	Parus caeruleus	5	0
	Parus major	1	0
ACCIPITRIDAE	Circus aeruginosus	1	0
PASSERIDAE	Passer montanus	2	0
MUSCICAPIDAE	Ficedula albicollis	1	0
EMBERIZIDAE	Emberiza schoeniclus	5	0
COLUMBIDAE	Streptopelia turtur	1	0

Table 3

Viraemia and neutralizing antibodies in young geese and ducks subcutaneously inoculated by Lednice virus

Animal	Viraemia										Antibodies in log LD_{50}				
	Days past infection										Days past infection				Days past reinfection
	1	2	3	4	5	6	7	8	11	14	11	21	28	6 weeks	6 weeks
Goose	-	+[1]	+	-	-	-	-	-	-	-	0.8-1.8[2]	1.0-2.5	2.5-3.0	2.8-3.0	2.8 - 3.1
Duck	-	+	+	-	-	-	-	-	-	-	not done	2.7	1.3-2.7	0.3-2.7	2.7

1) + = <1.0 log LD_{50}/0.01 ml

2) range of neutralization index in 6 assayed birds

REPORT FROM THE DEPARTMENT OF VIROLOGY, NEUROLOGY CLINIC
UNIVERSITY OF COLOGNE, FEDERAL REPUBLIC OF GERMANY

Eyach 38 - a new arbovirus related to Colorado tick fever virus in Germany.

In the spring of 1972, in a natural TBE focus in Swabia, approximately 60 km southwest of Stuttgart, 492 ticks of the species Ixodes ricinus (L.) were collected. From these an agent pathogenic solely for suckling mice could be isolated. The average survival time past IC inoculation for the animals is about 6 - 8 days. After 10 IC passages the virus titer had reached $10^{5.6}$ LD_{50}. By the IP and SC routes, however, only 50 % of the mice died, even when the virus was given in high doses.

The agent passes membrane filters with a pore width of 200 nm, but not 100 nm. It is resistent to treatment with ether and sodium-desoxycholate, but highly sensitive to chloroform.

Serological comparisons (Dr. J. Casals, YARU) with 49 tick-borne viruses, except of those group B, showed in the CF test a close relationship to Colorado tick fever (CTF) virus. This relationship could be verified in a neutralization test, but a one sided relationship could be established only. The Eyach 38 virus could be neutralized by CTF antibodies. On the other hand neutralizing antibodies of Eyach 38 barely showed any reaction with CTF virus.

(R. Ackermann)

REPORT FROM THE INSTITUTE OF VIROLOGY,
VETERINARY FACULTY, UTRECHT, THE NETHERLANDS

Viral specific RNA in Semliki forest virus infected cells.

Neuroblastoma cells infected with Semliki forest virus (SFV) start synthesizing viral RNA 3 hrs p.i. and newly synthesized virus is detected 4 hrs p.i. Virus specific RNA was labeled with ^{3}H-uridine in the presence of actinomycin D and the polysomal fraction of SFV-infected cells 5 hrs p.i. which sedimented at $>$ 400 S was isolated. The polysomal character of this fraction was established by the fact that it disappears when EDTA is added or when puromycin-incubation is used. The density of this fraction in CsCl after glutaraldehyde fixation is characteristic for polysomes. RNA was extracted from the polysomes and analyzed on polyacrylamide-agarose gels with E.coli ribosomal RNA and TMV RNA as internal markers. The ^{3}H-uridine labeled, viral-specific mRNA migrated as one band with MW 4 x 10^{6} daltons which is the same as the MW of viral RNA. RNA was isolated too from infected cells between 3 and 9 hrs p.i. and the only detectable viral-specific RNA had a MW of about 4 x 10^{6} daltons. We therefore conclude that for a successful infection cycle in neuroblastoma cells SFV uses only 42 S RNA, unlike the results reported for SFV-infected BHK and CEF cells, where smaller (mainly 26 S) mRNA molecules were found.

(B.A.M. van der Zeijst, M.H. Pranger and M.C. Horzinek).

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUTE OF HYGIENE AND TROPICAL MEDICINE
LISBON, PORTUGAL

This report summarize the recent studies done in cooperation between our laboratory and Dr. M. Teresa Garea from the Facultad de Farmacia, Universidad de Santiago de Compostela, Spain.

Serological survey for arboviruses in north of Spain

The information concerning the presence and activity of arboviruses in Spain is very rare. However, this country with the large extension of its territory, the geographical contiguity to Africa, the existence of different kinds of climates and ecologic regions, offers all the necessary conditions to the survival and spread of several viruses transmited by arthropods.

In order to have some information about the arboviruses probably active in the country a survey for antibodies to arboviruses was made in the human population of the north of Spain (Galicia, Leon and Asturias).

Sera were obtained from 701 persons living in the studied area. The hemagglutination-inhibition (HI) test was made using 16 antigens, from group A, B and also Tahyna, Calovo, Sandflyfever, Sicily and Naples strains.

The hemagglutination-inhibition (HI) test showed that 16.5% of the studied population had antibodies to group B viruses. Neutralization tests done with some selected sera and several group B viruses confirmed an earlier infection with one or more viruses of this antigenic group.

(Armindo R. Filipe)

Table 1

Hemagglutination-Inhibition test results with human sera from some northern Provinces of Spain

Province	Number of the studied sera	Total nº of positive sera	Positive %
Coruña	362	62	17.1
Orense	14	1	-
Pontevedra	179	35	19.5
Asturias	85	11	12.9
Leon	61	7	11.4
Total	701	116	16.5

Table 2

Results of hemagglutination-inhibition tests to group B arboviruses distributed by age group and analysed by the Muench's simple catalytic curve

Birth date (year)	Age group	Number of the studied sera	Total of positive sera	positive %	center of the class	Fitted curve	
						%	Age
1971- -	0-4.99	10	0	0.00	2.5	1.09	2.5
						2.18	5
1961-1970	5-14.99	37	8	21.62	10	4.30	10
						6.39	15
1951-1960	15-24.99	105	7	6.67	20	8.42	20
						10.42	25
1941-1950	25-34.99	110	13	11.82	30	12.37	30
						14.27	35
1931-1940	35-44.99	136	21	15.44	40	16.14	40
						17.96	45
1921-1930	45-54.99	123	24	19.51	50	19.75	50
						21.49	55
1911-1920	55-64.99	94	14	14.89	60	23.20	60
						24.87	65
1900-1910	65-74.99	52	10	19.23	70	26.51	70
						28.11	75
						29.67	80
1885-1900	75-90.99	12	4	33.33	83	30.59	83
						31.20	85
						32.70	90

REPORT FROM THE VIRUS LABORATORY
EGYPTIAN ORGANIZATION FOR BIOLOGICAL AND VACCINE PRODUCTION
AGOUZA, EGYPT

Dugbe virus in Egypt

Dugbe virus was isolated in Nigeria 1971 from ticks picked from cattle. Also it was isolated from cattle blood. Specific antibodies were found in associated animals. The isolation of the virus from aman was also reported in 1969-1970.

The virus was found to be active in Nigeria, Uganda, Sengal, Central Africa and South Africa. This gives the possibility of its presence in Egypt. To answer this question, an antibody survey was done in cattle sera.

Attempts to isolate the virus from human and cattle sera are also being made in parallel with the antibody survey.

Positive sera were found in camels, cows and buffaloes thus far, as shown in the following table:

Kind of sera	Number tested	NO. positive
Cow	100	4
Buffaloes	196	3
Camels	100	2
Rodents	50	0

Sandfly fever

Sandfly fever virus was isolated repeatedly from patients from the fever hospital suffering from fever which could not be clinically diagnosed. Antibody neutralization tests in VERO cell cultures are being conducted. The seasonal epidemiology and the geographical distribution of sandfly fever in Egypt are also under investigation.

Mumps virus was isolated and identified from patients suffering from encephalitis.

(Imam Zaghlovl Imam)

REPORT FROM THE VIROLOGY AND ENTOMOLOGY SECTIONS
NAMRU-5, ADDIS ABABA, ETHIOPIA

In the past two years, NAMRU-5 has been asked to investigate several reported outbreaks of yellow fever for the Ethiopian Ministry of Public Health. Although no epidemics were demonstrated some evidence of yellow fever endemicity was found in two separate areas. In 1974, two trips were made to the original study site of Serie and co-workers about 60 km by air south of Jimma. The area included the villages of Chebara and Manera (now called Menorra). Serum specimens were obtained from 48 residents, including the entire mission school. Some adults giving serum specimens specifically remembered being vaccinated by Dr. Serie. Yellow fever neutralization indexes of five known vaccinated individuals remained better than 3.3 logs after 11 years. The pattern was cross reactive, however, and stimulation by another group B virus such as Zika could have occurred. A more interesting finding was specific yellow fever antibody in one of the school children age 8. The HAI titer was low, only 1 to 10 against West Nile, Zika and yellow fever. In the complement fixation test, the titer was 1/256 against yellow fever, 1/8 against Ntaya and negative for Zika and West Nile. In neutralization testing, the serum neutralized 2.6 logs of yellow fever virus but less than 1.3 logs of either West Nile or Zika virus. No history of illness or travel could be obtained. Since the area is so isolated, there have been no vaccinations performed since Dr. Serie. Therefore, it would seem that a recent, but subclinical, infection of yellow fever had occurred. Thirteen baboon and colobus monkey sera taken in the area had CF antibody and neutralizing antibody to yellow fever. However, inability to estimate the adult monkeys' age made it impossible to rule out infection during the original epidemic.

Adult mosquito collections on human bait, during October 1974, resulted in a few Ae. africanus biting at and near ground level in the forest; Eretmapodites chrysogaster were also biting in the forest; Ae. simpsoni were abundant in and near the false banana and Colocasia plantations. None of three species yielded viral isolates when later processed.

The second area visited has been the Anger Guten Agriculture scheme, north of the Anger River, 60 km north of Nekempt. This area has been opened for settlement for about three years. It is high grass wooded savannah but there is dense forest along the Anger River and its tributary streams. Thirty-six serum samples were taken from settlers; thirteen showed HAI antibody to yellow fever virus. Of these, eight neutralized more than 100 LD50's of yellow fever virus but less than 100 LD50's of either West Nile or Zika virus. Six sera were yellow fever positive by complement fixation while two were anticomplementary.

No history of vaccination or travel could be obtained. All of the settlers, prior to their stay at Anger Guten, had lived at a village 25 kms to the south on the edge of the valley. From serology evidence, it would seem that natural infection with yellow fever had occurred in this area. Arrangements are being made to bleed surrounding villages. A search for possible yellow fever vectors in the Anger Guten area has shown the presence of the following mosquitoes:

Aedes africanus - collected only as larvae, in small numbers in the forest.

Aedes simpsoni - collected as larvae from banana leaf axils on a small plantation.

Eretmapodites chrysogaster group - collected in the forest as larvae (abundant) and as adults.

In addition to yellow fever studies, NAMRU-5 routinely processes ticks for virus isolation. Amongst the ticks collected from cattle at the Addis Ababa abbatoir were Amblyomma variegatum, from which 6 isolates were obtained. These agents closely resemble Jos virus. An isolate of a virus resembling Dugbe was isolated from a pool of A. variegatum collected from cattle at Begi in western Ethiopia, near the Sudan border. A third isolate from Rhipicephalus evertsi, from cattle at Anger Guten, could not be identified. All three agents have been sent to YARU for identification and confirmation.

O.L. WOOD

V. LEE

REPORT FROM THE VIROLOGICAL SECTION OF THE DUTCH MEDICAL RESEARCH CENTRE/NATIONAL PUBLIC HEALTH LABORATORY SERVICES OF KENYA AT NAIROBI.

Isolates from mosquitoes collected in Kenya and mentioned on the pages 187 and 188 of Information Exchange number 27 of September 1974 were identified as follows:

Lake Naivasha area:

Ae.cumminsi pool: Pongola virus - Bwamba group
C.rubinotus pool: Germiston virus - Bunyamwera group

Ngong Forest:

Ae.cumminsi pool: Pongola virus -Bwamba group

Msambweni - Mrima - Coastal area:

Two M.uniformis pools, one M. africana pool: Bunyamwera virus - Bunyamwera group.

An.funestus - Simba group, species still to be identified by YARU

The identifications were done at the Pasteur Institute of Dakar (Dr. Y. Robin).

In the period 6 August to 15 November 1974, for a total of eight weeks 31,191 mosquitoes were collected on human baits in the Lake Naivasha area. The species best represented in the collections, in order of the numbers collected were: C. rubinotus, Ae. dentatus group, C.zombaensis, C. nakuruensis, M. fuscopennata, An. squamosus, C. aurantapex, Ae.lineatopennis group, M. africana. Of other species less than one hundred specimens came to bite. So far virus strains were isolated from eight Ae.dentatus and four C. rubinotus pools.

A report received in March 1975 that monkeys were dying near Shimoni at the Kenya Coast led to a safari to the area. We gained the impression that hunger because of extensive deforestation and not disease was the reason for the mortality amongst the primates. Organs and serum were taken from four Colobus and seven blue monkeys. Between 3 and 23 April 1975 a total of 4,397 mosquitoes were collected. The species of which more than one hundred specimens were amongst the catches, in order of the numbers were: Ae. pembaensis, C. tritaeniorhynchus group and Ae. albithorax.

Nairobi, August 1975

D. METSELAAR

REPORT FROM INSTITUT PASTEUR AND O.R.S.T.O.M. BANGUI
CENTRAL AFRICAN REPUBLIC

The identification of the six isolates obtained in September/November 1974 from wild caught mosquitoes, Aedes africanus and Aedes opok, as YELLOW FEVER virus has been confirmed by the WHO Collaborating Center for Arboviruses in Dakar. The existence of a focus of sylvatic yellow fever near the field station of Bozo (110 km North of Bangui) is confirmed. Epidemiological studies (entomological, virological and serological) in this area are being actively continued in 1975.

One isolate obtained from Aedes g. tarsalis caught at Bozo in November 1973 has been identified in Dakar as PATA. Two isolates obtained from Aedes g. tarsalis and Culex g. perfuscus caught at Bozo in February 1974, and previously reported as PONGOLA (Arbo-Info n°28, p.106) have eventually been identified in Dakar as being WESSELSBRON.

One isolate obtained from ticks (Amblyomma variegatum) collected from cattle at Bangui in June 1973, has been identified in Dakar as being BHANJA virus.

The isolate obtained in 1973 from a bat (Roussettus aegyptiacus) (see Arbo-Info n°26, p.43) has been identified in Dakar as YOGUE virus.

The isolate obtained from man (laboratory contamination) in January 1974, and previously reported as KOUTANGO (Arbo-Info n°28, p.106) has been identified in Dakar as WESSELSBRON virus.

The following additions should be made to the 1975 issue of the International Catalogue of Arboviruses, regarding the viruses isolated in the Central African Republic:

BHANJA. Natural Host Range and Known Geographical Distribution: add Amblyomma variegatum (1/557 pools) Central African Rep. (Ref. Institut Pasteur Bangui, Ann.Rep.1974).

BOTEKE. Remarks: read Dak An B 1245 instead of Dak Ar B 1245.

CONGO. Natural Host Range and Known Geographical Distribution: add Hyaloma nitidum (1/3 pools) Central African Republic. (Ref. Institut Pasteur Bangui Ann.Rep. 1974).

INGWAVUMA. Natural Host Range: add Numida meleagris, Hyphanturgus brachypterus, and Ploceidae sp. Central African Rep. (Ref. Institut Pasteur Bangui, Ann.Rep. 1973).

KAMESE. Natural Host Range: add Culex pruina and Culex tigripes, Central African Republic (Ref. Institut Pasteur Bangui Ann.Rep. 1972 and 1973).

MIDDELBURG. see Ref.24. about Natural Host Range and Known Geographical Distribution: the mosquitoes from which MIDDELBURG virus has been isolated had been received from Brazzaville, Congo (Mansonia africana and M. uniformis) and from Yaounde, Cameroon (Aedes simulans). Actually, no isolate of MIDDELBURG virus has yet been obtained in Central African Republic.

MOSSURIL. Natural Host Range: add Culex weschei (1969) and Culex telesilla (1970), Central African Republic (Institut Pasteur Bangui, Ann.Rep. 1969 and 1970).

Natural Host Range: Coliuspasser macrourus: read 1 isolate (not 4).

M'POKO. Natural Host Range: add Culex decens (1973) and Turdus lybonianus (1972), Central African Republic. (Institut Pasteur Bangui, Ann.Rep. 1972 and 1973).

PATA. Natural Host Range: add Aedes g. tarsalis, Central African Rep. (Institut Pasteur Bangui, Ann.Rep. 1973).

SANDJIMBA. Time of Collection, Month: fill in January.

SINDBIS. Natural Host Range: add 1 isolate from man, Central African Rep. (Institut Pasteur Bangui, Ann.Rep. 1974).

TATAGUINE. Natural Host Range: add 2 isolates from man, Central African Rep. (Institut Pasteur Bangui, Ann.Rep. 1972 and 1973).

USUTU. Natural Host Range, see Ref.5: read Bycanistes sharpii instead of Andropadus virens.

Natural Host Range: add Culex pruina (1970), C. duttoni (1972) and C. nebulosus (1973), Central African Republic. (Institut Pasteur Bangui, Ann.Rep. 1970, 1972, 1973).

WESSELSBRON. Natural Host Range: add 1 isolate from man (laboratory contamination) and Aedes g. tarsalis and Culex g. perfuscus. Central African Rep. (Institut Pasteur Bangui, Ann.Rep. 1974).

YELLOW FEVER. Natural Host Range: add Aedes opok and Aedes africanus, Central African Republic. (Institut Pasteur Bangui, Ann.Rep. 1974).

YOGUE. Natural Host Range and Known Geographical Distribution: add Roussettus aegyptiacus, Central African Republic (Institut Pasteur Bangui, Ann.Rep. 1974).

ZINGA. Natural Host Range: add 2 isolates from man, Central African Republic. (Institut Pasteur Bangui, Ann.Rep. 1973 and 1974).

(P. Sureau, Institut Pasteur Bangui and M. Germain, O.R.S.T.O.M. Bangui)

REPORT FROM THE FEDERAL MINISTRY OF AGRICULTURE AND NATURAL RESOURCES, DIVISION OF VETERINARY RESEARCH, VOM, NIGERIA

Bluetongue

An agar gel diffusion test using sonicated mouse brain antigen has detected evidence of past infections in 49.7% of cattle, 28.9% of sheep and 29.4% of goats. Details are shown in the accompanying table. An alternative (but weaker) antigen prepared in Vero Cells gave a 70% correlation with brain antigen results. These figures establish for the first time the high level of involvement of Nigerian livestock species in the maintenance of Bluetonge virus. No information is available on the total number of types present or their relative distribution.

Serological conversions have been seen in a local herd of dairy cattle under both wet and dry season conditions. Virus activity in this group of animals is being monitored.

An ongoing study in conjunction with Animal Virus Research Institute, Pirbright, U.K. is aimed at identifying Culicoides species occurring in Vom and also determining the year round frequency of potential vectors.

We would be pleased to hear from any laboratory in a position to supply us with different strains of Bluetongue virus.

African Horse Sickness

Between September and October, 1974 an outbreak occurred in Kano City, Northern Nigeria. Some 20 imported horses died with clinical signs and post-mortem lesions of acute AHS. Type 9 virus was recovered. Spleen emulsion from one case contained $10^{7.5}$ suckling mouse LD_{50}/g and when inoculated into a susceptible locally bred horse produced no more than a mild pyrexia of 48 hours duration; Type 9 virus was reisolated.

In collaboration with the AVRI, Pirbright, periodic serum samples from 40 sentinel horses are being examined for evidence of virus activity. So far there is clear evidence that one subclinical infection with type 9 occurred in the Jos area between October 1974 and April, 1975.

(W.P. Taylor)

Precipitating Antibody to Bluetongue antigen in Nigerian cattle, sheep and goats.

Source of material	Ox Sample No.	% +tve	Sheep Sample No.	% +tve	Goat Sample No.	% +tve
East Central State	-	-	40	27.5	60	26.7
Jos	100	40.0	72	51.4	100	44.0
Kaduna	109	52.5	40	32.5	52	50.0
Kano	124	51.6	100	26.0	100	20.0
Maiduguri	82	31.7	15	73.3	18	27.8
Mambilla	47	34.0	80	6.3	-	-
Sokoto	96	79.2	114	34.2	59	16.9
Vom	77	68.8	53	11.3	-	-
Zaria	171	40.3	-	-	20	40.0
Totals	806	49.7	514	28.9	429	29.4

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUT PASTEUR AND ORSTOM, DAKAR, SENEGAL

Surveillance for arbovirus activity in Senegal was concentrated, during the first half of 1975, in two areas : Bandia and Kedougou.

1. Virological Studies

1.1 Human blood samples

43 blood specimens collected from febrile patients, mostly children in Dakar, Bandia and Kedougou were processed for virus isolation. One strain of Bunyamwera virus was isolated from the blood of a mosquito catcher working in the field near Kedougou. This is the first time that Bunyamwera virus is isolated in Senegal.

1.2 Wild vertebrate samples

64 specimens from monkeys captured in Kedougou were investigated for the presence of virus without success.

1.3 Arthropods

14026 mosquitoes were processed in 527 pools. 16 virus strains were isolated. From mosquitoes of the species Aedes dalzieli 14 isolates were recovered, the majority of which have now been identified : Wesselsbron virus (2), Zinga virus (3), Simbu virus (1), Bunyamwera virus (3), Middelburg virus (1), Eretmapodites 147 virus (3); one isolate is still in investigation.

From Aedes furcifer taylori mosquitoes, 2 strains were isolated : one has been identified to Bunyamwera virus and further work is on to type the second isolate.

Most of these viruses had never been isolated in Senegal before.

2. Serological Studies

2.1 Human sera

2.1.1. Senegal - A multipurpose serological survey was initiated in 1972 by a WHO-VDT team (WHO/IR 051 project) . Serum samples from various zones of Senegal have been tested for arbovirus antibobies. Results concerning fleuve Senegal region and Casamance region were reported previously.

250 sera were collected in Diourbel region, Linguere district. In this area, arbovirus endemicity is at a low level. Serological positive reactions increase with age, reaching 50% in the adult group.

2.1.2. Togo - 750 sera collected in Togo from school children 5 to 15 years old were examinated for HI, CF and neutralizing (plaque reduction test) antibodies. 50% show HI antibodies for group A and group B arboviruses. Some sera show CF antibodies titer compatible with a recent yellow fever virus infection. 17% only had yellow fever virus neutralizing antibodies.

On 188 sera collected in the same area from children under 5 years, only 7% neutralize yellow fever virus.

2.1.3. Madagascar - Isolation of 2 strains of Dakar bat virus and results of studies on fruit bat sera were reported previously (A.B.V.I.E 1974, n°27 p 183). Following these finding 190 human sera were examinated for HI and neutralizing antibodies. Hi antibodies were found for Sindbis (17%) and chikungunya (1%) antigens.

89% of the sera have HI antibodies to yellow fever antigen and 63% of these positive sera neutralize yellow fever virus.

As most of the sera showed a broad group B response (Zika, Ntaya, Wesselsbron) the specificity of the yellow fever antibodies has to be supported by other studies.

2.2. Wild vertebrate sera

200 sera collected from monkeys in Kedougou, Senegal have been examinated for HI and CF antibodies. They are to be tested for neutralizing antibodies.

(Ch. Jan and Y. Robin, Institut Pasteur and J. Coz and M. Cornet, ORSTOM Dakar, Senegal).

REPORT FROM ARBOVIRUS RESEARCH UNIT,

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG

Rift Valley Fever

Following the same pattern observed in recent years extensive outbreaks of RVF occurred among sheep and cattle in the Highveld region of South Africa during the summer and autumn of 1974/75. Collections of mosquitoes, Culicoides and Simulium were made on several farms where outbreaks occurred to obtain information on the epizootic vector. Isolations of RVF virus were only obtained from mosquitoes. There were 12 isolations from Culex theileri, 3 from Aedes juppi, 2 from Aedes lineatopennis and 1 from Anopheles squamosus. While these isolations tend to implicate theileri, thus agreeing with earlier observations, the actual situation is still uncertain. While theileri is a proven vector it apparently only possesses moderate vector capability and on some farms infection in theileri could not be demonstrated despite tests on large numbers of this species. It does, however, occur in vast numbers so it is possible that infection rates are being lowered by recently emerged individuals. Its feeding habits, geographical distribution and lengthy persistence of the adult female population, all tend to also implicate theileri and on existing evidence we have to assume that it is the main epizootic vector, probably assisted at times by several Aedes species.

Despite some work in recent years we still have to admit complete ignorance on methods of viral maintenance in nature. It seems possible in theory that adult theileri or some Anopheles species could carry virus through the Highveld winter but this is unlikely to