

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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IMPORTANT NOTICE: This exchange is issued for the sole purpose of timely exchange of information among investigators of arthropodborne viruses. It contains reports, summaries, observations, and comments submitted voluntarily by qualified agencies and investigators. The appearance of any information, data, opinions, or views in this exchange does not constitute formal publication. Any reference to or quotation of any part of this exchange must be authorized directly by the person or agency which submitted the text.

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COMMENTS FROM THE EDITOR

The American Committee on Arthropod-borne Viruses is pleased by your interest in the Information Exchange, and with the high quality of the reports that have been submitted. The mailing list has reached 360, and it continues to grow.

With such a large number of names on the list, it becomes necessary to constantly cull out those individuals who may no longer have a need for the Information Exchange. If your interests have changed and you find that you no longer derive benefit from the Information Exchange, I'd appreciate a brief letter to that effect so that your name can be removed from the list. Conversely, new collaborators are welcome, provided that they are actively engaged in some aspect of arbovirus work and sincerely intend to contribute a report of their ongoing studies occasionally.

Many thanks for following the standard format for your reports; most of the reports could be reproduced without retyping. In the interest of economy, please type single space, with double space between paragraphs.

Reports for the fall issue (No. 35) will be due September 1, 1978. Please mark your calendars. The address, as usual:

Roy W. Chamberlain, Editor
Arthropod-borne Virus Information Exchange
Virology Division
Center for Disease Control
Atlanta, Georgia 30333 U.S.A.



DR. CARL M. EKLUND

December 5, 1903 - November 25, 1977

Dr. Carl M. Eklund, former head of the Arbovirus and Chronic Viral Disease section of the Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases, died November 25 in Hamilton, Montana.

Dr. Eklund had retired in 1967 from the U.S. Public Health Service after 22 years of service but continued his association with the Rocky Mountain Laboratory as a consultant.

In addition, after his retirement, Dr. Eklund became a staff member at the University of Minnesota.

Except for his first year with the Public Health Service, Dr. Eklund's career was spent at the Rocky Mountain Laboratory. In 1968, he was awarded the Department of Health, Education and Welfare Distinguished Service Medal for his pioneer investigations of slow viral infections.

During his early years at Rocky Mountain Laboratory, Dr. Eklund was instrumental in developing much of our knowledge on the ecology of mosquito- and tick-transmitted virus diseases, especially the encephalitides and Colorado tick fever.

Subsequently, with Dr. William Hadlow, he developed methods to study the pathogenesis of slow viral diseases of animals as experimental models for possible counterpart diseases of man, particularly those involving the central nervous system,

Born on December 5, 1903, in Moorhead, Minnesota, Dr. Eklund received his B.A. and M.D. degrees from the University of Minnesota. Before beginning his Public Health Service career in 1945, Dr. Eklund worked for 8 years as an epidemiologist for the Minnesota Department of Health.

6th FEMS SYMPOSIUM
"ARBOVIRUSES IN THE MEDITERRANEAN COUNTRIES"
(in collaboration with the World Health Organization,
organized by the Association of Yugoslav Societies
for Microbiology)

Supetar (Island of Brac), Yugoslavia
8-10 September 1978

Official language: ENGLISH

PRELIMINARY PROGRAMME

8 September
Morning:

- Opening
- Papers by invited speakers:
 - Dr. P. Bres (WHO). Development of the WHO Programme in the Field of Arboviruses and Allied Viruses.
 - Prof. N. Oker-Blom (Medical Faculty, Helsinki). Arboviruses in Europe (Mediterranean countries not included).
 - Dr. C.H. Calisher (CDC, Fort Collins). Arboviruses in the Americas.

Afternoon:

- Reports of the delegates of the Mediterranean countries
- Papers on various aspects of Arboviruses, including virological, epidemiological, clinical, entomological, phytocenological, pathological, and other aspects, presented in the form of poster sessions with subsequent discussions.

9 September
Morning:

- Papers by invited speakers:
 - Dr. Akira Oya (National Institute of Health, Tokyo). Studies on Epidemics and Epizootics of Some Arboviruses in the East Asia.
 - Dr. D.K. Lvov (Institute of Poliomyelitis and Viral Encephalitis, Moscow). Arboviruses in the USSR (to be confirmed).
 - Dr. H. Hoogstraal (NAMRU 3, Cairo). Established and Emerging Concepts regarding Tick Associated Viruses, and Unanswered Questions.
 - Prof. C.E. Gordon Smith (London School of Hygiene and Tropical Medicine, London). Priorities in Arbovirus Research.

Afternoon:

- Reports of the delegates of the Mediterranean countries.
- Papers on various aspects of Arboviruses (as above).

10 September

Morning:

- Papers by invited speakers:
Dr. J.S. Porterfield (Sir William Dunn School of Pathology, Oxford). Arboviruses in perspective.
- Round Table Discussion and Conclusions.

Afternoon:

- Visit to natural foci of TBE and Bhanja viruses on the island of Brac

Social programme

8 September:

- Sightseeing round the island of Brac (for accompanying members)
- Cocktail

9 September:

- Sightseeing in the town of Split (accompanying members)

10 September:

- Picnic in the folklore style following visit to natural foci

- - - -

Further information for delegates and invited speakers will be given directly by the organizer.

Summaries up to 150 words are requested to be sent to the organizer by 15 June 1978. The size of the posters: 1 x 1 m. In extenso texts for subsequent publication are to be handed over on arrival.

The registration fee is US \$ 30.-

Supetar can be reached either by plane or train to Split and from Split by a ferry-boat (5 times daily) to Supetar. The ferry-boat is within walking distance from the railway station and the bus terminal.

Accommodation:

	<u>Single rooms</u>	<u>Double rooms</u>
	US \$	US \$
<u>Hotel "Kaktus":</u>		
Full pension	19.00	15.50 per person
Half pension	18.00	14.50 " "
Garni	16.00	12.50 " "
Without any meal	14.50	21.50 per 2 persons
<u>Hotel settlement "Palma":</u>		
Full pension	13.00	11.00 per person
Half pension	12.00	10.00 " "
Garni	9.50	8.00 " "
Without any meal	8.00	14.00 per 2 persons

Reservations: Directly to: "Jadran", Hotel Agency
58400 Supetar, Yugoslavia
not later than 20 August 1978, indicating "ARBO".

For accommodation in private rooms (about US \$ 3.50 single)
write to "Tourist Office"
58400 Supetar, Yugoslavia

The participation of experts from non-Mediterranean countries is also welcome.

All further necessary information can be obtained from the organizer.

Organizer: Professor J. Vesenjāk-Hirjan
Association of Yugoslav Societies for Microbiology
Rockefellerova 4, P.O. Box 770
41000 ZAGREB, Yugoslavia

THE RHABDOVIRIDAE FAMILY

This table on the Rhabdoviridae family was updated by Dr. Frederick A. Murphy and Mrs. Alyne K. Harrison, CDC Virology Division, and Dr. Robert E. Shope, YARU, for the International Committee on the Taxonomy of Viruses, which will meet in late August, 1978 at the Hague, in association with the 4th International Congress of Virology. It is reproduced here for your information.

Rhabdoviruses of Vertebrates and Invertebrates^(a)

Genus ^(b) or Serologic Group ^(c)	Virus Name (Species) (Serotype)	Source of ^(d) Isolates	Geographic ^(e) Distribution	Disease in Nature (Experimental Animals)	Ultrastructural Characteristics			
					Length of Virus Particles in nm	Shape of Virus Particles ^(f)	In vivo Site of Budding	Inclusion ^(h) Bodies
A. Vesiculovirus Genus	1. Vesicular stomatitis—Indiana (prototype species of family)	man, cattle, horses, sandflies, mosquitoes	New World	vesicular disease (encephalitis, hepatitis, vesicular disease)	180	B	PM	±
	2. Cocal (VSV-Argentina)	horses, rodents, mites, mosquitoes	Trinidad, Argentina, Brazil	vesicular disease (encephalitis, hepatitis, vesicular disease)	180	B	PM	±
	3. Alagoas (VSV-Brazil)	horses	Brazil	vesicular disease (encephalitis, hepatitis, vesicular disease)	180	B	PM	NA
	4. Vesicular stomatitis—New Jersey	man, cattle, horses	New World	vesicular disease (encephalitis, hepatitis, vesicular disease)	180	B	PM	±
	5. Chandipura	man hedgehogs, sandflies	India, Nigeria	(encephalitis, hepatitis, vesicular disease)	180	B	PM	±
	6. Piry	opossum	Brazil	(encephalitis, hepatitis, vesicular disease)	180	B	PM	±
	7. Isfahan	(man) (gerbil) sandflies	Iran (USSR)	(encephalitis, hepatitis, vesicular disease)	180	B	PM	NA
B. Lyssavirus Genus	1. Rabies	man, bats, carnivores, etc.	Worldwide with known exceptions	rabies	180	B	ER/PM	++
	2. Lagos bat	bats	Nigeria, Central African Empire	(encephalitis)	180	B	ER/PM	++
	3. Mokola	man, shrews	Nigeria, Cameroon	rabies-like disease (encephalitis)	180	B	ER/PM	++
	4. Duvenhage	man (bat)	South Africa	rabies-like disease (encephalitis)	180	B	ER/PM	++
C.	5. Kotonkan	(cattle), culicoides	Nigeria	ephemeral fever-like disease (encephalitis)	180	C	PM	+
D.	6. Obodhiang	mosquitoes	Sudan	(encephalitis)	180	C	PM	+
E.	Bovine ephemeral fever	cattle, culicoides	Japan, Australia, Africa, Iran	ephemeral fever (encephalitis)	185	C/B	PM	+
F.	Marco	lizards	Brazil	(encephalitis)	180	C	PM/ER	—
G.	Kern Canyon	bats	California	(encephalitis)	132	SB	PM	±
H.	Klamath	rodents	California, Alaska	(encephalitis)	167	SB	ER	++
I.	Barur	rodents, bats, ticks, mosquitoes	India, Kenya	(encephalitis)	149	SB	PM	—
J.	1. Flanders	birds, mosquitoes	North America	(encephalitis)	218	LB	PM	+
	2. Hart Park	birds, mosquitoes	North America	(encephalitis)	218	LB	PM	+
K.	Mount Elgon bat	bats	Kenya	(encephalitis)	230	LB	PM	++
L.	Joinjakaka	mosquitoes	New Guinea	(encephalitis)	197	LB	PM	++
M.	Navarro	birds	Colombia	(encephalitis)	220	LB	ER/PM	++
N.	1. Kwatta	mosquitoes	Surinam	(encephalitis)	230	LB	ER/PM	+
	2. BeAn 157575	birds	Brazil	NA	NA		NA	NA
O.	1. Mossuril	birds, mosquitoes	Africa	(encephalitis)	246	LB	PM	NA
	2. Kamese	mosquitoes	Africa	NA	NA		NA	NA
P.	1. Sawgrass	ticks	Florida	(encephalitis)	220	LB	PM	±
	2. New Minto	ticks	Alaska	(encephalitis)	220	LB	NA	NA
Q.	Chaco	lizards	Brazil	(encephalitis)	202	LB	ER	—

R.	S-1643	mosquitoes	Sarawak	NA	NA	NA	NA	NA
S.	Grey Lodge	mosquitoes	California	NA	NA	NA	NA	NA
T.	Oita - bat	bats	Japan	NA	NA	NA	NA	NA
U.	Kimberley	mosquitoes	Australia	NA	NA	NA	NA	NA
V.	Parry Creek	mosquitoes	Australia	NA	NA	NA	NA	NA
W.	Kununurra	mosquitoes	Australia	NA	130	SB	NA	NA
X.	Yata	mosquitoes	Central African Empire	NA	185	B	NA	NA
Y.	Keuraliba	gerbils, Mastomys	Senegal	NA	195	B	NA	NA
Z.	Egtvad (viral hemorrhagic septicemia)	trout	Europe	hemorrhagic septicemia	180	B	PM	NA
AA.	Infectious hematopoietic necrosis virus	salmon and trout	United States	hematopoietic necrosis	188	B	PM	NA
BB.	Spring viremia of carp	cyprinids	Europe	systemic hemorrhagic disease	180	B	PM	NA
CC.	Red disease of Pike (Pike fry rhabdovirus)	esocids	Europe	hemorrhagic septicemia hydrocephalus	160	B	PM	NA
DD.	Rhabdovirus of eels	anguillids	Japan	NA	173	B	NA	NA
EE.	Rhabdovirus of grass carp	grass carp	Europe	systemic hemorrhagic disease	180	B	PM	NA
FF.	Sigma	Drosophila		(CO ₂ Sensitivity)	160	SB	PM	NA
GG.	Rhabdovirus of entamoeba	<i>E. histolytica</i> , <i>E. invadens</i>						
HH.	Rhabdovirus of blue crab	crab	Canada		115	SB	ER	NA

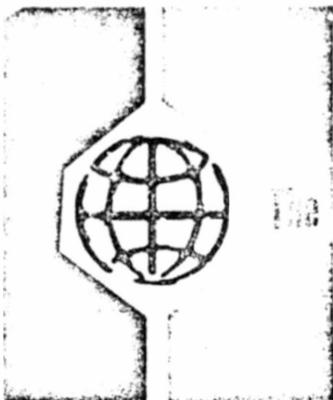
- a. This table has been compiled from data obtained from many sources, and has been assembled in the Viral Pathology Branch, Center for Disease Control, Atlanta, Georgia, and the Yale Arbovirus Research Unit, New Haven, Connecticut. Listing of previously undescribed viruses in this table is not intended to constitute priority description. Several viruses isolated only from arthropods are listed with the viruses from vertebrates; this is done arbitrarily because such viruses are infectious, usually lethal, to newborn mice when inoculated intracerebrally.
- b. Horizontal lines have been used to distinguish subgroupings. With the vesiculoviruses and lyssaviruses this distinction is at the genus level. In other cases subgroupings are informal and primarily based upon clusterings of morphologic and morphogenetic characteristics. The dotted horizontal line separates lyssaviruses from two viruses which have distinct morphologic characteristics, but a reproducible serologic relationship to lyssaviruses. The doubled horizontal lines separate the fish rhabdoviruses (which do not infect mice) and the invertebrate viruses.
- c. Serogroups are designated by capital letters at the left margin. Members of serogroups are designated with numbers.
- d. Parentheses indicate serologic evidence only.
- e. Tabulated from Annual Report of Yale Arbovirus Research Unit, New Haven.
- f. Abbreviations are: B, typical rhabdovirus particle morphology - bullet shaped with parallel sides and length of approximately 180nm. SB, typical morphology with parallel sides, but characteristically shorter than prototype VSV. LB, typical morphology with parallel sides, but characteristically longer than prototype VSV. C, conically shaped virus particles and conically wound nucleocapsids. NA, data not available.
- g. Abbreviations are: PM, plasma membrane. ER, endoplasmic reticulum (and other intracytoplasmic membranes).
- h. Symbols are: ++, inclusion bodies (massed nucleocapsid material) are prominent - large and widespread in infected cells. +, inclusion bodies are present in most instances, but not too prominent. ±, inclusion bodies present under some circumstances, but not characteristic of the infection. -, inclusion bodies not found.

Report of Subcommittee on Interrelationships Among
Catalogued Arboviruses - November 1977

Robert E. Shope, Chairman; C.H. Calisher; J. Casals;
J. Dalrymple; F.A. Murphy; M. Wiebe

The subcommittee met once during 1977. Eight viruses, mostly non-group B tick-borne agents, were titered in suckling mice and several cell cultures in an attempt to develop high enough infectivity titers to study morphology and viral protein composition. By these means it was hoped that the agents could be classified to family in the Universal System. A satisfactory growth system has not yet been developed for these viruses.

A decision was made to publish SIRACA reports on arbovirus groups. The initial manuscript will concern group A.



THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1977 ANNUAL REPORT ON THE CATALOGUE OF ARTHROPOD-BORNE AND
SELECTED VERTEBRATE VIRUSES OF THE WORLD*

by

THE SUBCOMMITTEE ON ARTHROPOD-BORNE VIRUS
INFORMATION EXCHANGE

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I. Objectives:

The objectives of the Catalogue are to register data concerning occurrence and characteristics of newly recognized arthropod-borne viruses and other viruses of vertebrates of demonstrated or potential zoonotic importance, and to disseminate this information at quarterly intervals to participating scientists in all parts of the world; to collect, reproduce, collate, and distribute current information regarding registered viruses from published materials, laboratory reports, and personal communications; and to prepare and distribute an annual summary of data extracted from catalogued virus registrations.

II. Materials and Methods:

Viruses are registered and information supplied on a voluntary basis, usually by scientists responsible for their isolation and identification. New registration cards, information concerning registered viruses, and pertinent abstracts of published literature are distributed at quarterly intervals to participating laboratories. Abstracts of published articles dealing with catalogued viruses are reproduced by special arrangements with the editors of Biological Abstracts, Abstracts on Hygiene, and the Tropical Diseases Bulletin.

*The Catalogue is supported by the Center for Disease Control, Atlanta, Georgia.

NOTE: This report is not a publication and should not be used as a reference source in published bibliographies.

Distribution of Catalogue Material: At the start of 1977, 160 mailings of Catalogue material were being made. During the year, 4 participants were dropped and 6 new participants were added to the mailing list. At the end of the year, 162 mailings of Catalogue material were being made, including 59 within the U.S.A. and 103 to foreign addresses. Distribution by continent was: Africa 15, Asia 20, Australasia 7, Europe 33, North America 71, and South America 16.

Abstracts and Current Information: A total of 610 abstracts or references were coded by subject matter and distributed to participants during 1977. Of this total, 484 were obtained from Biological Abstracts, 122 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 4 from current journals, personal communications, or other sources. A total of 10,832 references or units of information have been issued since the start of the program.

Registration of New Viruses: Seven viruses were accepted for registration during 1977. As of December 1976, there were 381 registered viruses in the Catalogue. With the 7 registrations during 1977, the total number of registered viruses stood at 388 as of December 1977. The viruses registered during 1977 are listed below.

<u>Virus Name</u>	<u>Recommended Abbreviation</u>	<u>Country</u>	<u>Source</u>	<u>Antigenic Group</u>
Kununurra	KNA	Australia	Mosquitoes	
Rio Grande	RG	U.S.A.	Rodent	PHL
Rocio	ROC	Brazil	Human	B
Saumarez Reef	SRE	Australia	Argasid ticks	B
Ebola	EBO	Zaire	Human	MBG
Kaikalur	KAI	India	Mosquitoes	SIM
Urucuri	URU	Brazil	Rodent	PHL

The above viruses all were isolated between 1966 and 1976. One was isolated in 1966 (URU), one in 1971 (KAI), one in 1973 (KNA), two in 1974 (RG, SRE), one in 1975 (ROC), and one in 1976 (EBO).

Six of these recently registered viruses were evaluated as possible arboviruses by the SEAS* Subcommittee. Ebola was evaluated as probably not arbovirus.

The two viruses from man (EBO,ROC) were associated with severe human disease.

Antigenic Grouping: The Marburg (MBG) serogroup has been formed as a result of the recent registration of Ebola virus and the presentation of data demonstrating its antigenic relationship to Marburg virus.

* Subcommittee on Evaluation of Arthropod-Borne Status. T.H.G. Aitken (Chairman), R.W. Chamberlain, D.B. Francly, J.L. Hardy, D.M. McLean, and J.P. Woodall.

At the request of the Catalogue editor, the SIRACA* Subcommittee has reviewed the status of the Hart Park and Flanders virus registrations. Both are listed as ungrouped viruses in the Catalogue, although there is ample evidence to indicate that they are closely related antigenically. The SIRACA Subcommittee has concluded that "Hart Park and Flanders are separable by conventional CF and N tests and are at least as different as snowshoe hare and LaCrosse viruses. Therefore, they constitute the Hart Park group with Hart Park being the type virus with 2 subtypes, Hart Park and Flanders."

Information from R.W. Emmons and R.E. Shope, in the form of a personal communication, indicated that Bocas virus is probably a coronavirus, closely related or possibly identical to mouse hepatitis virus (MHV). In light of these findings, the Bocas virus registration will no longer be listed as a member of the California group. Furthermore, it is anticipated that the Bocas virus registration will be eventually withdrawn. In order for this event to occur, the original registrant will voluntarily request withdrawal of the registration from the Catalogue.

Taxonomic Status of Registered Viruses: During 1977, electron microscopic studies have provided possible taxonomic information about a number of registered viruses. Yata, Sawgrass, Marco, Keuraliba, and Chaco were observed to be rhabdoviruses. Timbo virus also should be classified as a rhabdovirus by virtue of its antigenic relationship to Chaco virus. Bobaya, Trinita, and Zinga were found to be spherical bunyavirus-like particles, measuring 90-100 nm in diameter. Pending a complete review by SIRACA of the taxonomic status of all registered viruses, the above viruses will be provisionally listed in the appropriate taxon.

Supplement to the Second Published Edition of the Catalogue: A manuscript, representing a supplement to the Catalogue, was prepared and submitted on 1 October 1977 to the editor of the American Journal of Tropical Medicine and Hygiene. It will be published in March 1978; and it will contain information on 30 registered viruses, all of which were accepted for registration subsequent to the publication of the second edition of the Catalogue. Two of the registered viruses (EBO,ROC) appearing in the supplement were isolated from man and were associated with severe human disease. An arthropod isolate (ORU) and a virus isolated from a mule (VSA) have also been implicated in human disease, while SSH virus from a hare has been noted to cause subclinical infections in man. Most of these registered viruses were rated by SEAS as possible arboviruses. One registered virus (SSH) was rated as a proven arbovirus; another (ORU) was upgraded to probable arbovirus; a third (EBO) as probably not arbovirus.

* Subcommittee on Interrelationships Among Catalogued Arboviruses.
R.E. Shope (Chairman), C.H. Calisher, J. Casals, J.M. Dalrymple,
F.A. Murphy, and M. Wiebe.

Synopsis of Information in Catalogue: This synopsis has been compiled primarily to provide a short review of the viruses included in the Catalogue. The following tabulations are designed to draw together groups of viruses showing certain characteristics in common, listing viruses according to their demonstrated serological relationships and known taxonomic status and, where appropriate, by principal arthropod vector. Isolations from arthropod and animal hosts, continental distribution, involvement in human disease, and arbovirus status are indicated. Other tables summarize numbers of viruses assigned to presently recognized antigenic groups; chronology and areas of isolations of registered viruses; continental distribution by groups; numbers of viruses recovered from naturally infected arthropods and vertebrates; association with human disease; and evaluation of arthropod-borne status of members in various serogroups.

Table 1. Alphabetical listing of registered viruses. Table 1 presents an alphabetical listing of the 388 viruses registered in the Catalogue as of December 1977. Also, a recommended abbreviation is given for each virus, which has been formulated according to the guidelines established by the American Committee on Arthropod-Borne Viruses (11). All too often, abbreviations are employed in publications which are of the author's choosing and which do not conform to the recommended abbreviation. Their use is confusing, contrary to established guidelines, and erodes a portion of the effort of the Arbovirus Information Exchange program. All arbovirologists who plan to employ abbreviations in print should make every effort to use the recommended abbreviations.

Antigenic groups to which viruses have been assigned also are shown in this table. If no antigenic group is given, the virus is ungrouped and indicates that it has not been demonstrated to be serologically related to any other known arbovirus.

Table 2. Antigenic groups of registered viruses. The originally described antigenic groups of arboviruses were designated by letters A, B, and C, but in present practice, the first discovered virus of a newly recognized serogroup lends its name to the antigenic cluster. Before a virus can be assigned to an antigenic group, it must be shown to be serologically related to, but clearly distinguishable from a previously isolated virus.

The listing in Table 2 shows that 51 antigenic groups have been established for viruses registered in the Catalogue. There are several instances in which only a single virus is shown in an antigenic group. That is so because one or more antigenic relatives of that virus have not been registered.

It is also noted that the Bunyamwera Supergroup consists of 11 distinct antigenic groups as well as a collection of viruses (Bunyamwera Supergroup Unassigned) which antigenically fall into the Supergroup but which lack a close antigenic relationship to any other virus in the Supergroup. The Bunyamwera Supergroup was formulated to reflect low level but reproducible intergroup relationships usually by complement-fixation and/or hemagglutination-inhibition reactions. It has been demonstrated that all

Supergroup viruses examined possess similar, if not identical, morphologic and morphogenetic characteristics (8,9) as well as other biochemical properties. In accordance with the present international taxonomic scheme, Supergroup viruses have been designated to form the Bunyavirus genus within the family Bunyaviridae by the International Committee on Taxonomy of Viruses (ICTV) (9,12).

Table 3. Initial isolations by decade and country of origin. Table 3 lists the initial isolation of specific registered viruses by the decade of discovery and according to the continent or subcontinent and country in which each was first discovered. Because of the large number of virus names involved, abbreviations are employed. These abbreviations and the associated complete names of the respective viruses may be found in Table 1.

Table 4. Initial isolation of viruses by continent, country, and chronological period. Similar data were utilized in Tables 3 and 4, though they were subjected to slightly different analyses and were presented in a different format. Periods or locations which show high numbers of virus isolations undoubtedly reflect the net effect of a number of contributing factors such as the change in emphasis of field programs from a search for viruses causing specific diseases to a systematic search for viruses, new or known, in their natural ecological niche in a given geographical area, refinements in isolation and identification techniques, improved communication between arbovirus laboratories, and more rapid dissemination of new information, as well as the presence in a given area of an arbovirus laboratory with highly active and effective field programs.

Tables 5 through 27 list registered viruses by serogroup with information regarding isolations from arthropod vectors and vertebrates, and geographic (by continent) distribution based on virus isolation. Data also are presented regarding production of disease in man in nature or by laboratory infection, evaluation of arbovirus status, and proved or provisional taxonomic status. Where possible, sets of viruses were grouped additionally according to their actual or suspected principal arthropod vector and by taxonomic status.

The data presented in these tables clearly illustrate the salient features characteristic of each set or subset of viruses. Thus, the reader is urged to carefully examine the tables for information that may be of specific interest or that will provide an overview of the general characteristics of a given group of viruses.

Table 5. Group A arboviruses. This serogroup presently consists of 20 registered members. All but three are rated as arboviruses or probable arboviruses. Group A arboviruses are clearly mosquito associated although a few have been isolated from other arthropods. One-half of the registered members have been shown to produce human disease.

Taxonomically, group A arboviruses constitute the genus Alphavirus in the family Togaviridae (12).

Tables 6, 7, and 8. Group B viruses. The group B tick-borne viruses (Table 7) contain four registered viruses, Absettarov, Hanzalova, Hypr, and Kumlinge, which are very closely related or indistinguishable by conventional serological techniques, though they are said to be clearly differentiated on the basis of clinical, epidemiological, and ecological markers from RSE and other members of the same complex.

Saumarez Reef (SRE), a recently registered virus, has been added to the list of group B arboviruses which are tick-associated (Table 7). This virus was isolated from ticks in Australia, and it has been rated as a possible arbovirus.

Rocio (ROC) virus, isolated in Brazil, joins the list of group B viruses with no proven arthropod vector (Table 8). This agent has been implicated in extensive outbreaks of encephalitis in human beings and has also been recovered from birds.

With the addition of these two viruses, there are now 60 registered group B viruses.

Tables 9, 10, 11, 12, 13, and 14. Bunyamwera Supergroup. The 11 antigenic sets of viruses plus the unassigned viruses, all in the Supergroup, have been formally accorded taxonomic status as the Bunyavirus genus in the family Bunyaviridae (9,12).

Table 9. Bunyamwera group. SIRACA considers Calovo and Batai viruses to be serologically indistinguishable. Although Bunyamwera group viruses are widely distributed and are found on every continent except for Australia, only three registered viruses, Batai, Guaroa, and Wyeomyia have been isolated on more than one continent, though not more than two continents.

Prior to the registration of Anhemi, Birao, and Northway, SIRACA examined the serological relationships of Bunyamwera group viruses in 1971 and concluded that there were five complexes within the group. Two of the complexes consisted of a single virus each. The subsets consisted of the following:

1. Bunyamwera (Bunyamwera, Germiston, and Ilesha).
2. Cache Valley (Cache Valley, Batai-Calovo, Lokern, and Main Drain).
3. Wyeomyia (Wyeomyia and Sororoca).
4. Kairi.
5. Guaroa.

Table 10. Bwamba and Group C viruses. Utilizing available data on the antigenic relationships of group C viruses, SIRACA examined these relationships in 1968 and 1970 and determined that group C arboviruses consist of three complexes, each containing two or more viruses.

1. Caraparu (Caraparu, Apeu, and Madrid).
2. Marituba (Marituba and Nepuyo).
3. Oriboca (oriboca and Itaqui).

Ossa virus was judged to be a subtype of Caraparu; Murutucu, and Restan subtypes of Marituba virus; and Gumbo Limbo a subtype of Nepuyo virus.

Table 11. California and Capim group viruses. As the result of findings publicized this past year, Bocas virus has been removed as a member of the California serogroup. Prior to the registration of snowshoe hare and Inkoo viruses, SIRACA examined the antigenic relationships of nine other California group viruses in 1969 and 1970. It was suggested that there were three complexes within the group, each complex consisting of a single virus or type.

1. California encephalitis (Subtypes: Jamestown Canyon, Keystone, LaCrosse, San Angelo, and Tahyna).
2. Trivittatus.
3. Melao.

All the subtypes of California encephalitis were considered to be distinguishable from each other, while Jerry Slough was judged to be indistinguishable, or nearly so, from Jamestown Canyon virus.

Lumbo virus is now regarded as a strain of Tahyna virus, and its registration was withdrawn from the Catalogue some time ago.

All the California group viruses are associated with mosquito vectors and four members have been recovered from naturally infected rodents. Nine of the present eleven members have been rated as arboviruses or probable arboviruses. In the past year, SEAS upgraded the status of Trivittatus virus to a proven arbovirus.

The Capim group viruses have been isolated only in North and South America, and they have not been implicated in human disease.

Table 12. Guama, Koongal, Olifantsvlei, and Patois group viruses. Guama group viruses have been found only in the western hemisphere. Two of the members, Catu and Guama, have been implicated in human disease.

Both Koongal group viruses were isolated in Australia and very little is known about them.

Olifantsvlei virus has an African relative, Bobia virus, which is unregistered at present.

Viruses of the Patois group have been isolated only in North America. Three of the four members also have been recovered from rodents and sentinel animals.

Table 13. Simbu group viruses. Kaikalur virus, isolated from mosquitoes in India, has been added to the list of Simbu group members. Kaikalur is a very close antigenic relative of Shuni virus.

Table 14. Tete group and unassigned (SBU) viruses. Bahig virus of the Tete group has been recently upgraded by SEAS to a probable arbovirus. All four members were recovered initially from birds, and subsequently Bahig and Matruh were isolated from ixodid ticks.

Of the unassigned viruses, Kaeng Khoi recently has been recovered from cimicid bugs associated with bats.

Table 15. Phlebotomus fever group viruses. Thus far, intergroup antigenic relationships have not been demonstrated between the PHL group and members of the Bunyamwera Supergroup. However, representative members of the PHL group have been examined by electron-microscopy and they have been found to be identical in morphology and morphogenesis to Bunyamwera virus. They have been designated as bunyavirus-like though their precise taxonomic status is officially unresolved at present.

The addition of Urucuri and Rio Grande viruses increases the number of PHL group viruses to 22. Urucuri was isolated from rodents in Brazil while Rio Grande was recovered from rodents in Texas, USA. Because of insufficient information, both are rated as possible arboviruses.

Table 16. Tick-borne groups other than group B viruses. Members of these five minor antigenic groups also have been characterized as being bunyavirus-like.

Congo virus is an extremely important causative agent of human disease, especially in the USSR.

Table 17. Tick-borne groups other than group B viruses. While the viruses in Table 17 also are tick-borne agents, they differ taxonomically from those in Table 16 in that they have been classified as orbiviruses in the family Reoviridae. The orbiviruses are relatively resistant to lipid solvents, are inactivated at acid pH, and possess a double stranded RNA genome.

Table 18. Tick-borne groups other than group B viruses. Members of these five minor antigenic groups have not been classified taxonomically. Within the DGK serogroup, numerous isolations of Kao Shaun have been reported from Argas ticks collected in Australia. Previously found in Asia, the distribution of this virus is now extended to Australasia. Soldado virus of the Hughes group has been upgraded by SEAS to proven arbovirus.

Tables 19, 20, 21, and 22. Minor antigenic groups of viruses. All the viruses listed in these tables are members of minor antigenic groups. Viruses of the serogroups listed in Table 19 are characterized as bunyavirus-like and thus are in the same situation as viruses of the PHL group as well as members of the tick-borne serogroups shown in Table 16.

Table 20. Minor antigenic groups of viruses. Members of these minor antigenic groups have been characterized and taxonomically classified as orbiviruses.

Table 21. Minor antigenic groups of viruses. Members of the serogroups listed in this table possess a "bullet-shaped" morphology and are classified as rhabdoviruses.

Two additional serogroups have been added to this table. Hart Park and Flanders viruses, of the Hart Park group, were previously listed as ungrouped viruses in spite of adequate evidence which indicated that they were closely related. After official review, SIRACA has amended the listings so that they more accurately reflect currently available information.

Members of the Timbo group, consisting of Chaco and Timbo viruses, are now taxonomically classified as rhabdoviruses following examination of Chaco virus by electron microscopy.

Neutralizing antibody to Isfahan virus of the VSV serogroup has been reported in human beings residing in the USSR. The suspected geographic distribution now includes the USSR.

Table 22. Minor antigenic groups of viruses. These antigenic groups consist of members which are taxonomically unclassified. This table now includes the newly formed Marburg serogroup consisting of Ebola and Marburg viruses. Both Marburg virus and the recently registered Ebola virus cause human disease in nature and have been associated with laboratory infections.

Table 23. Tacaribe group viruses. Tacaribe group viruses are serologically related to lymphocytic choriomeningitis virus, and they are classified taxonomically in the Arenavirus genus. They are primarily rodent viruses, and there is little or no evidence that they are associated with an arthropod vector in nature. SEAS has judged all members to be non-arthropod-borne.

Three members of this group have been implicated in severe, often fatal human disease. These include Junin (Argentine hemorrhagic fever), Machupo (Bolivian hemorrhagic fever) and Lassa (Lassa disease).

Table 24. Ungrouped mosquito-associated viruses. The viruses in this table are serologically ungrouped, though they have been clustered together according to taxonomic designation including those which have been provisionally designated as bunyavirus-like. Of this latter category, Rift Valley fever virus is best known. It causes serious and extensive disease in domestic animals such as sheep and cattle and may cause disease in veterinary personnel, field and laboratory workers, as well as herdsmen who handle infected animals.

On the basis of electron microscopic observations, Trinita and Zinga viruses have been added to the list of agents which are provisionally classified as bunyavirus-like. Witwatersrand virus has been upgraded to a proven arbovirus by SEAS.

Bocas virus has been removed from the CAL group and it is listed here pending final disposition of its status as a registered virus.

The picornavirus, Nodamura, was isolated from wild-caught mosquitoes in Japan, and it has been demonstrated to produce disease in moths and honey bees. While it has been shown that it replicates in mosquitoes and is experimentally transmitted by mosquitoes, consideration should be given to the possibility that it represents a true or an evolved form of insect pathogen.

None of the rhabdoviruses listed here have been implicated in disease of man or animals. Two of the viruses represent recent additions to this list. Kununurra virus was registered within the past year. It was isolated from mosquitoes collected in Australia. Yata virus has been registered for some time but recently was found to be a rhabdovirus by electron microscopy.

Table 25. Ungrouped mosquito-associated viruses. These serologically ungrouped viruses are clustered together because of their association with a mosquito vector and because they are taxonomically unclassified.

Table 26. Ungrouped tick-, culicoides-, or phlebotomus-associated viruses. The serologically ungrouped viruses listed in Table 26 appear to be primarily associated with non-mosquito vectors. The majority of them are taxonomically unclassified. Sawgrass is now added to the list of rhabdoviruses shown in this table. Recent electron microscopic investigations have shown that it possesses a "bullet-shaped" morphology.

Table 27. Ungrouped viruses, no arthropod vector known. As a result of recent electron microscopic observations, Bobaya virus has been classified provisionally as bunyavirus-like.

Both Keuraliba and Marco viruses have been observed to be "bullet-shaped," and they now are listed with the other ungrouped rhabdoviruses in this table. Lagos bat virus is antigenically related to rabies virus.

Table 28 gives continental distribution of viruses in different antigenic groups on the basis of virus isolation. Overall, the registered viruses are very limited in their distribution. Approximately 84% have been isolated on a single continent only, while 15 or 3.9% have been found on 3 or more continents. The largest number of viruses have been isolated in Africa.

Table 29 shows the number of viruses, according to antigenic group, which have been isolated from various classes of arthropods. Fifty percent have been recovered from mosquitoes, about 23% from ticks, and 15% from all other classes.

Table 30 presents a similar type of analysis in terms of virus isolations from various classes of vertebrates. Man and rodents have provided the largest number of virus isolations.

Table 31 lists the viruses in each antigenic group which cause disease in man. Approximately 26% of all registered viruses have been associated with human infection, either in nature or by laboratory infection, or both.

An analysis of the SEAS ratings for all registered viruses is presented in Table 32, and it shows that 207 (53%) registrations are rated as possible arboviruses. Clearly, additional data are required if we are to have a more precise rating of the arthropod-borne status of these viruses.

Of the 99 registered viruses listed in Table 31 as causing infection in nature, or in the laboratory, or both, 76 (77%) are rated as probable or proven arboviruses, while only 7 (7%) are rated as probably not or not arboviruses.

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Table 1

ALPHABETICAL LISTING OF 388 VIRUSES REGISTERED AS OF 31 DEC. 1977
WITH RECOMMENDED ABBREVIATIONS AND ANTIGENIC GROUPING

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
ABSETTAROV	ABS	B	AVALON	AVA	SAK
ABU HAMMAD	AH	DGK	BAGAZA	BAG	B
ACADO	ACD	COR	BAHIG	BAH	TETE
ACARA	ACA	CAP	BAKAU	BAK	BAK
AFRICAN HORSESICKNESS	AHS	AHS	BAKU	BAKU	KEM
AFRICAN SWINE FEVER	ASF		BANDIA	BDA	QYB
AGUACATE	AGU	PHL	BANGORAN	BGN	
AINO	AINO	SIM	BANGUI	BGI	
AKABANE	AKA	SIM	BANZI	BAN	B.
ALFUJ	ALF	B	BARUR	BAR	
ALMPIWAR	ALM		BATAI	BAT	BUN
AMAPARI	AMA	TCR	BATKEN	BKN	
ANHANGA	ANH	PHL	BATU CAVE	BC	B
ANHEMBI	AMB	BUN	BAULINE	BAU	KEM
ANOPHELES A	ANA	ANA	BEBARU	BEB	A
ANOPHELES B	ANB	ANB	BELMONT	BEL	
APEU	APEU	C	BERTIOGA	BER	GMA
APOI	APOI	B	BHANJA	BHA	
ARIDE	ARI		BIMBO	BBO	
ARKONAM	ARK		BIMITI	BIM	GMA
ARUAC	ARU		BIRAO	BIR	BUN
ARUMOWOT	AMT	PHL	BLUETONGUE	BLU	BLU
AURA	AURA	A	BOBAYA	BOB	

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
BOCAS	BOC		CHANDIPURA	CHP	VSV
BORACEA	BOR	ANB	CHANGUINOLA	CGL	CGL
BOTAMBI	BOT	SBU	CHARLEVILLE	CHV	
BOTEKE	BTK	BTK	CHENUA	CNU	KEM
BOUBOUI	BOU	B	CHIKUNGUNYA	CHIK	A
BOVINE EPHEMERAL FEVER	BEF		CHILIBRE	CHI	PHL
BUJARU	BUJ	PHL	CHOBAR GORGE	CG	
BUNYAMWERA	BUN	BUN	CLO MOR	CM	SAK
BURG EL ARAB	BEA	MTY	COCAL	COC	VSV
BUSHBUSH	BSB	CAP	COLORADO TICK FEVER	CTF	CTF
BUSSUQUARA	BSQ	B	CONGO	CON	CON
BUTTONWILLOW	BUT	SIM	CORRIPARTA	COR	COR
BWAMBA	BWA	BWA	COTIA	COT	
CACAO	CAC	PHL	COWBONE RIDGE	CR	B
CACHE VALLEY	CV	BUN	D'AGUILAR	DAG	PAL
CAIMITO	CAI	PHL	DAKAR BAT	DB	B
CALIFORNIA ENC.	CE	CAL	DENGUE-1	DEN-1	B
CALOVO	CVO	BUN	DENGUE-2	DEN-2	B
CANDIRU	CDU	PHL	DENGUE-3	DEN-3	B
CAPE WRATH	CW	KEM	DENGUE-4	DEN-4	B
CAPIM	CAP	CAP	DERA GHAZI KHAN	DGK	DGK
CARAPARU	CAR	C	DHORI	DHO	
CAREY ISLAND	CI	B	DUGBE	DUG	NSD
CATU	CATU	GMA	EBOLA	EBO	MBG
CHACO	CHO	TIM	EAST. EQUINE ENC.	EEE	A
CHAGRES	CHG	PHL	EDGE HILL	EH	B

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
ENTEBBE BAT	ENT	B	HUGHES	HUG	HUG
EP. HEM. DIS.	EHD	EHD	HYPR	HYPR	B
EUBENANGEE	EUB	EUB	IBARAKI	IBA	
EVERGLADES	EVE	A	ICOARACI	ICO	PHL
EYACH	EYA	CTF	IERI	IERI	
FLANDERS	FLA	HP	ILESHA	ILE	BUN
FRIJOLES	FRI	PHL	ILHEUS	ILH	B
GAMBOA	GAM	SBU	INGWAVUMA	ING	SIM
GANJAM	GAN	NSD	INKOO	INK	CAL
GARBA	GAR	MTY	IPPY	IPPY	
GERMISTON	GER	BUN	IRITUIA	IRI	CGL
GETAH	GET	A	ISFAHAN	ISF	VSV
GOMOKA	GOM		ISRAEL TURKEY MEN.	IT	B
GORDIL	GOR	PHL	ISSYK-KUL	IK	
GOSSAS	GOS		ITAPORANGA	ITP	PHL
GRAND ARBAUD	GA	UUK	ITAQUI	ITQ	C
GREAT ISLAND	GI	KEM	JAMESTOWN CANYON	JC	CAL
GUAJARA	GJA	CAP	JAPANAUT	JAP	
GUAMA	GMA	GMA	JAPANESE ENC.	JE	B
GUARATUBA	GTB	SBU	JERRY SLOUGH	JS	CAL
GUAROA	GRO	BUN	JOHNSTON ATOLL	JA	QRF
GUMBO LIMBO	GL	C	JOINJAKAKA	JOI	
HANZALOVA	HAN	B	JUAN DIAZ	JD	CAP
HART PARK	HP	HP	JUGRA	JUG	B
HAZARA	HAZ	CON	JUNIN	JUN	TCR
HUACHO	HUA	KEM	JURONA	JUR	SBU

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
JUTIAPA	JUT	B	KUMLINGE	KUM	B
KADAM	KAD	B	KUNJIN	KUN	B
KAENG KHOI	KK	SBU	KUNUNURRA	KNA	
KAIKALUR	KAI		KWATTA	KWA	KWA
KAIRI	KRI	BUN	KYASANUR FOR. DIS.	KFD	B
KAISODI	KSO	KSO	LA CROSSE	LAC	CAL
KAMESE	KAM	MOS	LAGOS BAT	LB	*
KAMHAVANPETTAI	KMP		LA JOYA	LJ	
KANNAMANGALAM	KAN		LANDJIA	LJA	
KAO SHUAN	KS	DGK	LANGAT	LGT	B
KARIMABAD	KAR	PHL	LANJAN	LJN	KSO
KARSHI	KSI	B	LASSA	LAS	TCR
KASBA	KAS	PAL	LATINO	LAT	TCR
KEMEROVO	KEM	KEM	LEBOMBO	LEB	
KEKŃ CANYON	KC		LE DANTEC	LD	
KETAPANG	KET	BAK	LIPOVNIK	LIP	KEM
KETERAH	KTR		LOKERN	LOK	BUN
KEURALIBA	KEU		LONE STAR	LS	
KEYSTONE	KEY	CAL	LOUPING ILL	LI	B
KHASAN	KHA		LUKUNI	LUK	ANA
KLAMATH	KLA		MACHUPO	MAC	TCR
KOKOBERA	KOK	B	MADRID	MAD	C
KOLONGO	KOL		MAGUARI	MAG	BUN
KOONGOL	KOO	KOO	MAHOGANY HAMMOCK	MH	GMA
KOUTANGO	KOU	B	MAIN DRAIN	MD	BUN
KOWANYAMA	KOW		MALAKAL	MAL	MAL

*Rabies related

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
MANAWA	MWA	UUK	MUCAMBO	MUC	A
MANZANILLA	MAN	SIM	MURRAY VALLEY ENC.	MVE	B
MAPPUTTA	MAP	MAP	MURUTUCU	MUR	C
MAPRIK	MPK	MAP	NAIROBI SHEEP DIS.	NSD	NSD
MARBURG	MBG	MBG	NARIVA	NAR	
MARCO	MCO		NAVARRO	NAV	
MARITUBA	MTB	C	NDUMU	NDU	A
MATARIYA	MTY	MTY	NEGISHI	NEG	B
MATRUH	MTR	TETE	NEPUYO	NEP	C
MATUCARE	MAT		NGAINGAN	NGA	
MAYARO	MAY	A	NIQUE	NIQ	PHL
MELAO	MEL	CAL	NKOLBISSON	NKO	
MERMET	MER	SIM	NODAMURA	NOD	
MIDDELBURG	MID	A	NOLA	NOLA	SIM
MINATITLAN	MNT	SBU	NORTHWAY	NOR	BUN
MINNAL	MIN		NTAYA	NTA	B
MIRIM	MIR	SBU	NUGGET	NUG	KEM
MITCHELL RIVER	MR	WAR	NYAMANINI	NYM	
MODOC	MOD	B	NYANDO	NDO	NDO
MOJU	MOJU	GMA	OKHOTSKIY	OKH	KEM
MONO LAKE	ML	KEM	OKOLA	OKO	
MONT. MYOTIS LEUK.	MML	B	OLIFANTSVLEI	OLI	
MORICHE	MOR	CAP	OMSK HEM. FEVER	OMSK	B
MOSSURIL	MOS	MOS	O'NYONG NYONG	ONN	A
MOUNT ELGON BAT	MEB		ORIBOCA	ORI	C
M'POKO	MPO	TUR	OROPOUCHE	ORO	SIM

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
ORUNGO	ORU		RAZDAN	RAZ	
OSSA	OSSA	C	RESTAN	RES	C
OUANGO	OUA		RIFT VALLEY FEVER	RVF	
OUBANGUI	OUB		RIO BRAVO	RB	B
PACORA	PCA		RIO GRANDE	RG	PHL
PACUI	PAC	PHL	ROCIO	ROC	B
PAHAYOKEE	PAH	PAT	ROSS RIVER	RR	A
PALYAM	PAL	PAL	ROYAL FARM	RF	B
PARAMUSHIR	PMR		RUSS. SPR. SUM. ENC.	RSSE	B
PARANA	PAR	TCR	SABO	SABO	SIM
PATA	PATA	EUB	SABOYA	SAB	B
PATHUM THANI	PTH	DGK	SAGIYAMA	SAG	A
PATOIS	PAT	PAT	SAINTE-FLORES	SAF	
PHNOM-PENH BAT	PPB	B	SAKHALIN	SAK	SAK
PICHINDE	PIC	TCR	SALANGA	SGA	
PIRY	PIRY	VSV	SALEHABAD	SAL	PHL
PIXUNA	PIX	A	SAN ANGELO	SA	CAL
PONGOLA	PGA	BWA	SANDFLY F. (NAPLES)	SFN	PHL
PONTEVES	PTV	UUK	SANDFLY F. (SICILIAN)	SFS	PHL
POWASSAN	POW	B	SANDJIMBA	SJA	SIM
PRETORIA	PRE	DGK	SANGO	SAN	SIM
PUCHONG	PUC	MAL	SATHUPERI	SAT	SIM
PUNTA SALINAS	PS	HUG	SAUMAREZ REEF	SRE	B
PUNTA TORO	PT	PHL	SAWGRASS	SAW	
QALYUB	QYB	QYB	SEBOKELE	SEB	
QUARANFIL	QRF	QRF	SELETAR	SEL	KEM

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
SEMBALAM	SEM		TANJONG RABOK	TR	
SEMLIKI FOREST	SF	A	TATAGUINE	TAT	
SEPIK	SEP	B	TEMBE	TME	
SHAMONDA	SHA	SIM	TEMBUSU	TMU	B
SHARK RIVER	SR	PAT	TENSAW	TEN	BUN
SHUNI	SHU	SIM	TETE	TETE	TETE
SILVERWATER	SIL	KSO	TETTANG	TET	
SIMBU	SIM	SIM	THIMIRI	THI	SIM
SIMIAN HEM. FEV.	SHF		THOGOTO	THO	THO
SINDBIS	SIN	A	THOTTAPALAYAM	TPM	
SIXGUN CITY	SC	KEM	TIMBO	TIM	TIM
SNOWSHOE HARE	SSH	CAL	TLACOTALPAN	TLA	BUN
SOKOLUK	SOK	B	TOURE	TOU	
SOLDADO	SOL	HUG	TRIBEC	TRB	KEM
SOROROCA	SOR	BUN	TRINITI	TNT	
SPONDWENI	SPO	B	TRIVITTATUS	TVT	CAL
ST. LOUIS ENC.	SLE	B	TRUBANAMAN	TRU	MAP
STRATFORD	STR	B	TSURUSE	TSU	
SUNDAY CANYON	SCA		TURLOCK	TUR	TUR
TACAIUMA	TCM	ANA	TYULENIY	TYU	B
TACARIBE	TCR	TCR	UGANDA S	UGS	B
TAGGERT	TAG	SAK	UMATILLA	UMA	
TAHYNA	TAH	CAL	UMBRE	UMB	TUR
TAMDY	TDY		UNA	UNA	A
TAMIAMI	TAM	TCR	UPOLU	UPO	
TANGA	TAN		URUCURI	URU	PHL

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
USUTU	USU	B	ZIKA	ZIKA	B
UUKUNIEMI	UUK	UUK	ZINGA	ZGA	
VELLORE	VEL	PAL	ZINGILAMO	ZGO	BTK
VEN. EQUINE ENC.	VEE	A	ZIRQA	ZIR	HUG
VENKATAPURAM	VKT				
VS-ALAGOAS	VSA	VSV			
VS-INDIANA	VSI	VSV			
VS-NEW JERSEY	VSNJ	VSV			
WAD MEDANI	WM				
WALLAL	WAL				
WANOWRIE	WAN				
WARREGO	WAR	WAR			
WESSELSBRON	WSL	B			
WEST. EQUINE ENC.	WEE	A			
WEST NILE	WN	B			
WHATAROA	WHA	A			
WITWATERSRAND	WIT				
WONGAL	WON	KOO			
WONGORR	WGR				
WYEMYIA	WYO	BUN			
YAQUINA HEAD	YH	KEM			
YATA	YATA				
YELLOW FEVER	YF	B			
YOGUE	YOG				
ZALIV TERPENIYA	ZT	UUK			
ZEGLA	ZEG	PAT			

Table 2. Antigenic Groups of 388 Viruses Registered in Catalogue.

Antigenic Group	Abbreviation	No. Registered	
		Viruses	in Group
A	A	20	5.2
African horsesickness	AHS	1	0.3
Anopheles A	ANA	3	0.8
Anopheles B	ANB	2	0.5
B	B	60	15.5
Bakau	BAK	2	0.5
Bluetongue	BLU	1	0.3
Boteke	BTK	2	0.5
Bunyamwera Supergroup		88	22.7
Bunyamwera	BUN	18	
Bwamba	BWA	2	
C	C	11	
California	CAL	11	
Capim	CAP	6	
Guama	GMA	6	
Koongol	KOO	2	
Olifantsvlei	OLI	1	
Patois	PAT	4	
Simbu	SIM	16	
Tete	TETE	4	
Unassigned	SBU	7	
Changuinola	CGL	2	0.5
Colorado tick fever	CTF	2	0.5
Congo	CON	2	0.5
Corriparta	COR	2	0.5
Dera Ghazi Khan	DGK	5	1.3
Epizootic hemorrhagic disease	EHD	1	0.3
Eubenangee	EUB	2	0.5
Hart Park	HP	2	0.5
Hughes	HUG	4	1.0
Kaisodi	KSO	3	0.8
Kemerovo	KEM	16	4.1
Kwatta	KWA	1	0.3
Malakal	MAL	2	0.5
Mapputta	MAP	3	0.8
Marburg	MBG	2	0.5
Matariya	MTY	3	0.8
Mossuril	MOS	2	0.5
Nairobi sheep disease	NSD	3	0.8
Nyando	NDO	1	0.3
Palyam	PAL	4	1.0
Phlebotomus fever	PHL	22	5.7
Qalyub	QYB	2	0.5
Quaranfil	QRF	2	0.5
Sakhalin	SAK	4	1.0
Tacaribe	TCR	9	2.3
Thogoto	THO	1	0.3
Timbo	TIM	2	0.5
Turlock	TUR	3	0.8
Uukuniemi	UUK	5	1.3
Vesicular stomatitis	VSV	7	1.8
Warrego	WAR	2	0.5
Ungrouped viruses		88	22.7
		388	

Table 3. Initial Isolations of Viruses by Decade and Country of Origin.

Decade	Continent	Country	Virus
1900-09	Africa	South Africa	BLU
1910-19	Africa	Kenya	ASF, NSD
1920-29	Africa	Nigeria	YF
	Europe	Scotland	LI
	North America	U.S.A.	VSI
1930-39	Africa	Kenya	RVF
		S. Africa	AHS
		Uganda	BWA, WN
	Asia	Japan	JE
		U.S.S.R.	RSSE
	N. America	U.S.A.	EEE, SLE, WEE
S. America	Venezuela	VEE	
1940-49	Africa	Uganda	BUN, NTA, SF, UGS, ZIKA
	Asia	Japan	NEG
		U.S.S.R.	OMSK
	Australasia	Hawaii	DEN-1*
		New Guinea	DEN-2*
	Europe	Czechoslovakia	HAN
		Italy	SFN*, SFS*
	N. America	U.S.A.	CE, CTF, TVT
	S. America	Brazil	ILH
		Colombia	ANA, ANB, WYO
	1950-59	Africa	Egypt
Nigeria			ILE, LB
South Africa			BAN, GER, ING, LEB, MID, MOS, NDU, NYM, PGA, SIM, SPO, TETE, USU, WIT, WSL.
Asia		Uganda	CHIK, CON, ENT, NDO, ONN, ORU
		India	ARK, BHA, GAN, KAS, KSO, KFD, MIN, PAL, SAT, VKT, UMB, WAN.
		Israel	IT
		Japan	AKA, APOI, IBA, NOD, SAG, TSU
		Malaya	BAK, BAT, BEB, GET, KET, LGT, TMU
		Australasia	MVE
Europe		Philippines	DEN-3*, DEN-4*
		Czechoslovakia	HYPR, TAH
		Finland	KUM
N. America		U.S.S.R.	ABS
		Canada	POW
		Panama	BOC, LJ, PCA
S. America		U.S.A.	CV, EHD, HP, MML, MOD, RB, SA, SSH, TUR, VSNJ
		Argentina	JUN
		Brazil	APEU, AURA, BSQ, CAP, CAR, CATU, GJA, GMA, ITQ, MAG, MIR, MOJU, MTB, MUC, MUR, ORI, TCM, UNA.
			GRO, NAV
			ARU, BIM, BSB, IERI, KRI, LUK, MAN, MAY, MEL, NEP, ORO, TCR, TNT.
		Colombia	
Trinidad			

* Isolated in U.S.A. Laboratory

Table 3. (Continued)

Decade	Continent	Country	Virus	
1960-69	Africa	Cameroon	NKO,OKO	
		Cent.Afr.Rep.	BAG,BCN,BIR,BOT,BOU,BTK,MPO,PATA,YATA,ZGA	
		Egypt	ACD,AMT,BAH*,BEA,MTR,MTY,RF	
		Kenya	THO	
		Nigeria	DUG,LAS*,SABO,SAN,SHA,SHU	
		Senegal	BDA,DB,GOS,KEU,KOU,LD,SAB,TAT,TOU,YOG	
		South Africa	OLI	
		Sudan	MAL***	
		Uganda	KAD,KAM,MEB,TAN	
		Asia	Cambodia	PPB
			India	BAR,CHP,DHO,KAN,KMP,SEM,THI,TPM,VEL
			Iran	KAR*,SAL*
			Japan	AINO
			Malaysia	JUG,KTR,LJN,PUC,TR
	Pakistan (West)		DGK,HAZ,MWA	
	Persian Gulf		ZIR	
	Singapore		SEL	
	Thailand		KK	
	U.S.S.R.		OKH,SAK,TYU,ZT	
	Australasia	Australia	ALF,ALM,BEF,BEL,CHV,COR,DAG,EH,EUB,JAP, JOI,KOK,KOO,KOW,KUN,MAP,MPK,MR,RR,SEP, STR,TRU,UPO,WAR,WON	
		New Zealand	WHA	
		Pacific Island	JA*	
		Europe	Czechoslovakia	CVO,KEM,LIP,TRB
			Finland	INK,UUK
	France		GA,PTV	
	N. America	West Germany	MBG	
		Canada	SIL	
		Guatemala	JUT*	
		Mexico	MNT,TLA*	
		Panama	AGU,CHG,CHI,CGL,FRI,GAM,JD,LAT,MAD, MAT,OSSA,PAR,PAT,PT*,ZEG	
		U.S.A.	BUT,CR,EVE,FLA,GL,HUG,JC,JS,KC,KEY,KLA, LAC,LOK,LS,MER,MD,MH,ML,PAH,SAW,SC,SHF, SR,TAM,TEN,UMA	
	S. America	Bolivia	MAC**	
		Brazil	ACA,AMA,AMB,ANH,BER,BOR,BUJ,CDU,CHO, COT,GTB,ICO,IRI,ITP,JUR,MCO,PAC,PIRY, PIX,SOR,TIM,TME,URU,VSA	
		Colombia	PIC	
		Peru	HUA*,PS*	
		Surinam	KWA	
		Trinidad	COC,MOR,NAR,RES,SOL	

* Isolated in U.S.A. laboratory

** Isolated in Panama laboratory

*** Isolated in Egypt laboratory

Table 3. (Continued)

<u>Decade</u>	<u>Continent</u>	<u>Country</u>	<u>Virus</u>
1970-76	Africa	Cent.Afr.Rep.	BBO,BGI,BOB,GAR,GOM,GOR,IPPY,KOL,LJA, NOLA,OUA,OUB,SAF,SEB,SGA,SJA,ZGO
		Egypt	AH,KS,PTH
		Seychelles	ARI***
		S. Africa	PRE***
		Zaire	EBO
		India	CG,KAI
	Asia	Iran	ISF*
		Malaysia	BC,CI
	Australasia	U.S.S.R.	BKN,IK,KHA,KSI,PMR,RAZ,SOK,TDY
		Australia	KNA,NGA,NUG,SRE,TAG,WAL,WGR
	Europe	Germany	EYA,TET
		Scotland	CM,CW
	N. America	U.S.S.R.	BAKU
		Canada	AVA,BAU*,GI*
		Panama	CAC,CAI,HIQ
	S. America	U.S.A.	NOR,RG,SCA,YH
		Brazil	ROC

* Isolated in U.S.A. laboratory

** Isolated in Panama laboratory

*** Isolated in Egypt laboratory

Table 4. Initial Isolation of 388 Registered Viruses by Continent, Country, and Chronological Period

Continent	Country or Area	Before 1930	1930-39	1940-49	1950-59	1960-69	1970-77	Totals
AFRICA	Cameroon					2		2
	Cent. Afr. Rep.					10	17	27
	Egypt				5	7	3	15
	Kenya	2	1			1		4
	Nigeria	1			2	6		9
	Senegal					10		10
	Seychelles						1	1
	S. Africa	1	1		15	1	1	19
	Sudan					1		1
	Uganda		2	5	6	4		17
Zaire						1	1	
	Totals	4	4	5	28	42	23	106
ASIA	Cambodia					1		1
	India				12	9	2	23
	Iran					2	1	3
	Israel				1			1
	Japan		1	1	6	1		9
	Malaysia				7	5	2	14
	W. Pakistan					3		3
	Persian Gulf					1		1
	Singapore					1		1
	Thailand					1		1
	U.S.S.R. (East)		1	1		4	8	14
	Totals	0	2	2	26	28	13	71
AUSTRAL-ASIA and PACIFIC ISLANDS	Australia				1	25	7	33
	Hawaii			1				1
	Johnston Island					1		1
	New Guinea			1				1
	New Zealand					1		1
	Philippines					2		2
	Totals	0	0	2	3	27	7	39
EUROPE	Czechoslovakia			1	2	4		7
	Finland				1	2		3
	France					2		2
	West Germany					1	2	3
	Italy			2				2
	Scotland	1					2	3
	U.S.S.R. (West)				1		1	2
	Totals	1	0	3	4	9	5	22
NORTH AMERICA	Canada				1	1	3	5
	Guatemala					1		1
	Mexico					2		2
	Panama				3	15	3	21
	U.S.A.	1	3	3	10	26	4	47
	Totals	1	3	3	14	45	10	76
SOUTH AMERICA	Argentina				1			1
	Bolivia					1		1
	Brazil			1	18	24	1	44
	Colombia			3	2	1		6
	Peru					2		2
	Surinam					1		1
	Trinidad				13	5		18
	Venezuela		1					1
	Totals	0	1	4	34	34	1	74
	Grand Totals	6	10	19	109	185	59	388

TABLE 5. GROUP A ARBOVIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE	SEAS RATING*	TAXONOMIC STATUS					
	ARTHROPODS						VERTEBRATES					Africa	Asia	Aust:talasia	Europe	North America	South America			Natural Infection	Lab Infection			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sentinels
		Culicine	Anopheline																					
Aura	+																		22	Alphavirus				
Bebaru	+																		22	"				
Chikungunya	+																		20	"				
Eastern equine enc.	+																		20	"				
Everglades	+																		20	"				
Getah	+																		20	"				
Mayaro	+																		20	"				
Middelburg	+																		20	"				
Mucambo	+																		20	"				
Ndumu	+																		21	"				
O'nyong-nyong	+																		20	"				
Pixuna	+																		22	"				
Ross River	+																		20	"				
Sagiyama	+																		21	"				
Semliki Forest	+																		20	"				
Sindbis	+																		20	"				
Una	+																		21	"				
Venezuelan equine enc.	+																		20	"				
Western equine enc.	+																		20	"				
Whataroa	+																		20	"				

- * 20 = Arbovirus
 21 = Probable Arbovirus
 22 = Possible Arbovirus
 23 = Probably not Arbovirus
 24 = Not Arbovirus

TABLE 6. GROUP B ARBOVIRUSES, MOSQUITO-BORNE

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats										
Culicine	Anopheline	Ixodid	Argasid																		
Alfuy	+																		20	Flavivirus	
Bagaza	+																		22	"	
Banzi	+						+											+	20	"	
Bouboui	+	+					+												22	"	
Bussuquara	+							+											20	"	
Dengue-1	+						+											+	20	"	
Dengue-2	+						+											+	20	"	
Dengue-3	+						+											+	20	"	
Dengue-4	+						+											+	20	"	
Edge Hill	+																		20	"	
Ilheus	+																	+	20	"	
Japanese encephalitis	+																	+	20	"	
Jugra	+																		22	"	
Kokobera	+																		21	"	
Kunjin	+																	+	20	"	
Murray Valley enceph.	+																	+	20	"	
Ntaya	+																		21	"	
Sepik	+																	+	21	"	
St. Louis encephalitis	+																	+	20	"	
Spondweni	+																	+	20	"	
Stratford	+																		22	"	
Tembusu	+																		21	"	
Uganda S	+																		20	"	
Usutu	+																		22	"	
Wesselsbron	+																		20	"	
West Nile	+																	+	20	"	
Yellow fever	+																	+	20	"	
Zika	+																	+	20	"	

*See footnote Table 5

TABLE 7. GROUP B ARBOVIRUSES, TICK-BORNE

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS					
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection				
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds											Bats	Marsupials	Other	Sentinels
		Anopheline	Ixodid																					
Culicine																								
Absettarov																			20	Flavivirus				
Hanzalova																			20	"				
Hypr																			20	"				
Kadam																			21	"				
Karshi																			22	"				
Kumlinge																			20	"				
Kyasanur Forest disease																			20	"				
Langat																			20	"				
Louping ill																			20	"				
Omsk hem. fev.																			20	"				
Powassan																			20	"				
Royal Farm																			22	"				
RSSE																			20	"				
Saumarez Reef																			22	"				
Tyuleniy																			21	"				

*See footnote Table 5

TABLE 8. GROUP B-VIRUSES, NO ARTHROPOD VECTOR DEMONSTRATED

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS				
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection			
	Hosp.	Ticks		Palaetotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sentinels
		Culicine	Anopheline																					
Apoi								+										+	22	Flavivirus				
Batu Cave																			22	"				
Carey Island										++									22	"				
Cowbone Ridge								+										+	23	"				
Dakar bat						+				++									24	"				
Entebbe bat										++									24	"				
Israel turkey meningo.									+										21	"				
Jutiapa								++											22	"				
Koutango								++										+	21	"				
Modoc								++											24	"				
Montana myotis leuko.										+									24	"				
Negishi						+												+	22	"				
Phnom-Penh Bat										++									23	"				
Rio Bravo										++								+	24	"				
Rocio						+			+									++	22	"				
Saboya								+										+	22	"				
Sokuluk														+					22	"				

*See footnote Table 5

TABLE 9. BUNYAMWERA SUPERGROUP: BUNYAMWERA GROUP VIRUSES

VIRUS	ISOLATED FROM										ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS					
	ARTHROPODS					VERTEBRATES					South America	North America	Europe	Australasia	Asia	Africa	Lab Infection	Natural Infection							
	Mosq.	Ticks				Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels												
Anhembí																									Bunyavirus
Batai	+	+																							"
Birao																									"
Bunyamwera																									"
Cache Valley	+	+																							"
Calovo**	+	+																							"
Germiston																									"
Guarao																									"
Ilesha																									"
Kairi																									"
Lokern																									"
Maguari																									"
Main Drain																									"
Northway																									"
Sororoca																									"
Tensaw																									"
Tlacotalpan																									"
Wyeomyia																									"

* See footnote Table 5

** May be strain of Batai

TABLE 10. BUNYAMWERA SUPERGROUP: BWAMBA GROUP AND GROUP C VIRUSES

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection
	Mosp. Culicine.	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Fodents										
<u>BWAMBA GROUP</u>																				
Bwamba																				
Pongola	+	+						+												
<u>GROUP C</u>																				
Apeu		+						+											+	20
Caraparu		+						+	+										+	20
Gumbo Limbo		+						+	+										+	21
Itaqui		+						+	+										+	20
Madrid		+						+	+										+	20
Marituba		+						+	+										+	20
Murutucu		+						+	+					+					+	20
Nepuyo		+						+	+										+	20
Oriboca		+						+	+				+						+	20
Ossa		+						+	+										+	20
Restan		+						+	+										+	20

*See footnote Table 5

TABLE 12. BUNYAMWERA SUPERGROUP: GUAMA, KOONGOL, OLIFANTSVLEI,
AND PATOIS GROUP VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS				
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sentinels
		Culicine	Anopheline																					
<u>GUAMA GROUP</u>																								
Bertioga																				22	Bunyavirus			
Bimiti	+							+						+						20	"			
Catu	+	+					+	+		+										20	"			
Guama	+			+						+										20	"			
Mahogany Hammock	+						+	+		+										22	"			
Moju	+							+		+										20	"			
<u>KOONGOL GROUP</u>																								
Koongol	+	?																						
Wongal	+																			21	Bunyavirus			
																				21	"			
<u>OLIFANTSVLEI GROUP</u>																								
Olifantsvlei	+													+							22	Bunyavirus		
<u>PATOIS GROUP</u>																								
Pahayokee	+																				22	Bunyavirus		
Patois	+																				20	"		
Shark River	+	+																			21	"		
Zegla								+													22	"		

*See footnote Table 5

TABLE 15. PHLEBOTOMUS FEVER GROUP VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE	SEAS RATING*	TAXONOMIC STATUS					
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America	South America			Natural Infection	Lab Infection			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sentinels
		Culicine	Anopheline																					
Aguacate																					21	Bunyavirus-like		
Anhanga																						22	"	
Arumowot																						22	"	
Bujaru	+																					22	"	
Cacao																						21	"	
Caimito																						22	"	
Candiru																						22	"	
Chagres																						21	"	
Chilibre																						21	"	
Frijoles																						22	"	
Gordil																						22	"	
Icoaraci																						21	"	
Itaporanga		+																				20	"	
Karimabad																						22	"	
Nique																						22	"	
Pacui																						21	"	
Punta Toro																						21	"	
Rio Grande																						22	"	
Salehabad																						22	"	
SF-Naples																						20	"	
SF-Sicilian																						20	"	
Urucuri																						22	"	

*See footnote Table 5

TABLE 16. TICK-BORNE GROUPS OTHER THAN GROUP B VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS				
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sertinejs
		Culicine	Anophelinae																					
<u>CHF-CONGO GROUP</u> Congo Hazara				+		+						+						+	+	20 22	Bunyavirus-like "			
<u>KAISODI GROUP</u> Kaisodi Lanja Silverwater									+											22 22 21	Bunyavirus-like " "			
<u>NAIROBI SHEEP DISEASE</u> Dugbe Ganjam Nairobi Sheep Disease				+		+		+				+						+	+	22 22 20	Bunyavirus-like " "			
<u>THOGOTO GROUP</u> Thogoto						+						+						+		22	Bunyavirus-like			
<u>UUKUNIEMI GROUP</u> Grand Arbaud Manawa Ponteves Uukuniemi Zaliv Terpeniya																				20 22 22 21 22	Bunyavirus-like " " " "			

*See footnote Table 5

TABLE 17. TICK-BORNE GROUPS OTHER THAN GROUP B VIRUSES

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS		
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection	
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds											Bats
Culicine	Anopheline	Ixodid	Argasid																		
<u>COLORADO TICK FEVER</u> Colorado Tick Fever Eyach			+	+		+		+				+					+	+	20 22	Orbivirus "	
<u>KEMEROVO</u> Baku Bauline Cape Wrath Chenuda Great Island Huacho Kemerovo Lipovnik Mono Lake Nugget Okhotskiy Seletar Sixgun City Tribec Wad Medani Yaquina Head				+																22 22 22 22 22 22 21 22 22 22 22 22 22 21 22 22	Orbivirus " " " " " " " " " " " " " " " " "

*See footnote Table 5.

TABLE 19. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM										ISOLATED IN						HUMAN DISEASE	SEAS RATING*	TAXONOMIC STATUS			
	ARTHOPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America						
	Mosq.	Ticks	Other			Man	Other Primates	Rodents	Birds	Bats							Marsupials	Other		Sentinels		
			Culicine	Anopheline	Ixodid						Argasid	Culicoides										
<u>ANOPHELES A GROUP</u> Anopheles A Lukuni Tatajuma																		21	22	21	Bunyavirus-like	
		+	+	+								+						22	22		"	
		+	+																			"
<u>ANOPHELES B GROUP</u> Anopheles B Boracea																						Bunyavirus-like
		+	+																			"
		+	+																			"
<u>BAKAU GROUP</u> Bakau Ketapang																						Bunyavirus-like
																						"
																						"
<u>MAPPUTA GROUP</u> Mapputta Maprik Trubanaman																						Bunyavirus-like
																						"
																						"
																						"
<u>TURLOCK GROUP</u> M'Poko (=Yaba-1) Turlock Umbre																						Bunyavirus-like
																						"
																						"

*See footnote Table 5

TABLE 21. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS				
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australia	Europe	North America	South America	Natural Infection			Lab Infection			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sentinels
		Culicine	Anopheline																					
<u>HART PARK GROUP</u> Hart Park Flanders	+	+							+	+									21 22	Rhabdovirus "				
<u>KWATTA GROUP</u> Kwatta	+																+		22	Rhabdovirus				
<u>MOSSURIL GROUP</u> Kamese Mossuril	+	+							+					+	+				22 22	Rhabdovirus "				
<u>TIMBO GROUP</u> Chaco Timbo													+	+				+	22 22	Rhabdovirus "				
<u>VESICULAR STOMATITIS GR.</u> Chandipura Cocal Isfahan Piry VS-Alagoas VS-Indiana VS-New Jersey				+		+			+				+		+			+	20 20 22 22 22 20 22	Rhabdovirus " " " " " "				

*See footnote Table 5

TABLE 22. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS				
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sentinels
		Culicine	Anopheline																					
<u>BOTEKE GROUP</u> Boteke Zingilamo	+									+									22 22	Unclassified "				
<u>MALAKAL GROUP</u> Malakal Puchong	+	+																	22 22	Unclassified "				
<u>MARBURG GROUP</u> Ebola Marburg							+											+	+	23 23	Unclassified "			
<u>MATARIYA GROUP</u> Burg el Arab Garba Matariya										+										22 22 22	Unclassified " "			
<u>NYANDO GROUP</u> Nyando		+					+													+	21	Unclassified		

*See footnote Table 5

TABLE 23. TACARIBE (LCM) GROUP VIRUSES

VIRUS	ISOLATED FROM										ISOLATED IN		HUMAN DISEASE	SEAS RATING*	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					South America	North America				
	Mosq.	Ticks				Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Lab Infection	Natural Infection	
Amapari						+		+				+		+	+	+
Junin						+		+						+	+	+
Lassa																
Latino																
Machupo																
Parana																
Pichinde																
Tacaribe																
Tamiari																

*See footnote Table 5

TABLE 24. UNGROUPED MOSQUITO-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM				ISOLATED IN							HUMAN DISEASE	SEAS RATING*	TAXONOMIC STATUS
	ARTHIPODS		VERTEBRATES		Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			
	Mosq.	Ticks	Man	Other								Rodents	Birds	
					Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides				
Rift Valley fever													20	Bunyavirus-like
Tataguine													21	"
Trinit													21	"
Witwatersrand													20	"
Zinga													22	"
Bocas													22	Coronavirus
Japanaut													21	Orbivirus
Lebombo													21	"
Orungo													21	"
Umattilla													20	"
Nodamura													23	Picornavirus
Cotia													24	Poxvirus
Joinjakaka													22	Rhabdovirus
Kununurra													22	"
Yata													22	"

*See footnote Table 5

TABLE 25. UNGROUPED MOSQUITO-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM										ISOLATED IN						SEAS RATING*	TAXONOMIC STATUS			
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America			HUMAN DISEASE		
Mosq.		Ticks		Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials							Other	Sentinels		Natural Infection	
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other														
Kowanyama		+																		22	Bunyavirus-like
Arkonam	+	+																		22	Unclassified
Aruac	+																			21	"
Bangoran	+																			22	"
Belmont	+																			22	"
Gomoka	+							+												22	"
Ieri	+																			22	"
La Joya	+																			22	"
Minnal	+																			22	"
Nkolbisson	+																			22	"
Okola	+																			22	"
Oubangui	+																			22	"
Pacora	+																			22	"
Tanga	+																			22	"
Tembe		+																		22	"
Venkatapuram		+																		22	"
Wongorr		+																		22	"

*See footnote Table 5

TABLE 26. UNGROUPED TICK-, CULICOIDES-, OR PHLEBOTOMUS-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS				
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other	Sentinels
		Culicine	Anopheline																					
Bhanja						+		+					+	+					+	22	Bunyavirus-like			
Lone Star																				22	"			
Sunday Canyon				+																22	"			
African swine fever				+									+						+	20	Iridovirus			
Barur				+				+					+	+						22	Rhabdovirus			
Bovine ephemeral fever					+															22	"			
Sawgrass				+															+	22	"			
Arde				+									+	+						22	Unclassified			
Batken	+			+									+	+						22	"			
Charleville					+							+								22	"			
Chobar Gorge																				22	"			
Dhori																				22	"			
Issyk-Kul																				22	"			
Keterah																				21	"			
Khasan																				22	"			
Matucare																			+	22	"			
Ngaingan																				22	"			
Nyamanini																				21	"			
Paramushir																				22	"			
Razdan																				22	"			
Tamdy																				22	"			
Tettnang																			+	22	"			
Upolu																				22	"			
Wallal																				22	"			
Wanowrie	+																			22	"			

* See footnote Table 5

**Cuba

TABLE 27. UNGROUPED VIRUSES: NO ARTHROPOD VECTOR KNOWN

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS					
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection				
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds											Bats	Marsupials	Other	Sentinels
		Culicine	Anopheline																					
Bobaya										+									22	Bunyavirus-like				
Ibaraki																			22	Orbivirus				
Nariva																			23	Paramyxovirus				
Kern Canyon																		+	23	Rhabdovirus				
Keuraliba																		+	22	"				
Klamath																		+	22	"				
Lagos Bat																			24	"				
Marco																		+	22	"				
Mount Elgon Bat																			23	"				
Navarro																		+	22	"				
Almpiwar																			21	Unclassified				
Bangui																		+	22	"				
Bimbo																			22	"				
Gossas																			23	"				
Ippy																			22	"				
Kannavanpettai																			22	"				
Kannamangalm																			22	"				
Kolongo																			22	"				
Landjia																			22	"				
Le Dantec																			22	"				
Ouango																			22	"				
Saint-Floris																			22	"				
Salanga																			22	"				
Sandjinba																			22	"				
Sebokele																			22	"				
Sembalam																			22	"				
Simian Hemorrh. fever																		+	24	"				
Tanjong Rabok																			22	"				
Thottapalayam																			22	"				
Toure																			22	"				
Yogue																			22	"				

*See footnote Table 5

TABLE 28. CONTINENTAL DISTRIBUTION OF GROUPED AND UNGROUPED VIRUSES

Antigenic Group	Total in Group	Africa	Asia	Australia	Europe	North America	South America	No. of Continents involved				
								1	2	3	4	5
A	20	6	6	5	1	5	8	13	5	1	0	1
AHS	1	1	1	0	1	0	0	0	0	1	0	0
ANA	3	0	0	0	0	0	3	3	0	0	0	0
ANB	2	0	0	0	0	0	2	2	0	0	0	0
B	60	18	24	13	7	10	7	46	10	3	1	0
BAK	2	0	2	0	0	0	0	2	0	0	0	0
BLU	1	1	1	0	1	1	0	0	0	0	1	0
BTK	2	2	0	0	0	0	0	2	0	0	0	0
BUN	18	4	1	0	2	8	6	15	3	0	0	0
BWA	2	2	0	0	0	0	0	2	0	0	0	0
C	11	0	0	0	0	5	8	9	2	0	0	0
CAL	11	1	0	0	2	8	1	10	1	0	0	0
CAP	6	0	0	0	0	3	5	4	2	0	0	0
GMA	6	0	0	0	0	2	5	5	1	0	0	0
KOO	2	0	0	2	0	0	0	2	0	0	0	0
OLI	1	1	0	0	0	0	0	1	0	0	0	0
PAT	4	0	0	0	0	4	0	4	0	0	0	0
SIM	16	9	6	2	0	2	2	9	6	0	0	0
TETE	4	3	1	0	2	0	0	2	2	0	0	0
SBU	7	1	1	0	0	2	3	7	0	0	0	0
CGL	2	0	0	0	0	1	1	2	0	0	0	0
CTF	2	0	0	0	1	1	0	2	0	0	0	0
CON	2	1	2	0	1	0	0	1	0	1	0	0
COR	2	1	0	1	0	0	0	2	0	0	0	0
DGK	5	2	3	0	0	0	0	5	0	0	0	0
EHD	1	0	0	0	0	1	0	1	0	0	0	0
EUB	2	1	0	1	0	0	0	2	0	0	0	0
HP	2	0	0	0	0	2	0	2	0	0	0	0
HUG	4	0	1	0	0	1	3	3	1	0	0	0
KSO	3	0	2	0	0	1	0	3	0	0	0	0
KEM	16	3	4	1	4	6	1	14	1	1	0	0
KWA	1	0	0	0	0	0	1	1	0	0	0	0
MAL	2	1	1	0	0	0	0	2	0	0	0	0
MAP	3	0	0	3	0	0	0	3	0	0	0	0
MBG	2	2	0	0	0	0	0	2	0	0	0	0
MTY	3	3	0	0	0	0	0	3	0	0	0	0
MOS	2	2	0	0	0	0	0	2	0	0	0	0
NSD	3	2	1	0	0	0	0	2	0	0	0	0
NEO	1	1	0	0	0	0	0	1	0	0	0	0
PAL	4	0	3	1	0	0	0	4	0	0	0	0
PHL	22	4	4	0	2	9	7	20	0	2	0	0
QYB	2	2	0	0	0	0	0	2	0	0	0	0
QRF	2	1	0	1	0	0	0	2	0	0	0	0
SAK	4	0	1	1	1	2	0	3	1	0	0	0
TCR	9	1	0	0	0	1	7	9	0	0	0	0
THO	1	1	0	0	1	0	0	0	1	0	0	0
TIM	2	0	0	0	0	0	2	2	0	0	0	0
TUR.	3	1	1	0	0	1	1	2	1	0	0	0
UUK	5	0	2	0	3	0	0	5	0	0	0	0
VSV	7	1	2	0	0	2	5	4	3	0	0	0
WAR	2	0	0	2	0	0	0	2	0	0	0	0
Ungrouped	88	37	23	12	4	11	11	78	3	3	0	0
Totals	388	116	93	45	33	89	89	324	43	12	2	1

TABLE 29. NUMBER OF VIRUSES ISOLATED FROM WILD CAUGHT ARTHROPODS

Antigenic Group	Total in Group	Isolated From						No. of Classes involved		
		Mosq.	Ticks	Phlebotomine	Culicoides	Mites	Other	1	2	3
				Flies						
A	20	20	0	0	1	4	2	16	3	1
AHS	1	0	0	0	1	0	0	1	0	0
ANA	3	3	0	0	0	0	0	3	0	0
ANB	2	2	0	0	0	0	0	2	0	0
B	60	28	16	0	0	1	1	40	3	0
BAK	2	2	1	0	0	0	0	1	1	0
BLU	1	0	0	0	1	0	0	1	0	0
BTK	2	1	0	0	0	0	0	1	0	0
BUN	18	17	0	0	2	0	0	17	1	0
BWA	2	2	0	0	0	0	0	2	0	0
C	11	11	0	0	0	0	0	11	0	0
CAL	11	11	0	0	0	0	1	10	1	0
CAP	6	5	0	0	0	0	0	5	0	0
GMA	6	5	0	1	0	0	0	4	1	0
KOO	2	2	0	0	0	0	0	2	0	0
OLI	1	1	0	0	0	0	0	1	0	0
PAT	4	3	0	0	0	0	0	3	0	0
SIM	16	10	0	0	8	0	0	8	5	0
TETE	4	2	0	0	0	0	0	1	0	0
SBU	7	5	0	0	0	0	0	5	0	0
CGL	2	0	0	1	0	0	0	1	0	0
CTF	2	0	2	0	0	0	0	2	0	0
CHF-CON	2	0	2	0	1	0	0	1	1	0
COR	2	2	0	0	0	0	0	2	0	0
DGK	5	0	5	0	0	0	0	5	0	0
EHD	1	0	0	0	0	0	0	0	0	0
EUB	2	2	0	0	0	0	0	2	0	0
HP	2	2	0	0	0	0	0	2	0	0
HUG	4	0	4	0	0	0	0	4	0	0
KSO	3	0	3	0	0	0	0	3	0	0
KEM	16	0	16	0	0	0	0	16	0	0
KWA	1	1	0	0	0	0	0	1	0	0
MAL	2	2	0	0	0	0	0	2	0	0
MAP	3	3	0	0	0	0	0	3	0	0
MBG	2	0	0	0	0	0	0	0	0	0
MTY	3	0	0	0	0	0	0	0	0	0
MOS	2	2	0	0	0	0	0	2	0	0
NSD	3	2	3	0	1	0	0	1	1	1
NDO	1	1	0	0	0	0	0	1	0	0
PAL	4	3	0	0	1	0	0	4	0	0
PHL	22	4	0	13	0	0	0	15	1	0
QYB	2	0	2	0	0	0	0	2	0	0
QRF	2	0	2	0	0	0	0	2	0	0
SAK	4	0	4	0	0	0	0	4	0	0
TCR	9	1	1	0	0	3	0	3	1	0
THQ	1	0	1	0	0	0	0	1	0	0
TIM	2	0	0	0	0	0	0	0	0	0
TUR	3	3	0	0	0	0	0	3	0	0
UUK	5	0	5	0	0	0	0	5	0	0
VSV	7	2	0	3	0	1	1	3	2	0
WAR	2	0	0	0	2	0	0	2	0	0
Ungrouped	88	34	21	2	3	0	1	51	4	0
Totals	388	194	88	20	21	9	6	277	25	2

TABLE 30. NUMBER OF VIRUSES ISOLATED FROM NATURALLY INFECTED VERTEBRATES

Anti- genic Group	Total in Group	Man	Other Pri- mates	Ro- dents	Birds	Bats	Marsu- pials	Live- stock	All others	Number of Classes Involved					
										1	2	3	4	5	6
A	20	8	2	6	7	2	5	5	3	6	2	1	3	1	1
AHS	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
ANA	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	60	27	3	17	15	14	1	5	6	27	7	5	4	2	1
BAK	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0
BLU	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
BTK	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
BUN	18	5	1	3	0	0	0	1	3	9	2	0	0	0	0
BWA	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0
C	11	9	0	8	0	1	5	0	1	2	6	2	1	0	0
CAL	11	2	0	3	0	0	0	0	1	1	3	2	0	0	0
CAP	6	0	0	3	0	0	1	0	0	2	1	0	0	0	0
GMA	6	2	0	5	0	2	3	0	0	2	1	0	2	0	0
KOO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLI	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PAT	4	0	0	3	0	0	0	0	0	3	0	0	0	0	0
SIM	16	2	1	0	3	0	0	6	2	8	3	0	0	0	0
TETE	4	0	0	0	4	0	0	0	0	4	0	0	0	0	0
SBU	7	0	0	0	1	1	0	0	0	2	0	0	0	0	0
CGL	2	1	0	1	0	0	0	0	0	2	0	0	0	0	0
CTF	2	1	0	1	0	0	0	0	0	0	1	0	0	0	0
CON	2	1	0	0	0	0	0	1	1	0	0	1	0	0	0
COR	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
DGK	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EHD	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
EUB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HP	2	0	0	0	2	0	0	0	0	2	0	0	0	0	0
HUG	4	0	0	0	1	0	0	0	0	1	0	0	0	0	0
KSO	3	0	0	0	1	0	0	0	1	2	0	0	0	0	0
KEM	16	1	0	1	1	0	0	0	0	1	1	0	0	0	0
KWA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAL	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAP	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MBG	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
MTY	3	0	0	0	3	0	0	0	0	3	0	0	0	0	0
MOS	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
NSD	3	3	0	1	0	0	0	1	1	1	1	1	0	0	0
NDO	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0
PAL	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHL	22	5	0	7	2	0	1	0	2	11	3	0	0	0	0
QYB	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0
QRF	2	1	0	0	1	0	0	0	0	0	1	0	0	0	0
SAK	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0
TCR	9	3	0	8	0	1	0	0	1	6	2	1	0	0	0
THO	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0
TIM	2	0	0	0	0	0	0	0	2	2	0	0	0	0	0
TUR	3	0	0	0	2	0	0	0	1	1	1	0	0	0	0
UUK	5	0	0	1	1	0	0	0	0	0	1	0	0	0	0
VSV	7	4	0	1	0	0	1	4	1	2	4	0	0	0	0
WAR	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped	88	8	1	13	12	9	0	6	3	41	3	0	0	0	0
Totals	388	88	9	83	60	30	17	32	30	151	44	13	10	3	2

TABLE 31. NUMBER OF VIRUSES ASSOCIATED WITH NATURALLY OR LABORATORY ACQUIRED DISEASE IN MAN

Antigenic Group	Total in Group	In Nature	Lab Infection	Either or Both Number	Both Percent	
Group A	20	10	7	11	55.0	
Afr. horsesickness	1	0	0	0		
Anopheles A	3	0	0	0		
Anopheles B	2	0	0	0		
Group B	60	28	24	31	52.0	
Bakau	2	0	0	0		
Bluetongue	1	0	0	0		
Boteke	2	0	0	0		
Bunyamwera Supergroup	Bunyamwera	18	6	2	7	38.9
	Bwamba	2	1	0	1	50.0
	C	11	9	2	9	81.8
	California	11	4	0	4	36.4
	Capim	6	0	0	0	
	Guama	6	2	0	2	33.3
	Koongol	2	0	0	0	
	Olifantsvlei	1	0	0	0	
	Patois	4	0	0	0	
	Simbu	16	2	1	2	12.5
Tete	4	0	0	0		
SBU	7	0	0	0		
Changuinola	2	1	0	1	50.0	
Colorado tick fever	2	1	1	1	50.0	
CHF-Congo	2	1	1	1	50.0	
Corriparta	2	0	0	0		
Dera Ghazi Khan	5	0	0	0		
Epizoot. hem. dis.	1	0	0	0		
Eubenangee	2	0	0	0		
Hart Park	2	0	0	0		
Hughes	4	0	0	0		
Kaisodi	3	0	0	0		
Kemeroovo	16	1	1	1	6.3	
Kwatta	1	0	0	0		
Malakal	2	0	0	0		
Mapputta	3	0	0	0		
Marburg	2	2	2	2	100.0	
Mossuril	2	0	0	0		
Nairobi sheep dis.	3	3	2	3	100.0	
Nyando	1	1	0	1	100.0	
Palyam	4	0	0	0		
Phlebotomus fever	22	5	0	5	22.7	
Qalyub	2	0	0	0		
Quaranfil	2	1	0	1	50.0	
Sakhalin	4	0	0	0		
Tacaribe	9	3	3	3	33.3	
Thogoto	1	1	0	1	100.0	
Timbo	2	0	0	0		
Turlock	3	0	0	0		
Uukuniemi	5	0	0	0		
Vesicular stom.	7	4	3	5	71.4	
Warrego	2	0	0	0		
Ungrouped	88	6	2	7	8.0	
Totals	388	92	51	99	25.5	

TABLE 32. EVALUATION OF ARTHROPOD-BORNE STATUS
OF 388 REGISTERED VIRUSES (SEAS)

Antigenic Group	Total in Group	Arbovirus	Probably an Arbovirus	Possible Arbovirus	Probably not Arbovirus	Not an Arbovirus	Arbo or Probably Arbo		Not or Probably Not Arbo		
							No.	%	No.	%	
A	20	14	3	3	0	0	17	85.0	0		
AHS	1	1	0	0	0	0	1	100.0	0		
ANA	3	0	2	1	0	0	2	66.7	0		
ANB	2	0	0	2	0	0	0		0		
B	60	29	8	16	2	5	37	61.7	7	11.7	
BAK	2	0	1	1	0	0	1	50.0	0		
BLU	1	1	0	0	0	0	1	100.0	0		
BTK	2	0	0	2	0	0	0		0		
Bunyamvera Supergroup	BUN	18	9	3	6	0	0	12	66.7	0	
	BWA	2	1	1	0	0	0	2	100.0	0	
	C	11	10	1	0	0	0	11	100.0	0	
	CAL	11	8	1	2	0	0	9	81.8	0	
	CAP	6	3	1	2	0	0	4	66.7	0	
	GMA	6	4	0	2	0	0	4	66.7	0	
	KOO	2	0	2	0	0	0	2	100.0	0	
	OLI	1	0	0	1	0	0	0		0	
	PAT	4	1	1	2	0	0	2	50.0	0	
	SIM	16	3	3	10	0	0	6	37.5	0	
TETE	4	0	1	3	0	0	1	25.0	0		
SBU	7	1	1	5	0	0	2	28.6	0		
CGL	2	0	1	1	0	0	1	50.0	0		
CTF	2	1	0	1	0	0	1	50.0	0		
CON	2	1	0	1	0	0	1	50.0	0		
COR	2	0	0	2	0	0	0		0		
DGK	5	0	0	5	0	0	0		0		
EHD	1	0	1	0	0	0	1	100.0	0		
EUB	2	0	0	2	0	0	0		0		
HP	2	0	1	1	0	0	1	50.0	0		
HUG	4	1	1	2	0	0	2	50.0	0		
KSO	3	0	1	2	0	0	1	33.3	0		
KEM	16	0	2	14	0	0	2	12.5	0		
KWA	1	0	0	1	0	0	0		0		
MAL	2	0	0	2	0	0	0		0		
MAP	3	0	1	2	0	0	1	33.3	0		
MBG	2	0	0	0	2	0	0		2	100.0	
MTY	3	0	0	3	0	0	0		0		
MOS	2	0	0	2	0	0	0		0		
NSD	3	1	0	2	0	0	1	33.3	0		
NDO	1	0	1	0	0	0	1	100.0	0		
PAL	4	0	0	4	0	0	0		0		
PHL	22	3	7	12	0	0	10	45.5	0		
QYB	2	0	0	2	0	0	0		0		
QRF	2	2	0	0	0	0	2	100.0	0		
SAK	4	0	1	3	0	0	1	25.0	0		
TCR	9	0	0	0	0	9	0		9	100.0	
THO	1	0	0	1	0	0	0		0		
TIM	2	0	0	2	0	0	0		0		
TUR	3	1	1	1	0	0	2	66.7	0		
UUK	5	1	1	3	0	0	2	40.0	0		
VSV	7	3	0	4	0	0	3	42.9	0		
WAR	2	0	0	2	0	0	0		0		
Ungrouped	88	4	9	67	5	3	13	14.8	8	9.0	
Totals	388	103	57	202	9	17	160	41.2	26	6.7	

REPORT FROM THE ARBOVIRUS LABORATORY
 INSTITUT PASTEUR DE NOUMEA, NEW CALEDONIA

Epidemiological studies on dengue and hemorrhagic fever, initiated in 1975, were developed in 1977.

Virus isolations

1) From patients suffering dengue-like disease : 43 strains were isolated by intra-cerebral inoculation to suckling mice, from blood specimens taken in acute phase. 3 others were obtained by intra-thoracic inoculation to Aedes aegypti ; in one case, the serum of the patient exhibited dengue 1 antibodies as high as 1 : 40 960. According to complement-fixation tests already performed, dengue 1 appears to be the lone serotype involved.

2) From mosquitoes : 50 pools made from 315 specimens were tested for viral infection by intra-cerebral inoculation to suckling mice. 1 strain was isolated from Aedes vigilax (one female caught in Noumea, on 2/2/77) and 1 from Aedes polynesiensis (two females caught in Ono, Futuna, 11/11/76) as reported in 1976. Identification of the strains is under study.

Species tested for viral infection were :

<u>Genus</u>	<u>Species</u>	<u>Country</u>	<u>N° of pools tested</u>	<u>N° of Specimens</u>	<u>N° virus Isolation</u>
<u>Aedes</u>	<u>aegypti</u>	New Caledonia	18	28	0
	<u>aegypti</u>	Ouvéa	3	38	0
	<u>hebrideus</u>	New Hebrides	5	19	0
	<u>polynesiensis</u>	Wallis	7	99	0
	<u>polynesiensis</u>	Futuna	4	14	1
	<u>vigilax</u>	New Caledonia	5	14	1
<u>Anopheles</u>	<u>farauti</u>	New Hebrides	2	4	0
<u>Culex</u>	<u>annulirostris</u>	Futuna	1	18	0
	<u>p. fatigans</u>	New Caledonia	5	81	0
	<u>Total tested</u>		50	315	2

Serology

Monthly records of human sera from suspected cases, with significant serology are given below (positive/total tested by hemagglutination-inhibition).

	<u>JAN</u>	<u>FEB</u>	<u>MAR</u>	<u>APR</u>	<u>MAY</u>	<u>JUN</u>	<u>JUL</u>	<u>AUG</u>	<u>SEP</u>	<u>OCT</u>	<u>NOV</u>	<u>DEC</u>	<u>TOTAL</u>
positive	16	32	44	90	17	19	9	1	5	0	7	6	246
total tested	29	129	228	261	126	83	74	34	36	30	24	32	1 086

Results were interpreted as follows :

Primary responses.....	60
Secondary responses.....	49
Presumptive recent infection.....	137

Epidemiology

New Caledonia

Out of 1086 patients suspected of dengue, 263 cases were confirmed by sero-diagnosis and/or virus isolation. 164 cases (62,3 %) occurred in the main town, Nouméa (56 000) - 47 (17,9 %) in Ouvea island (2 800) - 15 (5,7 %) in Thio (2 900) - 14 (5,3 %) in Kouaoua-Canala (3 880) ; other localities, affected were : La Foa (3 cases), Koumac (2 cases), Poindimié (2 cases), Poro-Népoui (2 cases) and the surroundings of Nouméa, Dumbéa, Mt Koghi, Conception, Koutio, Tonghoué, Robinson, St Louis (12 cases in total) ; 1 case was imported from Tahiti to La Foa.

Two aerial ultra-low-volume sprayings of malathion (225 ml/héctare), over Nouméa, on 6 th and 19 th April, 1977, contributed to reduce the number of cases as indicated by the monthly record of positive laboratory diagnosis for patients infected in Nouméa, in regard of Aedes aegypti index.

	<u>JAN</u>	<u>FEB</u>	<u>MAR</u>	<u>APR</u>	<u>MAY</u>	<u>JUNE</u>	<u>JUL</u>	<u>AUG</u>	<u>SEP</u>	<u>OCT</u>	<u>NOV</u>	<u>DEC</u>	<u>TOTAL</u>
pos. cases	13	26	38	50	6	7	7	2	4	0	7	4	164
Br. I	49	67	80	67	50	48	43	40	54	40	42	54	
Pr. I	21	28	26	24	18	15	18	23	22	17	18	21	

(Br. I = Breteau Index - Pr. I = Premise Index)

New Hebrides

Sera from 9 suspected cases sent to the laboratory between september and december 1977, gave no evidence of a recent infection by a Flavivirus.

During a survey in the Catholic Mission of Wala Rano, Malekolo, 42 blood specimens from school children between 9 and 16 years were taken by Pr. RAOULT and Dr. RATARD of the Rural Health Service, in December 1976, after a small outbreak in March 1976 ; 40 (95 %) sera showed dengue HI antibodies titers from 1 : 10 to 1 : 80. A similar survey, at the fall of 1974, in Wala Rano, gave 59 positive results on 91 samples (64,8 %).

Attempts of virus isolation from Ae. hebrideus and An. farauti caught by Pr. RAOULT in Wala Rano, in December 1976 failed.

Routine mosquito control operations in Vila (Vató), by Rural Health Service team revealed high percentage of Ae. aegypti larvae in artificial breeding places (78 to 95 %).

There was no evidence of dengue transmission in New Hebrides ; during the year round.

Futuna, Horne Islands

According to informations from Dr. PUJOL, dengue 1 outbreak studied and reported in 1976, lasted till the beginning of 1977. On clinical basis, about 400 cases occurred from October 1976 to January 1977 (total population 2700). Ae. polynesiensis, the most common mosquito species in and around premises, was found naturally infected. Ae. aegypti has never been found in Futuna and Alofi.

Wallis

Only one sero-diagnosis was made on paired sera sent in September, with a negative result. There was no evidence of dengue transmission during the year.

(P. FAURAN, G. LE GONIDEC, H. SCHILL)

Bovine ephemeral fever virus.

Both mouse-adapted strains of bovine ephemeral fever virus and unadapted strains obtained from the blood of infected cattle multiplied in chicken embryos following intravenous inoculation. Bovine ephemeral fever virus was isolated from the blood and brain of infected embryos and from the brain of chickens that hatched from infected eggs. Bovine ephemeral fever virus obtained from infected calves retained virulence for cattle after a single passage in chick embryos. Duck embryos were also susceptible to intravenous inoculation with bovine ephemeral fever virus.

One-day old, 4-week old and adult chickens developed neutralizing antibody following intravenous inoculation with bovine ephemeral fever virus but only one-day old chickens developed a detectable viraemia. Ducklings and ducks developed neutralizing antibodies but no detectable viraemia.

Three sheep and 2 goats were inoculated with fresh calf blood shown to contain bovine ephemeral fever virus. All developed neutralizing antibody to bovine ephemeral fever virus, one sheep developed a transient lameness but none of the animals developed detectable viraemia. Rats developed viraemia after intravenous inoculation with bovine ephemeral fever virus but mice, guinea pigs, kittens and a rabbit did not. Three water buffaloes were inoculated intravenously with cattle blood containing virulent bovine ephemeral fever virus. None developed clinical disease but viraemia was demonstrated in two of the buffaloes, which also developed low levels of neutralizing antibodies.

Serial intracerebral inoculation of bovine ephemeral fever virus in suckling mice resulted in the production of strains that produced fatal encephalitis on intracerebral inoculation of adult mice. Three of these neurotropic strains were examined for plaque type in Vero cell cultures and large, medium and small plaques were selected. Clones obtained from large plaques were pathogenic for adult mice and clones from small plaques were not. Vaccines produced from suckling mouse-adapted and Vero cell-adapted strains of bovine ephemeral fever virus protected adult mice against intracerebral inoculation with neurotropic strains of bovine ephemeral fever virus.

Akabane virus.

A proportion of chicken embryos inoculated into the yolk sac with Akabane virus when 4-days old survived and developed abnormalities including arthrogryposis.

Older embryos inoculated by the yolk sac, the allantoic cavity, the chorioallantoic membrane or intravenously did not develop abnormalities. The recovery of Akabane virus from abnormal embryos and the absence of abnormalities in control embryos suggested that Akabane virus was teratogenic in the chicken embryos. Pregnant rabbits inoculated with Akabane virus aborted or produced abnormal fetuses and Akabane virus was isolated from the fetuses. Rabbits that had undergone abnormal pregnancies following infection with Akabane virus produced normal litters when reinfected with Akabane virus during a subsequent pregnancy. Pregnant guinea pigs infected with Akabane virus aborted or resorbed fetuses and Akabane virus was isolated from fetuses and placentome.

P. Spradbrow, M.A. Gaffar Elamin, P.L. Young
and A.H. Miah.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF
WESTERN AUSTRALIA, PERTH, WESTERN AUSTRALIA, AUSTRALIA

Arboviruses from North-West Australia continue to be isolated from mosquitoes.

Table I shows the current state of virus isolates and their characterisation. The predominant mosquito of the Kimberley *Culex annulirostris* consistently yields most of the viruses pathogenic for infant mice.

Table II shows the current state of the mosquito captures and identifications.

(N.F. Stanley, S. Anderson, A. Wright, K.H. Chan, D. Britten).

TABLE I

VIRUS ISOLATIONS FROM KIMBERLEY (ORD RIVER AND DERBY) MOSQUITOES:

CURRENT IDENTIFICATION OF 179 OF 208 ISOLATES FROM 5 VECTORS.

Vector	Groups																Total	
	Flavi-virus		Alphavirus		Koongol			Corri-parta	Ungrouped rhabdovirus			Poly Anoph. A	Unknown/ungrouped†					Unde-fined
	MVE	Kun-jin	Sindbis	?*	Koon-gol	Won-gal	To be typed	Corri-parta	KNA OR 194	PC OR 189	KIM OR 250	OR 540	OR 374	OR 379	OR 408	OR 512		Won-gorr
<u>Culex annulirostris</u>	32	20	11	1	3	8	69	1		2	1	6	1	2	2	2	3	164
<u>Aedeomyia catasticta</u>								9	1									10
<u>Aedes tremulus.</u>		1																1
<u>Aedes normanensis</u>			2														1	3
<u>Culex fatigans</u>	1																	1
Total:	33	21	13	1	3	8	69	10	1	2	1	6	1	2	2	2	4	179

*Non-haemagglutinating - possibly a variant of Ross River virus.

†Tested against world group antisera and Australian antisera and found to be non-reacting.

TABLE II

ORD RIVER MOSQUITO CAPTURES (1972-77)

Species	No. captured	% Total
<u>Culex (Culex) annulirostris</u>	71 045	83.17
<u>Aedeomyia catasticta</u>	5698	6.67
<u>Culex (Culex) fatigans</u>	4766	5.58
<u>Aedes (Ochlerotatus) normanensis</u>	892	1.04
<u>Aedes (Ochlerotatus) vigilax</u>	588	0.69
<u>Mansonia (Coquillettidia) xanthogaster</u>	514	0.60
<u>Anopheles (Cellia) annulipes</u>	423	0.50
<u>Culex (Culiciomyia) pullus</u>	252	0.29
<u>Mansonia (Mansonioides) uniformis</u>	237	0.28
<u>Aedes (Finlaya) notoscriptus</u>	219	0.26
<u>Aedes (Macleaya) tremulus</u>	210	0.25
Other species (twenty)	576	0.67
Total:	85 420	

Dengue Hemorrhagic Fever in Indonesia

Dengue fever is endemic in most provinces of Indonesia. In Jakarta, and other densely populated areas, both urban and rural, DHF occurs sporadically throughout the year. Other urban centers, such as Ujung Pandang in South Sulawesi, on the other hand, have never reported DHF even though dengue virus(es) are highly endemic. In recent years, epidemic DHF has been occurring more frequently in both urban and rural areas. Furthermore, it is occurring in areas where it has never been reported before. We have recently initiated a study to determine the serotypes and extent of dengue virus activity in selected areas of Indonesia and to determine whether there is any relationship between virus strain or serotype and severity of disease. During the past two years we have collected some preliminary data which we present here.

Table 1 shows the dengue viruses isolated in Indonesia during the past 2 years by serotype and location. All 4 serotypes have been isolated, but dengue 3 has been the predominant virus isolated in most areas studied. In Jakarta, where all 4 types are endemic, dengue 3 and dengue 2 are the predominant viruses isolated, with dengue 1 and 4 occurring less frequently. It will be noted that most of the dengue 1 and 2 isolates came from Jakarta whereas dengue 3 had a much wider distribution in the country. The isolates from Jogjakarta, Central Java, and West Kalimantan were associated with DHF epidemics, all of which were caused primarily by dengue type 3. The data suggest that epidemic dengue 3 is gradually moving throughout Indonesia. The viruses responsible for outbreaks in the past are not known, but dengue 2 apparently was the predominant virus in the 1973 epidemic in Semarang, Central Java. Thus the 2 to 4 year cycles of epidemic DHF which occur in many countries of Southeast Asia may be due to the introduction of new serotypes of virus.

Preliminary data on the relationship between severity of disease and virus serotype have been collected in Jakarta, an endemic area of continuous sporadic transmission. Table 2 shows this relationship for 109 DHF patients who were confirmed virologically and for whom adequate clinical data were available. Of 109 patients 66 or 60% were classified as dengue shock

syndrome (grades III and IV). Nearly one half (48%) of shock cases were associated with dengue 3, 32% with dengue 2, 17% with dengue 1 and 3% with dengue 4 infections. It will be noted that this distribution does not differ significantly from the expected distribution based on the total number of cases shown in Table 3. If only fatal cases are considered, however, there is a significant difference in the observed and expected distribution (Table 3). Of the 20 fatal cases from which virus was isolated, 13 (65%) were associated with dengue 3 infection, suggesting that this type may be more virulent than the others. At this time, we do not know anything about the actual frequency of transmission of the 4 serotypes in Jakarta because we cannot monitor all FUO infections. We are confident, however, that our data represent the distribution of the severe and fatal forms of dengue in the city.

Our new program will monitor the serotypes of dengue viruses being transmitted in selected cities in Indonesia from Medan, North Sumatra to Jayapura, Irian Jaya. Mild and non-specific febrile illness as well as DHF will be studied in an attempt to gain some knowledge of the relationship between virus strain and serotype and severity of disease. Furthermore, this study will provide baseline data which will be invaluable if and when epidemic DHF occurs in the future.

(D. J. Gubler, W. Soeharyono, I. Lubis)

Table 1. Distribution of dengue serotypes in Indonesia, 1976-1977.

<u>Location</u>	<u>D1</u>	<u>D2</u>	<u>D3</u>	<u>D4</u>	<u>Totals</u>
Jakarta	22	38	48	6	114
Jogjakarta	-	3	11	4	18
Central Java	8	-	28	9	45
East Java	1	-	1	-	2
West Kalimantan	-	-	5	-	5
South Sumatra	-	-	1	-	1
Northern Moluccas	-	1	-	-	1
Totals	31	42	94	19	186

Table 2. Relationship between infecting serotype and severity of disease* in dengue hemorrhagic fever patients, Jakarta, Indonesia.

<u>Severity of illness</u>	<u>Dengue Serotypes</u>				<u>Totals</u>
	<u>D1</u>	<u>D2</u>	<u>D3</u>	<u>D4</u>	
DF	6	6	9	3	24
II	5	7	6	1	19
III	6	14	13	1	34
IV	5	7	19	1	32
Totals	22	34	47	6	109

* Using the criteria outlined by the WHO Technical Guide for diagnosis and treatment of DHF, 1975. All patients with a positive tourniquet test as the only hemorrhagic manifestation were classified as DF.

Table 3. Frequency of fatal dengue infections compared to total cases by **serotype**, Jakarta, Indonesia.

Dengue serotype	Total No. of DHF infections	% of total	No. of fatal DHF infections	% of total
D1	22	20.2	3	15.0
D2	34	31.2	3	15.0
D3	47	43.1	13	65.0
D4	6	5.5	1	5.0
Totals	109	100.0	20	100.0

Surveillance of Japanese encephalitis in 1977 in Japan

Surveillance of Japanese encephalitis carried out jointly by Ministry of Health and Welfare, National Institute of Health and Prefectural Public Health Laboratories finally confirmed five human cases in 1977 in Japan. The number of human cases was the lowest since World War II. Geographical distribution of cases showed all from the west part of Japan, 2 from Kumamoto, 2 from Wakayama and 1 from Osaka Prefectures. Ages of cases, one male and 4 female, are 28, 41, 46, 59 and 61. There was no death however 3 cases showed sequelae 30 days after the onset of the disease. The first case was noted August 28 and the last case October 22.

Antibody survey of domesticated pigs revealed that the enzootic foci located in Kyushu island and the Pacific coast of main land of Japan. Pig infection started in Okinawa in June and spread in main land Japan from July to October. Human infection was reported from districts where pig infection was highly frequent. (A. Oya)

Antibody survey in school children

Antibody for Japanese encephalitis was examined in a primary school and a middle school in a prefecture where booster vaccination once for 3 years is programmed. Antibody positive rates for HI and NT were 316/371 (85.2%) and 349/354 (98.6%) in a primary school and 94/145 (64.8%) and 139/145 (95.9%) in a middle school respectively. Since natural infection rate is assumed low (less than 1%), the most of antibodies was considered to be the results of vaccination. (A. Oya)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
KOBE UNIVERSITY SCHOOL OF MEDICINE
IKUTA-KU, KOBE 650, JAPAN

Anti-dengue antibodies detected in sera from residents of
Nagasaki, Japan, at 32-34 years after the dengue epidemics.

The city of Nagasaki, Japan, experienced large epidemics of dengue during 1942-44. Sera were taken from persons who suffered from dengue at that time and who have resided there since then without any experience of entering dengue-endemic areas. Anti-DEN plaque reduction neutralization (PRNT) titers were determined using DEN-1 virus Mochizuki strain which was isolated during the 1943 Nagasaki epidemic. Individuals who are receiving regular follow-up medical checking by the Radiation Effects Research Foundation of Japan were subjects of the present study. No disease suspected of DEN or JE has been recorded in their protocols.

Of 3 individuals, anti-DEN-1 50% PRNT titers of sera were 155, 978, and 219, respectively, which were detected entirely in the IgG fractions. The anti-DEN antibodies were clearly distinguished from anti-JE (G1 strain) antibodies of the same sera, 50% PRNT titers of which were 12, 22, and 15, respectively. It is to be added that no flavivirus infection excepting JE has been known in Japan. It was concluded from the data that the specific anti-DEN antibodies of considerably high level were maintained in human sera for 32-34 years after what is most probably a single attack of dengue.

(N. Fujita and K. Yoshida)

(The authors are indebted to Dr. S. Kawamoto, Dr. I. Nagai and the staff of the Radiation Effects Research Foundation of Japan for their kind cooperation in collecting the serum specimens.)

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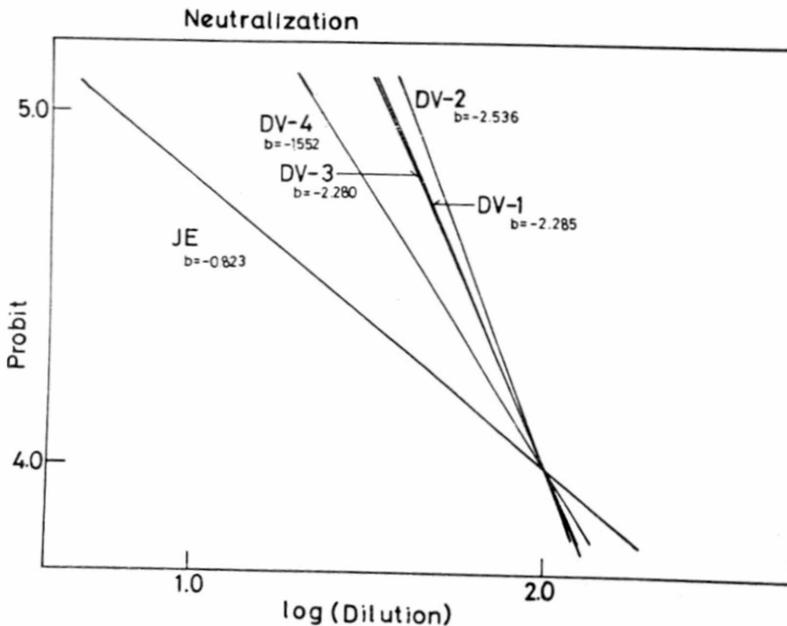
(S. Hotta)

1. Rapid neutralization test of dengue viruses by immunoperoxidase technique

As we reported previously, focus counting method either by peroxidase-anti-peroxidase (PAP) or indirect immunofluorescent (FA) technique was proved to be useful for the neutralization test of dengue viruses. Especially PAP technique has several advantages over FA for testing many specimens at the same time.

Several human sera of previous dengue histories were serially diluted and were incubated with a constant amount of each of the 4 types of dengue viruses at 28C for 2 hours. Then the serum virus mixtures were inoculated onto BHK-21 cells prepared in 8-chamber slides for focus formation, which were detected by PAP technique. Straight lines were obtained on a probit chart by plotting the focus reduction rates against the logarithm of the reciprocal of serum dilution. The inclination of the lines were almost the same and fell within a certain limit for several human sera for each of the 4 types of dengue viruses. Figure 1 shows the mean value of the inclination obtained for dengue and Japanese encephalitis (JE) viruses. Using these relationships, 50 % focus reduction neutralization titers could be estimated for test sera from the focus reduction rate at a single dilution of the sera, if the reduction rate is between 25 to 75 %. This method is especially useful for testing a number of specimens in sero-epidemiological surveillance or serodiagnosis on dengue infections.

Fig. 1



2. Dengue and Japanese encephalitis (JE) antibody profile before and after JE vaccination

Serological studies before and after JE vaccination as measured by dengue and JE hemagglutination-inhibition (HI) antibody and neutralizing (N) antibody were undertaken in a group of individuals comprising of various ethnic groups. The study population consisted of 35 persons (18 from Southeast Asia, 5 from South America, 3 from Africa, and 9 from Japan).

Seroconversion (4 fold or more difference between pre- and post-vaccination titers) was around 45 % as measured by JE-HI test, and around 63 % as measured by JE-N test in all the ethnic groups. Seroconversion by dengue HI test was also detected but only in 14 % with 4 fold difference. Seroconversion by dengue N test was only in one case (3 %) with 4 fold difference.

The presence of JE and/or dengue antibody in the pre-vaccination sera had no significant influence on seroconversion rates of JE-HI and N antibodies after vaccination.

3. Observations on Singh's Aedes albopictus cell clones persistently infected with dengue and chikungunya viruses.

Virus-sensitive A. albopictus clone C6/36 cells were infected with each of the 4 types of dengue (DEN) viruses or chikungunya (CHIK) virus. Persistently infected (PI) cultures have been maintained over a year by weekly subculturing the infected cells. Assay of the infective virus from the CHIK-PI culture revealed that following the high yield of the acute infection (10^7 - 10^8 FFU/ml) virus titer fell to a level of 10^4 - 10^5 FFU/ml after 2 month of the initial infection. Plaques formed at 39°C gradually diminished their size 6-7 month after the initial infection and then no plaques were recorded after 8 month from the initial infection, although the virus from the PI culture still produced as large plaques as the standard CHIK virus at 34°C.

Observations on the DEN-PI culture did not show such a significant change in the titer of virus yield as in the case of CHIK. However, the viruses from PI culture were more or less temperature-sensitive.

4. Cystine deficiency inhibits DNA synthesis in Singh's A. albopictus clone C6/36 cells and chikungunya viral RNA synthesis in the infected cells

Deprivation of cystine from the culture medium markedly inhibited the growth of A. albopictus clone C6/36 cells and the production of chikungunya (CHIK) virus in the infected cells. The effects on cellular and viral macromolecular syntheses after cystine deprivation were examined. Incorporation studies using radioisotope labelled precursors revealed that the DNA synthesis was markedly inhibited 1 day after cystine deprivation.

While the RNA synthesis was declined gradually and the protein synthesis was not affected up to 2 days after deprivation of cystine from the medium.

Studies on the CHIK virus infected cells showed that the Actinomycin resistant, CHIK virus-specific RNA synthesis was virtually undetectable 1 day after cystine deprivation from the medium of the infected cells.

The results indicate that cystine deficiency inhibits the syntheses of cellular DAN and CHIK viral RNA preferentially, resulting in the inhibition of cellular growth and CHIK virus production. Addition of cystine to the medium reversibly restored these inhibited activities.

5. Production of hemagglutinin from Singh's A. albopictus cells infected with type 1 dengue virus

Stock of dengue virus type 1, Hawaiian strain, prepared in suckling mouse brains, was inoculated to A. albopictus clone C6/36 cells. After incubation at 28°C for 6-9 days under Eagle's medium supplemented with 2 % fetal calf serum and nonessential amino acids, significant amount of hemagglutination activity (titer between 64 to 256) was detected in the infected culture fluid. Pretreatment of the fetal calf serum with Kaolin enhanced the HA activity only slightly (2 fold at most). Addition of $MgCl_2$ to the medium or Tween 80-ether extraction of the infected culture fluid did not enhance the titer, although the latter treatment abolished the infectivity of the specimen. No significant HA was obtained from the infected cells after alkaline extraction.

Parallel titration of several human sera with the antigen prepared from infected A. albopictus cells (TC antigen) and with standard antigen prepared from infected suckling mouse brains showed that the TC antigen could successfully be used in routine serological tests in the case if standard antigen is not available.

(A. Igarashi, Y. Okuno, F. Sasao, M. A. Quina, Soe Thein, W. Auvanich and K. Fukai)

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

Investigations of the second outbreak of Japanese
Encephalitis in an area near Calcutta.

The first epidemic of Japanese encephalitis broke out in four districts (Burdwan, Bankura, Birbhum and Midnapur) in 1973 when 700 clinical cases with about 40 per cent mortality were reported. During 1974 and 1975 the disease was not detected. But in July 1976 it reappeared in one of the above districts, that is Burdwan, which is about 50 miles from Calcutta. The first case was recorded on 2nd May and the outbreak continued till October 1976. Altogether 277 clinical cases were reported.

A team from the Indian Council of Medical Research Virus Unit at Calcutta visited the area. The State Govt. staff helped us by sending specimens of blood, brain specimens from autopsy cases and mosquitoes. Subsequently teams from Vector Control Research Centre, Pondicheri, National Institute of Communicable Disease, New Delhi, and Virus Research Centre, Poona visited the area and the former two teams supplied us a number of mosquito pools. Altogether, 215 acute samples of blood, 76 convalescent samples, 90 c.s.f., 81 autopsy brain samples, 7 cattle sera, 47 pig sera, 4 goat sera and 1475 mosquitoes (in 72 pools containing *A.hyrceanus*, *A.barbirostris*, *C.fatigans*, *C.gelidus*, *C.pseudovishnui*, *C.vishnui*, *C.tritaeni-orhyncus*) were processed.

J.E virus could be isolated from one mosquito pool (*C.vishnui*) and one autopsy brain tissue only. 33 out of 76 paired sera tested by HI & CF showed rise of J.E titre, 32 showed rise of Gr.B titre, and 11 did not show any definite rise. Of the 47 pig sera, 22 sera showed high titre of HI antibody (ranging from 80 to 2560) against JE. The cattle and goat sera were negative to JE antigen. All the other mosquitoes were inoculated in mice and tissue culture without any positive findings.

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

REPORT FROM THE VIRUS RESEARCH CENTRE
(INDIAN COUNCIL OF MEDICAL RESEARCH)
20-A, Dr. Ambedkar Road, P.O. Box No.11,
Poona - 411 001, India

Febrile illness in exotic and cross-bred sheep
due to Ganjam virus.

Commencing early 1977, sporadic cases of febrile illness of unknown aetiology, some of which terminated fatally, occurred in exotic and cross-bred sheep of the Sheep Farm, Palamner, Chittoor District, Andhra Pradesh. The clinical symptoms, which were more severe in exotic sheep than cross-breds, consisted of high temperature, rising to 107°F, dullness, depression, inappatance, rapid laboured breathing and occasional mucus or blood-tinged diarrhoea.

Two acute phase serum samples and two tick pools consisting of a total of 72 females of Haemaphysalis intermedia yielded virus isolates. All these isolates were identified as strains of Ganjam virus by complement fixation (CF) and neutralization (N) tests. In the CF test, titres of the immune serum to prototype strain of Ganjam virus (G619) with the antigen of these isolates were as high or only slightly lower than the titres with the homologous G619 antigen. The identity of one of the isolates was further confirmed as a strain of Ganjam virus by the neutralization test. The neutralization index of G619 immune serum with the isolate was 2.44 dex LD₅₀ in infant mice, inoculated intraperitoneally, when compared with normal non-immune mouse serum. Two convalescent-phase serum samples completely protected the mice when inoculated with the respective serum-virus mixtures against a dose of 2.3 dex LD₅₀ of the G619 strain of Ganjam virus.

In an N test, one hundred and twenty three serum samples from contact sheep were tested with 2.3 dex LD₅₀ of the G619 strain of Ganjam virus. N antibodies were detected in all 23 local sheep, 97 per cent of the 75 cross-bred sheep tested, and 64 per cent of the 25 exotic sheep tested. The difference in prevalence between the exotic sheep and the cross-bred sheep and that between the exotic sheep and the local sheep are both significant ($p < 0.001$ and $p < 0.001$, respectively).

(G.R. Ghalsasi, F.M. Rodrigues, C.N. Dandawate, N.P. Gupta, C.G. Khasnis, B.D. Pinto, Samuel George and V.N. Pargaonkar)

Genetics of Susceptibility of *Culex tritaeniorhynchus* to Infection with West Nile Virus.

Over 150 strains of *Cx. tritaeniorhynchus* are maintained in our laboratory including wild-type stocks from Pakistan, Bangladesh, Taiwan, and Japan, morphological and biochemical genetic marker strains, translocations, inversions, lethals, and temperature-sensitive conditional mutants. Using a membrane feeding technique, 20 laboratory colonies have been screened for differences in susceptibility to West Nile virus. A virus-defibrinated blood suspension infectious for about 90% of a reference stock (Balloki strain) of *Cx. tritaeniorhynchus* was used for feeding the mosquitoes at each test for comparative susceptibility. Engorged females from each strain were held at $28 \pm 2^{\circ}\text{C}$ for 10 days and individually tested for virus using a direct FA assay. The susceptibility of most of the mutant and geographic strains was very similar at this virus dose (Table 1). Only one strain, e ma (ebony body and maroon eye markers), has been found significantly less susceptible to infection with West Nile virus than the reference stock. This susceptibility difference remained when the e ma stock was compared to the reference strain at the approximate ID_{50} for the latter. At this dose, 28.5% of the e ma females became infected and 55.7% of the Balloki females were positive for virus.

Studies on the basic vector competence of Cx. tritaeniorhynchus (Balloki strain) for WN virus also have been conducted to provide background data for the experiments on the genetics of susceptibility. By membrane feeding and by intrathoracic inoculation, ca.100 and ca.0.06 SMICLD₅₀ of virus, respectively, were necessary to initiate infection in 50% of the females. When feeding on viremic mice, 100% of the mosquitoes become infected after imbibing a virus dose of about 100 SMICLD₅₀. Infected females could transmit as early as 7 days when held at $28 \pm 2^{\circ}\text{C}$ after imbibing $10^{3.9}$ SMICLD₅₀ of virus and by day 12, 100% of the infected females transmitted to baby mice (Table 2). These results and virus isolations from wild females indicate that Cx. tritaeniorhynchus is an important vector of WN virus in Pakistan.

(C. G. Hayes, R. B. Baker, and R. K. Sakai)

Table 1. Comparative susceptibility of colonized mutant and geographic strains of Culex tritaeniorhynchus to infection with West Nile virus.

Cx. <u>tritaeniorhynchus</u> strain	Virus* dose	No. infected		Reference strain	
		No. tested	(%)	No. infected No. tested	(%)
Okinawa	2.95	16/18	(88.9)	NT**	
dt ew	2.95	7/8	(87.5)	NT	
re e ae	2.95	18/23	(78.3)	NT	
Ball-5	3.08	20/21	(95.2)	30/31	(96.8)
go ^k	3.08	48/48	(100)	30/31	(96.8)
Sendai	3.08	45/52	(86.5)	30/31	(96.8)
Ball-1	3.08	13/13	(100)	30/31	(96.8)
e ma	3.03	10/19	(52.6)	NT	
Tulamba	3.03	48/50	(96)	NT	
Cy Lp ae	3.03	16/22	(72.7)	NT	
Karachi	3.03	30/32	(93.7)	NT	
Rs/Rs	2.70	18/20	(90.0)	23/23	(100)
dt w	2.70	23/27	(85.2)	23/23	(100)
Khulna	2.70	23/27	(85.2)	23/23	(100)
Ball-4	3.16	10/11	(90.9)	3/3	(100)
W ^{IV} Chittagong	3.16	8/9	(88.9)	3/3	(100)
Taiwan Amy-F	2.60	11/14	(78.6)	11/12	(91.7)
Lahore	2.60	33/38	(86.8)	11/12	(91.7)
Lp	2.84	26/32	(81.2)	20/22	(90.9)
re sw	2.84	10/13	(76.9)	20/22	(90.9)

*Log₁₀ of virus dilution (SMICLD₅₀) ingested/mosquito.

** Not tested.

Table 2. Transmission efficiency of Cx. tritaeniorhynchus for WN virus at different days post-infection.

Day post feeding	<u>No. transmitting</u> No. feeding	(%)	Avg. titer of individual females*	Titer range of females**
6	0/20	(0)	5.27	4.44 - 5.82
7	4/17	(23.5)	5.35	3.32 - 5.28
8	5/10	(50)	5.37	4.44 - 5.69
10	6/7	(85.7)	5.30	4.19 - 5.71
12	35/35	(100)	5.59	5.19 - 5.82
14	5/5	(100)	5.49	5.32 - 5.69
16	5/5	(100)	5.77	5.32 - 6.07
21	1/1	(100)	4.82	

* $\text{Log}_{10} \text{SMICLD}_{50}$ based on 5-7 individual females except for single female from day 21.

** $\text{Log}_{10} \text{SMICLD}_{50}$

REPORT OF THE SECTION VIROLOGY OF THE MEDICAL RESEARCH CENTRE
OF THE NETHERLANDS AND THE NATIONAL PUBLIC HEALTH LABORATORY
SERVICES OF KENYA AT NAIROBI

From 6 August till 15 November 1974, mosquitoes were collected in the Lake Naivasha area. Virus strains were isolated from several mosquito pools. (see Information Exchange No.29, p.152). Some of the isolates mentioned appeared not to be valid.

At the Pasteur Institute of Dakar (Director Dr. Y. Robin), three strains isolated from Ae.dentatus were identified as Pongola virus of the Bwamba group, two from Ae.dentatus as Shokwe virus of the Bunyamwera group, two from C.rubinothus as Germiston virus of the Bunyamwera group and one from C.rubinothus as Uganda S virus.

Apart from these, Dr. Robin identified for us some old isolates. Three of these latter isolates were Somone-like virus from respectively pools of M.africana, collected in 1968 in the Marsabit area, of Ae.pembaensis, collected in 1967 at Mtwapa and from An.coustani, collected in 1969 at Malindi.

One isolate was an Okola-like virus strain. It was isolated in 1967 from a pool of Eretmapodites semisimplicipes, collected in Kikambala.

Marsabit is situated in the interior, the other three places in the coastal area of Kenya.

Somone virus is an ungrouped virus, isolated for the first time in Dakar - Senegal from the tick Amblyoma variegatum (Annual Report 1971, Institut Pasteur de Dakar). The Kenya isolates, isolated from mosquitoes, can be easily differentiated from the Senegal strain. Okola virus was isolated once, in 1969, in Cameroon, from Eretmapodites chrysogaster. Antigenically the Kenya isolate clearly differs from the Cameroon strain.

Isolates obtained from serum of patients suffering from the dengue-like illness that hit the Seychelles islands in December 1976 and lasted for several months (Information Exchange No.33, p.138) were provisionally identified by Dr. Y. Robin of Dakar as belonging to the group of Flavi viruses and as dengue virus type 2 by Dr. D.I.H. Simpson of the London School of Hygiene and Tropical Medicine. The strains had been isolated in Nairobi.

Moreover, Dr. Rosen (pers.comm.) isolated dengue type 2 strains at Honolulu from sailors, coming from the Seychelles.

The problem

Dengue fever was first reported in Madagascar by Lindin (1) in 1898, when he noted a disease with dengue syndrome in 67 soldiers at Tamatave. Later, in 1938, Sanner and Destribats (2) observed some cases of pseudo-dengue in Diego-Suarez. In 1948, McCarthy and Bagster Wilson (3) reported on new epidemics that had taken place after the occupation of the port of Diego-Suarez in 1942 and 1943. More recently (1962 and 1963) an acute febrile dengue-like disease has been observed in Diego-Suarez.

The intervention of the Pasteur Institute of Madagascar

It was on the occasion of this last epidemic that the Pasteur Institute became interested in the problem of arboviruses as a result of a survey conducted by Sureau (4) on 11 cases, with inconclusive results. Subsequently, the Pasteur Institute conducted numerous serological surveys involving many hundreds of individuals coming from various regions of Madagascar. These surveys have shown an active circulation of dengue 2, West Nile, Wesselbron and Uganda S viruses, but an absence of members of the Bunyamwera group (5,6). In 1974 Coulanges and collaborators (7) isolated 2 strains of Dakar bat virus from microchiroptera, and by testing 101 sera from Pteropus rufus by the haemagglutination-inhibition test, obtained evidence of the circulation of Sindbis, chikungunya, West Nile and Wesselbron viruses.

New orientation of the Pasteur Institute of Madagascar

Since 1976, it has been decided to carry out a program of systematic study based on three points:

Entomological studies by missions of the viral ecology unit of the Pasteur Institute of Paris under Dr. Rodhain, with the support of a technician-entomologist of the Madagascar Ministry of Scientific Research.

Attempts to isolate arboviruses from arthropods, mainly ticks and mosquitoes, performed at the Pasteur Institute of Madagascar by inoculation into newborn mice (local breed).

Attempts to isolate viruses from the blood of vertebrates and man, performed likewise at the Pasteur Institute of Madagascar. In this respect it should be mentioned that the natural fauna of the island does not include any monkey species, but does include 41 species of Lemurids (prosimians).

The viruses which will be isolated will be sent to the Pasteur Institute of Dakar for study.

Four missions have already been carried out within the framework of this program:

1. Southeast region, Farafangana (8).
- 2,3. Northern region, Diego-Suarez and Nosy-Bé (9). This mission has studied in particular the distribution of Aedes aegypti. A second mission has involved the capture of larvae to be raised at the Pasteur Institute of Paris.
4. Region of the Upper Plateaus--primo-secondary forest of Analabe.

To date, these have been inoculated into mice, 28 lots of ticks, 76 lots of Culicidae, 7 human sera, 10 brains and salivary glands of bats, and 1 brain of a Lemurid. No viruses have yet been isolated, but we are optimistic.

(R. Coulanges and Y. Clerc)

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During the second half of 1977 virological and serological studies were carried out on samples from Senegal (Dakar Bandia, Kedougou).

I - Virological studies

1. Human blood samples

53 blood samples from febrile children seen at the clinic in Bandia were processed into suckling mice for virus isolation with negative findings.

2 samples from febrile technicians involved in arbovirus work were processed for virus isolation without success.

2. Wild vertebrate samples

26 blood samples collected from monkeys shot in Kedougou were inoculated into suckling mice : no isolate was recovered.

An additional strain of Bandia virus was recovered from an *Arvicanthis* sp caught in Bandia : this isolation raises to four the number of Bandia virus isolated from rodents caught in Bandia forest. These studies were initiated following a sudden reduction of the rodent population in Bandia area. Bandia virus, which is pathogenic for *Mastomys* new-born may be involved in this phenomenon.

3. Arthropods

3.1. Mosquitoes : During the second half of 1977 12531 mosquitoes caught in Kedougou during the 1977 rainy season were inoculated into suckling mice in 459 pools. Work is in progress but it seems that yellow fever virus has been very active in the area : several strains of yellow fever virus have been isolated from pools of *Aedes furcifer taylori* and *Aedes luteocephalus*. These findings confirm the activity of yellow fever in the Kedougou area which was proved by the isolation, in the first half of the year, of a strain of yellow fever virus from a pool of *Aedes furcifer taylori* collected in december 1976 near Kedougou.

3.2. Ticks : Pools of ticks collected in the Senegal river area were provided by a team of the Yale University, New Haven Connecticut, headed by Doctor W.G. Downs.

From 372 pools of Ixodids ticks, 9 strains of Wad Medani virus were recovered : 8 from *Rhipicephalus guilhoni* and 1 from *Rhipicephalus evertsi evertsi*.

From 20 pools of Argasids ticks, *Alectorobius capensis* 10 strains of Soldado virus were isolated.

It is the first time that these virus are isolated in Senegal.

II - Serological studies

1.Human sera

1.1. From Dakar and Bandia

61 serum specimens were examined for arbovirus antibodies by HI, CF and neutralization tests.No specific diagnosis was possible.

1.2. From Kedougou

243 serum specimens from children were studied by HI, CF and N tests for group A, group B and Bunyamwera antibodies.

Chikungunya, Zika and Yellow fever viruses were shown to have been active in a recent past in the human population.

Serological pattern of recent infection was found in 23.3% of the population for chikungunya, 14.5% for Zika and 2.5% for yellow fever. Last year 1.2% of recent infections were found for yellow fever. This may indicate a growing activity of yellow fever virus in the area.

2.Wild vertebrate sera

2.1. Sera from rodents

52 serum samples from rodents caught in Bandia were studied in CF test for antibodies to rodent viruses isolated in Senegal : Koutango, Saboya, Bandia, Eg 754, Touré, Keuraliba and Men-go viruses.

16 were anticomplementary and 28 negative. On the 8 positive samples, 5 were positive for Bandia, 2 for Saboya and 1 for Koutango viruses. These findings confirm the activity of Bandia virus in the area.

2.2. Sera from monkeys

23 sera from monkeys caught in Kedougou were tested for HI, CF, and N tests antibodies for group A, group B and Bunyamwera viruses.

11 have HI antibodies for chikungunya virus and 5 have CF antibodies too indicating a rather recent infection : 2 were positive for Zika virus only, one for yellow fever virus only and 8 had HI and CF antibodies for more than one flavivirus.

(J.Renaudet and Y.Robin, Institut Pasteur,
M.Cornet and J.L.Camicas, ORSTOM, Dakar, Senegal).

Rift Valley fever in the Arab Republic of Egypt. In October 1977 an outbreak of disease was noted in Sharqiya Governate northeast of Cairo. The illness affected thousands of persons, and as many as from 60 to 120 deaths with hemorrhage and jaundice were attributed to the malady. Virus was isolated from human sera, throat washings, and feces.

A rapid presumptive identification of strain ZF41 was made at YARU using mouse brain material from Cairo in a CF test. The test sera or ascitic fluids were: group A; group B; group Bunyamwera; group phlebotomus; polyvalent Bahig, Tete, Matruh, Matariya and Burg el Arab viruses; chikungunya; Rift Valley fever (RVF); Nairobi sheep disease; Germiston; and Eretmapodites 147. Only the RVF serum reacted positively with a titer of 1:16, against the suspension of ZF-41 diluted 1:4.

Newborn mice inoculated with second passage stock sickened and were moribund 36 hours later. Antigens were prepared by the sucrose acetone method and by the freezing and thawing method from brain and liver tissues; an antigen prepared by acetone precipitation from the sera of the same mice agglutinated goose RBC at 1:1024, pH 5.75.

A CF test with the brain and liver antigens gave the result shown in Table 1; the tentative identification of ZF-41 as RVF was, therefore, confirmed.

An HI test done simultaneously using serum antigens for ZF-41 and Germiston viruses, gave the result illustrated in Table 2; not only did the HI test result coincide with that of the CF test, but in addition gave evidence of the presence of antibodies against ZF-41 in the serum of a patient.

In all, seventy-one human sera from patients of the Zagazig Fever Hospital were acetone extracted and HI tested for RVF antibody. Most sera were from the acute phase of the disease and were later found viremic. Four (#'s 5, 15, 16, 63) had HI antibody, the rest were negative at 1:10. Thirty of these were tested with group A and group B antigens in the HI test. Results were as follows:

Semliki	0/30
Sindbis	1/30
Chikungunya	0/30
Getah	0/30
Murray Valley	24/30
Central European TB	15/30
Dengue 2	16/30
Wesselsbron	15/30
West Nile	26/30

Highest titers were to West Nile antigen and were interpreted as representing prior exposure to this virus.

Fifty-five of the sera were also tested by CF with RVF brain sucrose-acetone antigen. Two (#'s 5, 15) were positive. Sera 16 and 63, positive by HI, were negative by CF.

Electron microscopy of thin-sectioned infected mouse liver and BHK-21 cells revealed typical Bunyavirus morphology.

Table 1. Complement-fixation Test: identification of strain ZF-41 as presumptive Rift Valley fever virus.

Antigen	Serum			
	RVF, sheep *	RVF, mouse	EMC	CHIK
ZF-41, Brain, sucrose-acetone	>32/>16	>32/>16	0	0
ZF-41, Liver, sucrose-acetone	>32/>32	>32/>32	0	0
ZF-41, Brain, frozen-thawed	>32/ 8	>32/>16	0	0
ZF-41, Liver, frozen-thawed	>32/ 16	>32/ 64	0	0
Control, Brain, sucrose-acetone	0	0	0	0
Control, Liver, sucrose-acetone	0	0	0	0

First dilution of serum and antigen, 1:4.

*Supplied by U.S. Army, Fort Detrick, Maryland

Table 2. Hemagglutination-inhibition Test: identification of strain Eg-ZF-41 as presumptive Rift Valley fever virus.

Sera *	Antigen	
	Eg-ZF-41 [4 units]	Germiston [2 units]
RVF, sheep	>1:1280	0
RVF, mouse	1:160	0
Germiston	0	>1:640
Zagazig fever, conv.man [#5]	1:640	0
Control, sheep	0	0
Zagazig fever, acute man [#'s 2,9-13]	0	0

First dilution of serum, 1:10

*Acetone extracted.

The typical human illness included fever, rigors, malaise, headache, muscle pains, early flushed face and eyes, conjunctival congestion, nausea and sometimes vomiting. Illness lasted from 2 to 5 days. There were 3 forms of complications: hemorrhagic fever and jaundice, often ending in death, ocular exudates and macular degeneration leading to different grades of visual impairment, and encephalitis with mortality in children.

Abortions in sheep and cattle were common starting in July, although this had not been apparent on the sixth of October when human cases were first notified.

The epidemic was believed to be arthropod-borne on epidemiologic grounds. Human cases apparently stopped in November after mosquito control measures were instituted, although animal disease was still being observed.

[Imam Z. Imam, J.M.Meegan, O.L.Wood, J.D.Converse, J.Casals, and R.E.Shope].

REPORT FROM ARBOVIRUS UNIT
DEPARTMENT OF BACTERIAL AND VIRAL DISEASES
ISTITUTO SUPERIORE DI SANITA', ROME, ITALY

Serological studies with Phlebotomus-transmitted viruses in human population.

As reported in the Arbo Info.Exch.26:49,1974, a Phlebotomus fever group virus, closely related to Naples Sandfly virus, was isolated from Phlebotomus perniciosus collected in a central Italian region (Toscana) in 1971. Although previous surveys for antibodies to Sandfly Sicilian (SFS) and Sandfly Neapolitan (SFN) viruses gave evidence for the persistence of natural cycles of these viruses in Italy, notwithstanding the large-scale spraying with insecticide during malaria eradication programme, little is known about the current status of sandfly fever in Italy. The recent isolation of strains of a virus closely related to SFN virus prompted us to investigate this question and to evaluate the incidence of antibodies against the two sandfly viruses (SFN and SFS) and the SFN-like virus (Phl.3 strain) separately.

The present study was restricted to the region (Toscana) where Phl.3 virus was isolated. Between 1974 and 1977 a total of 355 sera were obtained from human residents in three different rural areas of the region. The results of HI test performed by micromethod are summarized in Table 1. The overall SFN, SFS and Phl.3 infection rates in the study population were 9.6%, 7.6% and 21.9% respectively, the highest infection rate being with Phl.3 virus.

Tables 2, 3 and 4 show the prevalence of SFN, SFS and Phl.3 HI antibodies by age group in the three localities under study. As expected, the highest antibody rates were in the older age groups (30 years and above). Although the age distribution of some sera from Firenze is not yet available and the sample sizes are not always adequate,

these preliminary results show that only a few persons less than 30 years old had antibodies against SFN and SFS viruses (Table 2,3), while reactors to Phl.3 virus were found also in the younger age groups (Table 4).

The specificity of HI antibodies against SFN and Phl.3 viruses was proven by a plaque reduction neutralization test (PRNT) in VERO microplate cell cultures using tragacanth gum in the overlay. Sera were first tested in pool of five diluted 1:2 and heat-inactivated at 56°C for 30 minutes. Neutralization of 90% or more of PFUs was regarded as positive. Pools showing this activity were separated into components and tested individually at 1:10. The comparison of the results obtained by HI and PRNT with some sera from Firenze are shown in Table 5. The incidence of positives by age group is similar in both tests with SFN and Phl.3 viruses. Two sera positive at 1:10 for SFN virus in HI test were found negative in PRNT, while 10 sera negative in HI test for Phl.3 virus were found positive in PRNT. These 10 sera were scored as negative in HI test although a partial inhibition was present at 1:10, the lowest dilution used.

The results of our study indicate that infection with SFN and SFS viruses became uncommon, at least in this Italian region, after insecticide spraying programme in the forties, while the SFN-like (Phl.3) virus seems to still occur in the study area.

Although antigenic relationship between SFN and Phl.3 viruses has been demonstrated by complement fixation, HI, immunodiffusion and neutralization tests, the two viruses do not appear to be absolutely identical. Furthermore, in the present survey the incidence of SFN and Phl.3 antibodies in the population sampled was different and only few of the Phl.3 positive sera reacted with SFN virus. In order to check the specificity of our results, we calculated the expected and observed frequencies of HI double reactors of these two viruses. 9.6% (34/355) of sera were positive to SFN and 21.9%

(78/355) were positive to Phl.3 virus. Assuming that infections with these two viruses occur independently, the expected frequency of double reactors should be 0.020 (0.096x0.219). The actual observed frequency of double positives was 0.034 (12/355), which is higher than the expected and may indicate either some cross-reactivity or a greater risk of some individuals of being infected with both viruses. When we calculated the expected and the observed frequency of dual infections with SFN and SFS viruses, which are considered to be distinct, we found an observed frequency of double reactors (0.011) higher than the expected (0.007), thus supporting the possibility of a greater risk of double infection for some individuals.

The reported serologic data confirmed an antigenic difference between the SFN-like and the prototype SFN virus, giving further importance to the use of both viruses in antibody survey. Besides, while the human pathogenicity of SFN and SFS viruses has been well documented, it is still to be determined the importance of the SFN-like virus to humans.

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Table 1 - HI antibodies to SFN, SFS and Ph1.3 viruses in sera from Toscana residents.

Locality	N° tested	SFN		SFS		Ph1.3	
		N°pos. (%pos.)	(%pos.)	N°pos. (%pos.)	(%pos.)	N°pos. (%pos.)	(%pos.)
Grosseto	55	5	(9.1)	1	(1.8)	4	(7.2)
Arezzo	63	10	(15.8)	3	(4.7)	14	(22.2)
Firenze	237	19	(8.0)	23	(9.7)	60	(25.3)
Total	355	34	(9.6)	27	(7.6)	78	(21.9)

Table 2 - Prevalence of SFN virus HI antibodies in the three localities under study.

Age group (years)	Grosseto		Arezzo		Firenze		Total	
	N° tested	%	N° tested	%	N° tested	%	N° tested	%
0-5	5	0	-	-	19	0	24	0
6-15	14	0	4	0	40	0	58	0
16-30	15	13.3	14	0	[40	7.3	[88	11.3
31-40	4	0	15	33.3				
41-60	8	37.5	19	15.7	39	7.6	66	13.6
> 60	9	0	7	28.5	37	20.5	53	18.8
unknown	-	-	4	0	62	8.4	66	7.5

Table 3 - Prevalence of SFS virus HI antibodies in the three localities under study.

Age group (years)	Grosseto		Arezzo		Firenze		Total	
	N° tested	%						
0-5	5	0	-	-	19	0	24	0
6-15	14	0	4	25.0	40	0	58	1.7
16-30	15	0	14	0	[40	4.8	[88	2.2
31-40	4	0	15	0				
41-60	8	0	19	5.3	39	17.9	66	12.1
> 60	9	11.1	7	14.2	37	23.0	53	20.7
unknown	-	-	4	0	62	8.4	66	7.5

Table 4 - Prevalence of Ph1.3 virus HI antibodies in the three localities under study.

Age group (years)	Grosseto		Arezzo		Firenze		Total	
	N° tested	%						
0-5	5	0	-	-	19	0	24	0
6-15	14	0	4	0	40	7.5	58	5.1
16-30	15	20.0	14	28.5	40	42.5	88	32.9
31-41	4	0	15	33.3				
41-60	8	0	19	21.0	39	33.3	66	25.7
> 60	9	11.1	7	0	37	37.8	53	28.3
unknown	-	-	4	25.0	62	20.9	66	21.2

Table 5 - Percentage of SFN and Ph1.3 antibodies as detected by HI and PRNT tests in Firenze province residents.

Age group (years)	SFN			Ph1.3		
	N° tested	HI	PRNT	N° tested	HI	PRNT
0-5	19	0	0	10	0	10.0
6-15	37	0	0	30	10.0	16.6
16-40	36	2.7	0	34	44.1	50.0
41-60	35	5.7	5.7	30	40.0	40.0
> 60	36	16.6	16.6	36	33.3	36.1
unknown	41	2.4	0	36	33.3	44.4
Total	204	4.9	3.9	176	30.6	36.3

REPORT FROM THE NATIONAL INSTITUTE OF HYGIENE, BUDAPEST, HUNGARY

In collaboration with the Forest Research Institute, Budapest investigation of a TBE natural focus was performed in a Transdanubian County of Hungary, in 1977.

In the spring 3602 I.ricinus and 453 D.marginatus ticks were collected from the vegetation and in the autumn 48 Apodemus spp. and 3 C.glareolus were trapped alive.

Virus isolation experiments were performed by intracerebral inoculation of suckling mice and serological investigations by HI test.

1. Virological studies.

1.1. Ticks. Two TBE virus strains were isolated from I.ricinus nymphae.

1.2. Small mammals

1.2.1. Blood. From the heparinized blood sample of an A.flavicollis a TBE virus strain was isolated.

1.2.2. Organs. From the pooled organs of two A.flavicollis and from those of a C.glareolus a total of three TBE virus strains were isolated.

2. Serological studies.

2.1. Serum sample of a C.glareolus inhibited the HA activity of the TBE antigen.

2.2. Eleven of 50 forest workers had HI antibodies against the TBE antigen.

/E.Molnár, K.Gerzsenyi/

REPORT FROM WHO COLLABORATING CENTER
FOR ARBOVIRUS REFERENCE AND RESEARCH

INSTITUTE OF VIROLOGY, BRATISLAVA, CZECHOSLOVAKIA

Chronic tick-borne encephalitis infection in the host
animals.

Tick-borne encephalitis /TBE/ infection has long been known to occur in two forms: the acute disease characterized by meningitis and/or meningoencephalitis, and the second inapparent or latent form.

The persistence of TBE virus in the latent form following virus inoculation has been experimentally produced in a wide range of host animals. The persistence of TBE virus in the latent form in the presence of antibodies was recognized in goats as early as in 1957, when TBE virus was isolated from the CNS of a goat 14 days p. i. Viral aetiology was confirmed by virus isolation from brain, cortex, cerebellum and medulla oblongata and by histological findings.

Chronic TBE infection was observed in hibernating animals: bats, hedgehogs and dormice. In infected bats, viraemia lasted for 26 days, including the hibernation period, but in the brain the virus persisted up to 31 days. In some cases the virus titre in the blood reached values

of $10^{6.5}$ mouse LD_{50} in 0.03 ml after hibernation.

In the hedgehogs, the duration of viraemia is also influenced by temperature; in the summer it lasts for 3-6 days, in the spring and autumn it persists for 8-14 days. If the hedgehog in viraemic phase enters hibernation, the virus persists during hibernation. In the posthibernation period, viraemia lasts for 8 days with a titre up to $10^{4.5}$ ic mouse LD_{50} per 0.03 ml. The TBE virus was demonstrated in hibernating hedgehogs for as long as 85 days after infection.

Among the birds, chronic latent infection was studied in the coots and in wild and domestic ducks,

The first model used was the coot /*Fulica atra*/ because TBE virus was isolated from this species in nature. After experimental infection, viraemia was detectable from the second up to the 10th day with the highest titre on the 4th to the 6th day. The virus was isolated from the brain 1 month after inoculation. Similar results were described later in young individuals of *Fulica atra*. Experimental infection resulted in viraemia lasting for 14 days.

Another model used were wild ducks /*Anas platyrhynchos*/. Viraemia lasting up to 6 days after inoculation, with a maximum titre of 10.000 LD_{50} /0.05ml/ intraperitoneally in mice / was found. Virus was isolated from the brain and other organs 8 and 14 days after inoculation.

The maximum titre of virus was in the brain 8 days after inoculation /100.000 LD₅₀ per 0.05 ml intraperitoneally in mice.

After infection of wild ducks with *Ixodes ricinus* ticks, the virus was isolated from the blood 9, 11, 14 and 16 weeks after infection. The virus was isolated from the brain on the 19 th day after infection and from the spleen and liver on the 20th day. One duck died on day 37 after tick bite and TBE virus was isolated from its spleen and mesenteric lymph node.

Chronic latent infection was also studied in domestic ducks. Viraemia was found up to the 36th week after virus inoculation.

All these experiments, however, were carried out with the prototype Hypr strain of TBE virus which had undergone many passages in mice.

Therefore we attempted to study chronic latent infection with a newly isolated strain of TBE. This virus strain was isolated from *Tringa erythropus* captured in 1974 in south Moravia.

Experimental pathogenicity of the freshly isolated strain and of the prototype strain of TBE virus was studied in juvenile ducks. It was found that these ducks were more susceptible to the infection with the freshly isolated strain than with the prototype strain. Subcutaneous inoculation of ducks with the freshly isolated

Tringa strain / $10^{4.5}$ LD₅₀ / 0.01 ml/ led to clinical symptoms of meningoencephalitis in all experimental animals on the 11th - 12th day post inoculation. TBE virus was isolated from the brains and meninges of infected ducks.

Out of 8 ducks, inoculated subcutaneously with the prototype strain, only 3 showed clinical symptoms of meningoencephalitis after 23-26 days post inoculation, i.e.: the infection was accompanied by a longer incubation period. In all experimentally inoculated animals, HI antibodies have been found 14 days after inoculation /Table 1/.

In studying chronic infection in domestic adult ducks inoculated with the freshly isolated strain, all attempts to detect TBE virus from organs, failed. Two methods have been used for isolation of TBE virus:

a/ direct isolation from the organs of inoculated animals in suckling mice;

b/ preparation of organ cultures from the spleen, liver, lymph nodes, muscles, brain and medulla spinalis. By this method, cell proliferation was obtained in the spleen, liver, lymph nodes as well as in the brain and medulla spinalis. All attempts to isolate TBE virus from the organ culture media in suckling mice and by plaques methods were negative. In the organ cultures, no replication of TBE virus was detected.

It is of interest that during the observation period of 5 months, IgM antibodies were detected in ducks, inoculated with the freshly isolated Tringa strain /Table 2/. The persistence of TBE virus in the latent form and the ability of this agent to emerge in the presence of antibodies has long be recognized but the mechanism of the persistence of the virus in the host organism has not yet been elucidated. According to preliminary results, freshly isolated strains of TBE virus may cause acute illness in susceptible juvenile host animals, while adapted laboratory strains may have the tendency to cause chronic infection in the host animals. These findings may be of importance also for the preparation of TBE vaccines.

/M. Grešíková and M. Sekyová: Presented at the symposium: "Chronic virus infections", Smolenice, October 10th - 14th, 1977./

Table 1

Haemagglutination-inhibiting /HI/ antibodies in juvenile ducks inoculated with the prototypic strain of TBE virus

Duck No.	HI antibody titre 2 weeks p.i.		HI antibody titre 4 weeks p.i.	
	Control HI titre	HI titre after ME treatment	Control HI titre	HI titre after ME treatment
1	320	40	-	-
2	640	40	320	40
3	640	80	320	80
4	640	80	80	40
6	40	40	-	-
7	40	80	-	-
8	640	40	-	-
9	640	80	320	40

ME = 2-mercaptoethanol

- = Not examined

Table 2

Haemagglutination-inhibiting /HI/ antibodies in adult ducks inoculated with "Tringa" strain of TBE virus

Duck No.	HI antibody titre /at months post inoculation/											
	1		2		3		4		5		6	
	A	ME	A	ME	A	ME	A	ME	A	ME	A	ME
61	160	40	40	0	80	20	20	0	-	-	-	-
62	80	20	40	0	80	20	-	-	-	-	-	-
64	80	10	40	0	-	-	-	-	-	-	-	-
65	20	0	20	0	20	0	10	0	10	0	0	0
69	80	20	20	0	40	20	10	0	40	0	10	0
70	20	0	0	0	10	0	-	-	-	-	-	-
71	80	20	-	-	-	-	-	-	-	-	-	-
73	80	40	40	0	320	10	20	0	40	0	0	0
75	80	20	40	0	80	10	20	0	40	0	10	10
76	20	0	10	0	10	0	0	0	20	0	-	-

A = Sera treated by acetone only

ME = Sera treated by 2-mercaptoethanol and then by acetone

- = Not examined

Isolation of the Tettang virus in Czechoslovakia (Bohemia)

On investigating diseases with possible natural fociety virological testing of ticks in Vltava valley (Middle Bohemia) was carried out in spring 1977. This region was chosen, as there are known numerous cases of human encephalopathy of unclear etiology often with tick bite in anamnestic data.

Ticks were collected by flagging method in the period from May 30 to June 8, 1977. In all, 10,644 nymphs and imagos of the tick *Ixodes ricinus* (L.) were collected and tested for virus in 134 pools. Isolation tests were done in one-two-day old suckling mice from live ticks stored at 4° C until used - at most 7 days. A total 9 virus strains were isolated from the ticks, as shown in Table 1. Three of the isolates were identified as TBE virus and other three as Uukuniemi virus by means of indirect immunofluorescent method and neutralization test. The last three isolated strains reacted neither with TBE and Uukuniemi virus antisera nor with Tribeč virus antiserum, i.e. with no tick-borne virus known in Czechoslovakia until now. In the next studies identity of all three unidentified strains and their correspondence to Tettang virus was proved. The identification was carried out in cross reaction between all three strains and Tettang virus and their antisera by means of indirect immunofluorescent method. The results were confirmed by CF test. The correspondence between the isolates and Tettang virus was also indicated by the course of low passages, incubation period, and illness of mice, which agreed with the results obtained in the isolation of Tettang virus in West Germany.

The results will be published more in detail in *Folia parasitol.* (Praha), 1978.

(V. Danielová, J. Holubová, D. Málková, F. Dusbábek, V. Bukva, J. Ryba)

Table 1. Survey of virus strains isolated in central Bohemia

No. of strain	Virus	Material
8622	Uukuniemi	33♂♂
8633	Tett nang	100nymphs
8634	Uukuniemi	16♀♀ + 29♂♂
8641	TBE	100nymphs
8656	TBE	100nymphs
8679	TBE	45♀♀
8704	Tett nang	21♂♂
8725	Uukuniemi	75nymphs
8726	Tett nang	5♀♀ + 5♂♂

STRUCTURE OF TOGAVIRUSES:
POLYPEPTIDES, RNA AND MORPHOLOGY OF HOG CHOLERA VIRUS
(GENUS PESTIVIRUS)

(P.-J. Enzmann and F. Weiland)

Hog cholera virus grown in PK-15 cells was labeled with (^{35}S)-methionine and purified by centrifugation through a sucrose cushion followed by sucrose gradient centrifugation. At least three polypeptides with molecular weights of 55,000, 46,000, and 36,000 were resolved by polyacrylamide gel electrophoresis. Analysis of virus labeled externally with (^3H)-sodium borohydride revealed two glycoproteins: gp 55 and gp 46. The third structural polypeptide, p36, seems not to be glycosylated. The gp 46 was also present in the virus-free supernatant of infected cells. It could be demonstrated by radioimmune precipitation of virus labeled with (^{35}S)-methionine that all three polypeptides are specific for hog cholera virions.

The molecular weight of the viral RNA was determined to be about 4×10^6 in polyacrylamide-agarose-gel electrophoresis. In sucrose gradients the RNA has a $S_{20,W}$ value of 40-45 S. By comparing hog cholera virus RNA and Sindbis virus RNA it was noted that the sedimentation coefficient of Sindbis virus RNA was slightly lower than that of hog cholera virus RNA. In contrast the molecular weight of Sindbis virus RNA determined in composite polyacrylamide-agarose-gel electrophoresis was slightly higher than that of hog cholera virus RNA. The RNAs of these viruses may therefore differ in their secondary structure.

Electron microscopically hog cholera virus appeared as a spherical particle with a diameter of 42 ± 8 nm. The virus particles frequently displayed a fringe of projections with a length of about 6-8 nm.

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Serosurvey on incidence of antibodies against various arboviruses and arenaviruses in small mammals of Tunisia.

This survey was performed in 1976-1977 in collaboration with the Department of Parasitology and Applied Zoology, University of Rennes, France [Pr. J.C. BEAUCOURNU].

Blood samples from 156 small mammals [152 rodents and 4 microchiroptera] were collected in Tunisia in 1976 and 1977. Those specimens were studied for antibodies against nine arboviruses and three arenaviruses : Sindbis (SIN), West-Nile (WN), Dengue type 2 (DEN 2), tick-bite encephalitis, european (TBE), Calovo (CVO), Tahyna (TAH), Uukuniemi (UUK), Bhanja (BHA), Tribec (TRB), LCM, Junin (JUN) and Tamiami (TAM).

By CF test, no antibody was found against TRB and the arenaviruses. Haemagglutination-inhibiting antibodies against WN virus were detected in 19,8 % of the examined sera, mainly from Mus sp., Rattus rattus, Eliomys tunetae in the northern part of the Tunisia and from Ctenodactylus gundi and Pipistrellus kuhli in the south. In addition, antibodies against UUK and BHA viruses were found in 3,2 % and 4,5 % respectively of the same sera.

Thus, evidence of active circulation of the WN virus in Tunisia, previously established by studies of human sera [NABLI and all. Bull. W.H.O., 1970, 48, 181], is corroborated and the activity of two tick-borne viruses, UUK and BHA, may be strongly suspected.

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Seven Bwamba (BWA) group isolations were recovered during the five year course of the joint project of the British Medical Research Council and the Kenya National Irrigation Board in western Kenya.

Six of the strains which came from mosquitoes were identified as Pongola (PGA) and a single isolation from human blood was identified as BWA virus. It was found that when the NY-45 strain of BWA was fed to Mansonia uniformis or Anopheles gambiae species B, both species were infected after ten days. In gel diffusion tests mouse brain suspensions of the mosquito passaged NY-45 virus exhibited a common line with PGA virus against PGA antiserum which was not evident with either the M-459 strain of BWA or NY-45 strain which had not been passaged through mosquitoes.

This tends to confirm the theory of Kokernot, et al. (South African Journal of Medical Sciences 1957, 22: 81-92) suggesting that BWA and PGA were a single virus which altered antigenically with passage through different hosts. Moreover, Tomori and Fabiyi (American Journal of Tropical Medicine and Hygiene 1976, 25: 489-493) found in gel-diffusion and immunoelectrophoretic tests that all BWA group strains originally coming from mosquitoes which they tested appeared to be PGA and all strains from vertebrates BWA.

Nearly 20,000 ixodid ticks (1,444 pools) collected largely from cattle and goats in western Kenya have been processed for virus isolation. Virus strains have been recovered from 60 pools of Amblyomma lepidum, A. variegatum, Rhipicephalus appendiculatus and immature Rhipicephalus species.

Preliminary screening of the isolates by complement-fixation test indicates that 43 of the strains are members of the Congo (CON) group. Five of these isolates appear to be most closely related to Dugbe (DUG) virus and the remainder appear to be similar to CON virus. Work is continuing on confirmation of the identification. One strain isolated from a pool of A. variegatum was identified as Thogoto (THO) virus, and an agent reacting weakly with NIH grouping fluid Poly 6 (which is comprised of New World viruses Marco, Timbo, Chaco and Pacui) was recovered from one pool of Rhipicephalus appendiculatus and from one pool of Amblyomma variegatum. Seven virus strains did not react with any of our grouping fluids or antisera.

Of 28 isolates from ticks which were tested for ability to form plaques in Xenopus laevis cells (Leake, Varma and Pudney, Journal of General Virology, 1977 35: 335-339) 25 formed plaques.

B.K.Johnson.

Arbovirus Unit.

The Responses of the Nude Athymic Mouse to a Nominally Avirulent Arbovirus Infection

It was reported in the March 1977 Information Exchange (Number 32, p 130) that the responses of nude athymic mice differed markedly from those of their normal litter mates when both were infected by defined avirulent strains of Venezuelan equine encephalomyelitis virus (VEEV) or Semliki Forest virus (SFV). In further studies with the A774 avirulent strain of SFV (Journal of General Virology 12, 141-160, 1971 : 28, 225-250, 1975) it has been found that both age and sex are important determinants of nude mouse response.

When attention is confined to young mature mice of 40 to 70 days old, the responses of nude mice and their normal litter-mates to i.p. infection by 10^4 p.f.u. of A774 SFV are as shown in Figure 1. Also shown in the figure are the responses to infection of nude mice that had received a transfer of about 10^6 spleen cells from normal litter mates of the same sex on the day before infection. In Figure 1 the lines D show the proportion of mice (in groups of about 25) that died following the primary infection. The lines P show the proportions of mice that survived to the 21st day and were subsequently protected against challenge i.p. by the lethally virulent strain L10 of SFV. The lines S show the proportions of mice that survived to the 21st day but were not protected against this lethal challenge.

Since all mice were positively infected, as confirmed by the detection of antibody on the 8th day, the lines D, S, P in Figure 1 show a clear progression of the extent of stimulation and response as the mice gain the T-component of their immunological competence in the order nude (μ), nude with transfer ($\mu+s$), sibling (SIB). Primary deaths (D) indicate a failure to mount an immediately effective immunological feed-back. Deaths on challenge (S) indicate an adequate immediate response that saves against primary death but wanes by the time of challenge. Protection against challenge (P) indicates a sustained and near-normal immunological stimulation and response.

Although these results show a clear T-cell dependence of the regulations that control the expression of virulence, this is not a total dependence since at least half the female nudes mount an effective response to primary stimulation that saves against challenge infection yet is not marked by maintained antibody synthesis. All nude mice,

regardless of sex, show an impaired antibody synthesis that is marked by a normal rise for about the first week following by a decline to negligible levels by the 14th to 21st day : this indicates the T-cell independence of early antibody stimulation and synthesis but the later T-cell dependence of the 'memory' that allows antibody synthesis to be maintained. All nude mice, regardless of sex, regain a normal and maintained antibody synthesis after spleen cell transfer and T-cell reconstitution, but, as shown in Figure 1, this is not associated with the uniform recovery of a normally protective response to the primary virus infection.

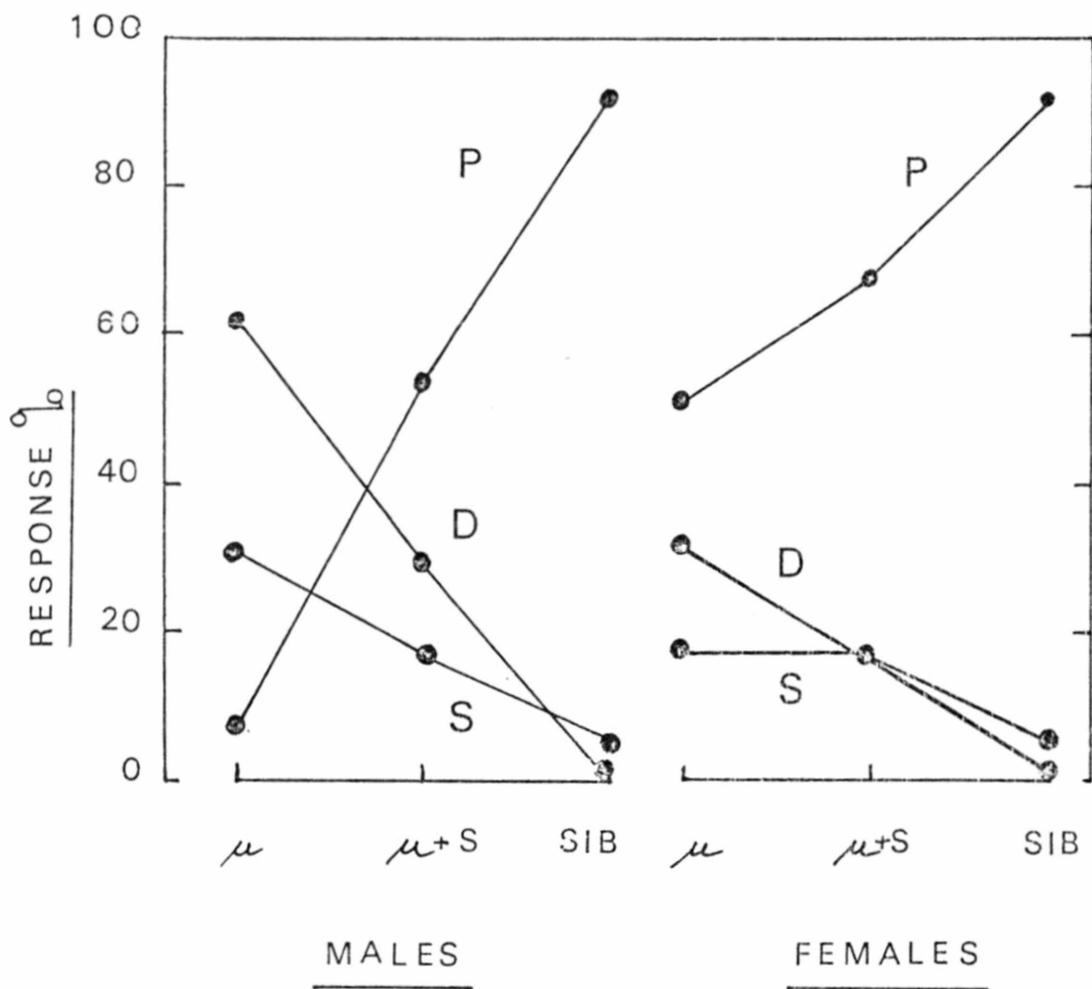
Since the earlier transfer of higher concentrations of sibling spleen cells has not improved this incidence of immunological recovery, it appears that up to half the recipient nudes may remain imperfectly receptive or responsive in T-cell or other functions. That the expressions of response and virulence, particularly in terms of clearance and persistence mechanisms, are not entirely T-cell dependent is indicated also by the following significant features:

- i) the elimination of viraemia is complete within 4 days of primary infection in female nudes and siblings but not until the 5th or 6th day in male nudes and siblings.
- ii) characteristic brain lesions are apparent by the 3rd day in nude mice and by the 4th to 5th day in siblings. Brain lesions in nudes and siblings are indistinguishable by the 6th to 7th day.
- iii) brain infectivity is eliminated by the 9th day in siblings and in nudes after spleen-cell transfer. Brain infectivity may persist at up to 10^4 p.f.u./brain in nude mice and may be associated with late deaths at up to five weeks or longer after primary infection. Thus these nude mice regard the A774 strain of SFV as persistent rather than virulent.

These observations will shortly be presented in greater detail elsewhere, particularly in comparison with the immunomodifications imposed by cyclophosphamide or γ -irradiation.

(C.J. BRADISH & R. FITZGEORGE)

FIG 1



TITRATION IN VITRO OF SILVERWATER VIRUS (SIL):

The Silverwater virus (SIL) could induce cytopathogenic effects (CPE) in a variety of cell lines including Vero cells. The results presented in this report show the advantage of the plaque forming assay and the TCID₅₀ for the titration of the SIL. This study also reports the effect of DEAE dextran and trypsin on the plaque formation by this virus.

Cell cultures.

The African green monkey kidney (Vero) cell line (ATCC CCL81) was used throughout these studies. It was maintained as a monolayer in Hanks 199 media (GIBCO), (Grand Islands N.Y.) containing HEPES buffer (Sigma Chemical, St. Louis, Mo.) and 2% fetal calf serum (GIBCO).

Silverwater virus was kindly provided by Dr. H. Artsob (National Arbovirus Reference Center, Toronto, Canada). Virus stocks were obtained in vivo, the following way: 2-4 days old mice were infected intracerebrally with the virus (0.02 ml) and 6 days later, the mice were sacrificed, the brain aspirated aseptically with a syringe and centrifuged at 4500 rpm (International Clinical Centrifuge) for 15 min. at 4°C. The resulting supernatant was collected and diluted a thousand fold with bovine albumine phosphate buffer saline 0.75% (BAPS) (GIBCO); the viral suspension obtained was kept at -80°C in 1 ml quantities.

Virus stocks were obtained in vitro, the following way: Falcon bottles (Fischer Scientific, Montreal) containing a monolayer of Vero cells, were washed with fresh medium and inoculated with one ml of virus obtained in vivo; after one hour adsorption at 37°C, the liquid in the bottles was discarded, the cell sheet washed again and fresh medium was added. After 4-5 days incubation at 37°C, 75-100% of the cells showed CPE: granulation, rounding and death of the cells. The bottles with the medium were frozen (-80°C) and thawed three times. The medium was centrifuged at 4500 rpm for 15 min. at 4°C to remove cell debris and the supernatant was kept at -80°C in 1-5 ml quantities. Four passages were made in vitro using the techniques described above and titrated by various methods for comparison.

Titration by the plaque-forming assay

The technique used was essentially the one described by Boisvert and Yamamoto (1).² Confluent monolayers of Vero cells in plastic bottles (Falcon 25 cm²) were inoculated with 0.5 ml of tenfold viral dilution made with BAPS. After an adsorption period of one hour at 37°C, the cells sheet was covered with 8 ml of Hanks 199 containing 1.5% Noble agar (Difco, Michigan). A second agar overlay containing 0.01% neutral red was added 24 h. later. Incubation at 37°C was continued for 24 h. in the dark, after which time the number and size of the plaque were determined.

The total time of incubation of 72 h. was found to be optimum for the number of plaques and the ease at which they were read when counting between 30-100 plaques were utilized to determine the number of plaque forming unit/ml (PFU/ml).

Effect of DEAE dextran and trypsin on the plaque forming assay

Plaque assay was performed as described earlier using the fourth in vitro passage. Trypsin (Sigma Chemicals, St. Louis, MO.) and DEAE dextran (kindly supplied by Dr. J. Arora, Institut Armand Frappier, Laval-des-Rapides, Quebec) were added to the viral inoculum at concentration of 25, 50, 100 and 200 µg/ml. Controls without DEAE dextran or trypsin were also made.

Titration by the TCID50 method

The titration of the successive passages of Silverwater virus was made with Vero cells using a micro culture technique in Cooke Microtiter plates (Fisher Scientific, Montreal). Each well received 0.15 ml of Vero cells in Hanks 199 with 2% calf serum (120,000 cells/ml) and 0.025 ml of tenfold viral dilutions made in BAPS. A total of 8 wells were inoculated with each viral dilution and two received BAPS as control. The plates were incubated at 37°C. After 8 days, the wells were examined for the presence of characteristic CPE of Silverwater virus. The titer of the viral suspension was determined by the Kärber method (2) and expressed as TCID50/ml.

Titration by the LD50 method

Suckling mice (3 days old) were inoculated intracerebrally with 0.02 ml of tenfold viral dilutions made with BAPS. A pool of nine mice were inoculated with each dilution and controls were inoculated with Hanks 199 and BAPS. Mortalities were read each day for a period of 15 days. The titer of the viral suspensions was determined by the Reed and Mñench method (3) and expressed as LD50/ml.

Results

Successive passages were made in Vero cells from the first passage in vitro as described above. Each passage was titrated for infectivity by the TCID50, LD50 and the plaque forming assay. As shown in table 1, there was a twofold increase in the titer obtained by the plaque forming assay (1.12×10^5 to 2.28×10^5) but no significant change of the titer by the TCID50 even after four successive passages in Vero cells. On the other hand, a significant increase (2.12 log unit) was noted when these passages were titrated by intracerebral infection of suckling mice (LD50). When the relationship between LD50 and infectious unit was examined (4), it was found that the ratio of PFU/ml over TCID50/ml was always inferior to 0.7 except for the last passage. The results were generally higher when the passages were titrated by the TCID50 (table 1). When the plaque forming assays were performed using DEAE dextran and trypsin as a mean of improving the titer, it was found that there was an increase in both the number of PFU/ml and the average diameter of individual plaques (table 2).

Both DEAE dextran and trypsin gave a slight increase (25 to 30%) in the number of PFU/ml. In both cases, the highest titer was obtained when 100 µg/ml was used. At 200 µg/ml there was destruction of the cell sheet when using either DEAE dextran or trypsin. The diameter of the plaques was increased in both cases; DEAE dextran gave the largest diameter of the plaques.

Discussion

The results presented in table 1 indicate that SIL can be titrated in vitro by either the plaque forming assay or the TCID50 using Vero cells. The analysis of the three modes of titration of SIL (table 1) shows that this Bunyalike virus can be titrated with success in vitro and in vivo. Plaque forming assay and TCID50 are methods which have numerous advantages over in vivo methods like the LD50. So either these methods should be more useful for the titration of the SIL.

When all three methods of assaying the virus were compared, it was found that the ratio of PFU/TCID50 was less than 0.7 indicating that the TCID50 assay was a more sensitive method of titration than the plaque forming assay for the SIL (4). On the other hand, the ratio of PFU/LD50 was always superior to 0.7 (except in one case) indicating that the plaque forming assay was a more sensitive method of titration of SIL than the LD50(4).

By order of sensitivity, the TCID50 was better than the plaque forming assay and in turn the latter was superior than the LD50. This would imply that titration of SIL by the usual LD50 assay was the least sensitive technique.

The use of DEAE dextran or trypsin did enhance the number of plaque and also the diameter of the plaques. The increase in the number of plaques was the same (25%) whether DEAE dextran or trypsin was used while the largest plaque diameter was obtained using DEAE dextran (table 1)

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J. Boisvert and S. Belloncik

TABLE I

Comparative assay between PFU, TCID₅₀ and LD₅₀ of Silverwater virus propagated in vitro

Passage number	TCID ₅₀ /ml	PFU/ml	LD ₅₀ /ml	PFU/DL ₅₀	PFU/TCID ₅₀
2	$4 \times 10^{6.75}$	1.12×10^5	$5 \times 10^{3.75}$	4.0	0.005
3	$4 \times 10^{6.175}$	1.8×10^5	$5 \times 10^{3.77}$	6.12	0.008
4	$4 \times 10^{6.50}$	2.37×10^5	$5 \times 10^{4.19}$	3.05	0.018
5	$4 \times 10^{6.63}$	2.28×10^5	$5 \times 10^{5.87}$	0.061	0.013

TABLE 2

Effect of DEAE dextran and trypsin on the plaquing efficiency of Silverwater virus in Vero cells

Concentration (µg/ml)	Titer (PFU/ml) ⁽¹⁾				
	0	25	50	100	200
DEAE-dextran	2.39×10^5 (0.5-2.0 mm) ⁽²⁾	2.95×10^5	2.95×10^5	3.10×10^5 (0.5-3.0 mm)	D ⁽³⁾
Trypsin	2.39×10^5 (0.5-2.0 mm)	2.64×10^5	2.64×10^5	2.98×10^5 (0.5-2.5 mm)	D

- (1) The fourth passage was used for this study
 (2) Range of the diameter for 100 plaques
 (3) Complete destruction of the cell sheet

DENGUE FEVER IN TRAVELLERS RETURNING TO CANADA

During 1977, outbreaks of dengue fever were reported in many Caribbean countries including Jamaica, Puerto Rico, Bahamas, Dominica and Haiti. Single or paired sera were received at the National Arbovirus Reference Service from twenty-two cases of suspect dengue fever in travellers returning to Canada with a history of recent travel to the Caribbean. Serological testing of these sera revealed diagnostic increases in titers to flavivirus antigens (dengue, St. Louis encephalitis, Powassan) by haemagglutination inhibition and/or complement fixation tests in seven patients. Four-fold declines in antibody titers indicative of recent flavivirus infections were demonstrated in sera of six patients while eight other patients demonstrated elevated flavivirus antibody levels.

An agent was isolated in *Aedes albopictus* cell culture from the acute serum of one patient who demonstrated a diagnostic flavivirus antibody increase. Neutralization tests performed in African green monkey kidney cells have shown the agent to be dengue virus. Studies to determine the exact serotype are currently underway.

Sera from suspect cases were submitted from laboratories in five provinces - Nova Scotia, Quebec, Ontario, Manitoba and British Columbia. All patients related a history of recent travel to Jamaica with the exception of two who had visited Haiti.

(H. Artsob, L. Spence, C. Th'ng and A. Gillani)

REPORT FROM

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An Ontario human isolate of St. Louis encephalitis (SLE) virus, strain Ont 327-75, was transmitted by bites of wild caught arctic Aedes communis mosquitoes after 13 to 76 days of extrinsic incubation at 13°C following intrathoracic injection with 300, 30 or 3 mouse LD₅₀ virus. Infective virus at titres 1.5 to 3.3 log mouse LD₅₀ for salivary gland were detected regularly after 27 to 76 days incubation at 13°C, but less frequently in those tested at 6 and 13 days. Although virus infectivity was demonstrated in 75% of mosquitoes, less than 35% of salivary glands showed antigen by indirect immunoperoxidase or immunofluorescence tests.

A. communis transmitted SLE virus after 13 days incubation at 13°C after imbibing 30 mouse LD₅₀ in blood meals, and after 27 days incubation at 13°C after feeding on 3 mouse LD₅₀. Although 16 of 17 salivary glands of mosquitoes incubated at 13°C yielded infectious virus, indirect immunoperoxidase or immunofluorescence tests showed virus antigen in only 4 of 15 glands.

Virus replication was demonstrated regularly in A. communis after 6 to 20 days of extrinsic incubation at 23°C after feeding of 3 mouse LD₅₀ or injection of 3, 30 or 300 mouse LD₅₀ SLE virus, but transmission experiments have not yet been attempted.

Domestic A. aegypti supported multiplication of SLE virus both after feeding and intrathoracic injection of 10, 100 or 1000 mouse LD₅₀, following incubation for 6 to 20 days at 13°C and 23°C. Although infective virus was detected in 71 of 84 salivary glands tested, indirect immunoperoxidase tests revealed antigen in 12 of 40 glands and indirect immunofluorescence tests showed antigen in 14 of 42 glands.

(D. M. MCLEAN)

Surveillance for arboviral disease in California during 1977 revealed only 1 confirmed human case of St. Louis encephalitis (SLE), out of 316 suspected cases tested serologically, and 1 confirmed equine case of western equine encephalomyelitis (WEE), out of 31 suspected cases tested serologically. The human case was a 42 year old woman most likely infected at the Salton Sea, Imperial County, July 16-17. Serum samples taken August 1, August 12 and October 31, showed SLE antibody titers as follows: (CF) 1:4, 1:16 and 1:32; (HAI) 1:160, 1:640 and 1:160 (Dr. W. C. Reeves' laboratory); (IFA) 1:128, 1:512 and 1:512; and (PRNT) 1:2048, 1:16,384 and 1:512. She recovered after a 2 week hospitalization for high fever, confusion, and coma. The unvaccinated 1½ year old equine which recovered after typical illness was from a ranch on the border of Riverside County and Yuma County, Arizona. The WEE CF antibody titer rose from <1:16 to ≥1:64 and the IFA antibody titer rose from <1:8 to 1:128. Brain samples from 16 other suspect equine cases, and 12 suspect human cases were tested in suckling mice but yielded no arboviruses. Seroconversion for SLE was shown in 2 of 30 chickens in a sentinel flock in Imperial County.

A total of 836 mosquito pools (33,649 mosquitoes) were tested in suckling mice, yielding 44 viruses: 19 WEE, 13 Turlock, 6 SLE, 5 Hart Park, and 1 California encephalitis (CE) group. This was the first CE group virus we have isolated since 1973, and the first WEE virus since 1974. Our largest surveillance effort was directed at Southern California Counties, since the severe drought conditions and more extensive mosquito control programs in the San Joaquin and Sacramento Valley areas of the State have helped minimize the problems there. All viruses were isolated from Culex tarsalis, except 3 strains of WEE from Aedes vexans, 1 strain each of WEE and SLE from Culex erythrothorax, and CEV-group virus from Aedes melanimon.

There was special interest in dengue during 1977, because of large outbreaks in the Caribbean, Pacific and South-East Asian regions. Eight cases of dengue in Californians exposed during travel were verified by serologic tests (IFA, CF, and PRNT): 5 from Jamaica, 1 from Puerto Rico, 1 from Tahiti, and 1 from the Philippines. Dengue type 1 was the apparent infecting serotype in all cases, based on the serologic findings.

There were 10 cases of Colorado tick documented during the year, 8 from exposure in known endemic areas of Northeastern California, 1 from Idaho, and 1 apparently from Colorado.

(R.W. Emmons)

REPORT OF THE ARTHROPOD-BORNE VIRUS RESEARCH UNIT,
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This report is the "Summary Statement" from a detailed research progress report that reviews field and laboratory studies on arboviruses and their vectors in California during the period May 1, 1974 through April 30, 1977. Extensive mailings have been made of the complete report. Copies are available upon request.

Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) have decreased to a level where there are only 1 or 2 cases per year in the state and no proven cases of WEE or SLE in man or horses occurred in our study areas in the San Joaquin and Sacramento Valleys during the period of this report. We believe this reflects a continuing effective level of control of the primary vector Culex tarsalis coupled with widespread occurrence of C. tarsalis populations that are relatively incompetent vectors. We have continued to monitor basic physical and biological factors in the environment that may influence the return of encephalitis to earlier epidemic proportions. Climatological factors such as temperature and rainfall have not deviated from normal except for a decreased rainfall in 1976. Elevated temperatures and rainfall commonly are associated with increased vector and viral activity in California.

We have continued to monitor vector populations and immunological conversions to WEE, SLE and Turlock (TUR) viruses in sentinel chickens that are exposed in representative areas of the San Joaquin (Kern County) and Sacramento Valleys (Butte, Glenn, Yuba, Sutter and Placer Counties). In Kern County, light trap indices of the C. tarsalis populations stayed low at all urban and most rural sites through the 3 years. Over 200 sentinel chickens were exposed each year. There was no evidence of SLE viral infection. Less than 1 percent of the birds converted to WEE in 1974, 20 percent in 1975 and none in 1976. The WEE viral activity in 1975 was all on the "West Side" of the Valley, an agricultural area produced by a new irrigation development. In contrast to WEE and SLE, TUR viral infection was detected in almost all sentinel flocks. The observed levels of viral activity are supportive of the hypothesis that TUR viral cycles can be maintained by very low C. tarsalis populations while the threshold levels to maintain WEE and SLE infections are considerably higher.

Parallel monitoring of viral activity in the Sacramento Valley revealed that WEE and SLE viral infections were practically absent from 1974-1976, except for a single conversion to WEE in over 200 sentinel chickens in 1974. As in Kern County, TUR viral infection occurred in almost all sentinel flocks. The overall levels of C. tarsalis populations are much higher in the northern area than in Kern County, and generally would be considered sufficient to maintain transmission cycles of WEE and SLE viruses at a low level. We continue to search for explanations of the lack of persistence of WEE and SLE viruses in their basic cycles in the Sacramento Valley. Suspected factors that we believe decrease viral transmission include high autogeny rates, frequent divergence of blood feeding from birds onto large mammals and general vector incompetence.

The search was continued for evidence of transovarial infection of California encephalitis (CE) virus in its primary vector Aedes melanimon. CE virus has continued to be active in the Sacramento Valley area as we isolated 5 strains from 10,369 female Ae. melanimon in 1974 and 6 strains from 6,732 females in 1975. No CE viral isolates were made from 7,512 male and 7,646 female Ae. melanimon reared from larvae and pupae collected in the same areas as the viral positive pools of adult females. Thus, there was no evidence of transovarial transmission.

We wished to compare WEE viral infection rates between Ae. melanimon and C. tarsalis collected in the Sacramento Valley but tests of the 17,101 Ae. melanimon females referred to above, failed to yield WEE virus, thus confirming the general absence of this virus. The only viral isolates in tests of C. tarsalis were 1 strain of TUR virus and 2 strains of Hart Park (HP) virus.

Paired serum samples from 647 human and 11 equine cases of suspect encephalitis that had onsets in the period 1973 through 1976 were screened for diagnostic rises in antibody titers to 11 arboviruses endemic in California. While a number of the sera contained HI neutralizing substances to WEE, Venezuelan equine (VEE), SLE, Powassan (POW), Modoc (MOD), Rio Bravo (RB), Lokern (LOK) and Main Drain (MD) viruses no diagnostic rises in titers were detected. The Group B reactors largely reflected high titered SLE antibodies and cross reactions with the related antigens.

We have established an extensive computer bank that includes field and laboratory data collected over more than a 20-year period. As examples, it encompasses temperature and rainfall data from our study areas, records of all confirmed cases of WEE and SLE in man and horse from 1953 to date, serological tests on domestic and wild animal sera, light trap indices for C. tarsalis and Ae. melanimon females from 1953 to date, blood meal identification data from 8,000 mosquito collections and most other records routinely collected and described in this report.

A major new objective for our research during this report period was to develop a working knowledge of the genetics of C. tarsalis and to evaluate if the insertion of genetically modified populations into field populations could contribute to an integrated program to control C. tarsalis. We have isolated 12 mutations of C. tarsalis and established them as laboratory colonies. Seven multiple-marker lines have been constructed that carry at least 1 marker on each of the chromosomes. The markers are being used extensively in genetic studies. As an example, with the multiple-marker lines 27 chromosomal genetic interchanges have been recognized and 3 autosomal and 9 sex-linked lines are established. Two sex-linked translocations are multiple, involving all 3 chromosomes, and 2 autosomal translocations are now homozygous. In several of the sex-linked interchanges 2 types of abnormal segregation have occurred on an irregular basis. Each type of segregation appeared to be a meiotic drive system that resulted in a surplus of recombinant progeny. In addition, a sex-switch mechanism occurred in several lines. These occurrences are the subject of continuing study.

Several of the mutants we have derived have the potential to be semi-lethal. One with markedly altered patterns of wing scale distribution designated as fringe (fr) interferes with emergence of adults from pupae. There is a 26-30

percent mortality. This and other mutants are now being evaluated as population control agents.

Preparations were begun in 1973 for field trials to determine if introductions of genetically modified populations would be effective for population control. Several isolated field areas were selected in the San Joaquin and Sacramento Valleys and basic studies were begun to establish for the C. tarsalis population: estimates of numbers of each sex, insecticide susceptibility profiles, autogeny rates and vector competence for WEE and SLE viruses. In addition, monitoring of WEE, SLE and TUR viral activity was begun at each site.

In 1974, we developed a new program that would allow us to rear C. tarsalis from egg raft introduction to adult emergence in small artificial outdoor ponds. Our purpose was to rear and release genetic stocks in the field rather than in the laboratory. Thus egg rafts of a desired stock would be produced in the laboratory and transferred to field rearing ponds where adults would emerge. Schedules were developed that produced 3,000 egg rafts, of 200 eggs each, in a single night's oviposition of females in a single cage. These females had been derived from only 150 egg rafts. When transferred to the field the time from egg placement to first adult emergence ranged from 10 days in summer to 25 days in the spring. Ponds produced a mean number of 9,041 and a maximum of 15,147 adults (52 percent males) per unit.

In 1976, a mark-release-recapture study of adult C. tarsalis was begun to provide better data on the actual size and fluctuations of populations at Poso West, the site selected for the first genetic releases in 1977. A total of 20,845 adult C. tarsalis were marked and released in 6 monthly studies. Collection of 132,719 C. tarsalis led to recovery of 52 marked males and 867 marked females. Daily survival estimates for females ranged from 64 to 77 percent. Estimates of the mean female population size in the study area ranged from 154,892 in June to 52,836 in September. Daily estimates of emergence of females ranged from 51,992 per day in June to 12,115 per day in September. The monthly estimates of population size that were derived now serve as guides in developing computer programs to simulate seasonal population fluctuations and to estimate the number of genetically modified mosquitoes to be introduced for a competitive field trial.

We selected 1 of the radiation induced sex-linked translocations of C. tarsalis, T(1;2;3)1A, for the first field trials to be done in 1977. The strain carries 2 interchanges that in pseudo-linkage bind the 3 chromosomes involved and transmits them as a set to male progeny. The mean percent of egg hatch of rafts fathered by this line ranges between 20 and 30 percent. The males of this heterozygote translocation line were competitive with males from several colony stocks in mating trials in small cages and with males from field populations in large outdoor cages. Two newly derived homozygous translocation lines are currently scheduled for competitive mating trials in 1978 in preparation for field releases.

A variety of biological factors are being monitored in the areas selected for pilot studies on genetic control. The purpose is to detect changes coincidental to introduction of genetically modified populations. Serological tests on

sentinel flocks at 4 field areas in 1975 and 1976 revealed no evidence of WEE or SLE viral transmission but relatively high levels of TUR viral infection. Identification of blood meals from C. tarsalis collected at the Poso West study site revealed the expected pattern of feeding principally on birds. Repeated tests were done to determine the vector competence for WEE and SLE virus of field populations of C. tarsalis collected from Poso West and occasional samples from 2 other field sites (McVans and Sheridan). Generally the populations were not very competent, having ID₅₀'s of 10^{3.0-5.4} PFU for WEE virus by plegget feeding and ID₅₀'s of 10^{3.8} PFU to greater than 10^{5.0} PFU for SLE virus. In addition, the majority of infected females could not transmit WEE virus after 13-14 days extrinsic incubation. Adult females that were reared from pupae or collected in light traps with CO₂ were compared for their susceptibility to WEE virus. The trap collected individuals (biting population) always were slightly more susceptible than those reared from pupae. Autogeny is monitored regularly and the rates range widely in different samples from the Poso West and McVan sites, 2 to 28 percent in adults reared from pupae and 15 to 60 percent in field collected newly emerged adult females. There was no correlation between autogeny rates and vector competence when fed on WEE or SLE virus. Tests of C. tarsalis larvae collected from Poso West and McVans in 1975 and 1976 consistently indicted they were susceptible to ethyl parathion.

A study was begun in 1976 to evaluate if density-dependence would be a significant factor that affected the aquatic populations of C. tarsalis and if so, how it would affect efforts to control this species. The study was carried out at Poso West to take advantage of the many other sets of data being collected at this site. Principal findings in the first year were that wild females produced 150-260 ova per raft, an average of 190 ova. There was a 90-100 percent egg hatch. The sex ratio of emergence from pupae was approximately equal. Three characteristics had approximately a negative exponential relationship to larval density and they were: size of adult females, survival from egg hatch to adult emergence and aquatic developmental time. Limited density-dependence, in conjunction with other effects such as water level and predation probably influence summer population limits and the studies are being extended to clarify these relationships.

A computer program was developed to simulate the effect when C. tarsalis that are carrying translocated chromosomes are released into wild populations of the same species. The model is based on the relative and absolute estimates of the 1975 and 1976 C. tarsalis population at Poso West. The model incorporates estimates of seasonal fluctuations in age and reproductive potential, density-dependent and density-independent factors and the genetic type and sex of mosquitoes that are released at any time. As information on each newly engineered genotype has become available we have incorporated data on its mating competitiveness, fecundity, sterility and survival rates. The model provides an essential implement in our planning for field trials on genetic control.

Aedes sierrensis (the western tree-hole mosquito) has been the subject of studies by this research unit for over 40 years. We continue to reexamine its relationships to arboviruses. This species is also a troublesome pest in urban-suburban and recreational areas. Our genetics staff has initiated laboratory studies to determine if the sterile male technique can be applied for control of A. sierrensis. Schedules have been established for the sterilization of males

by irradiation in normal and nitrogen atmospheres and procedures have been developed for comparative evaluations of mating competitiveness and determination of sterility rates of these populations. We expect to extend these studies into field trials by 1979.

Previous studies on the vector competence of C. tarsalis for arboviruses demonstrated for the first time that field and colonized strains varied significantly (1,500 fold) in their susceptibility to infection after ingestion of WEE virus and possibly SLE and TUR viruses. During the past 4 years we have attempted to identify and elucidate intrinsic factors that affect vector competence. The initial studies suggested that the susceptibility of C. tarsalis to infection with arboviruses was an inherited trait. An early study undertook to select for WEE viral susceptible (WS) and refractory (WR) strains. After prolonged selection from the Ft. Collins colony, and with outcrosses to the Knights Landing or Chico colonies 2 hybrid strains of C. tarsalis evolved that were highly refractory to infection following ingestion of WEE virus. When fed on viremic chicks, the WR strains, which had undergone 200 plus generations of selection, were at least 25,000 fold more resistant than the most susceptible parental strain (Knights Landing) and greater than 100,000 fold more resistant than the WS strain which was selected over 10 generations from the Knights Landing colony. Refractoriness was associated with a mesenteron barrier since both WR and parental strains were equally susceptible to infection by intrathoracic inoculation; but when virus was either inoculated intrathoracically or ingested from viremic chicks, the virus multiplied in the mesenterons of WS females but not in WR females. Susceptibility was dominant, possibly incompletely dominant, over resistance. Inheritance was probably polyfactorial but this was not certain since a small proportion of individuals appeared to become infected by nongentic mechanisms and/or allowed limited multiplication, possibly only in the mesenteron. The mean viral titer in individual WS females was $10^{5.9}$ PFU as compared to $10^{1.2}$ in WR females. It was of interest that viral titers in F_1 females that were derived from reciprocal mating between WS and WR parents were intermediate in susceptibility as compared to their parents. These observations suggested that viral multiplication after infection also was controlled genetically. Four of 13 WR females that became infected after feeding on high concentrations of virus and 13 of 27 WR females that were infected by intrathoracic inoculation transmitted virus by bite after an extrinsic incubation of 14 days. These data indicated that different mechanisms controlled the ability of C. tarsalis to become infected and their ability to transmit virus. There were no differences in the susceptibility of WR and unselected strains of C. tarsalis to infection with SLE virus and WR possibly were more susceptible to infection with TUR virus than were unselected strains of C. tarsalis. Thus, the susceptibility of C. tarsalis to WEE virus did not necessarily correlate with their susceptibility to other arboviruses.

Aedes melanimon, collected in the field, were found to be more competent vectors of WEE virus than most field populations of C. tarsalis, both in their susceptibility to infection and their ability to transmit virus. WEE virus was isolated as frequently from Ae. melanimon as from C. tarsalis collected in the Sacramento Valley from 1969 to 1974, so these results establish that Ae. melanimon is an important vector of WEE virus in California.

Four colonized strains of C. tarsalis (BFS-Winnipeg, Knights Landing, Frink and Manitoba) varied significantly in their susceptibility to infection by intrathoracic inoculation and/or plectet feeding with WEE, SLE and TUR viruses. A mutant strain of C. tarsalis (car-ble) was highly susceptible to infection after feeding on plectets soaked with WEE virus but had normal susceptibility to infection after ingestion of SLE virus.

BFS-Winnipeg, Knights Landing and Manitoba strains of C. tarsalis were evaluated for their capacity to transmit WEE virus 13-14 days after ingestion of WEE virus. The transmission rates to chicks were 68, 81-88, and 25-44 percent respectively. Viral titers in infected females of the Manitoba strain that failed to transmit virus were nearly 100 fold lower than those found in females that transmitted virus.

The Knights Landing strain of C. tarsalis, that were being reared in outdoor ponds during the summer of 1975, were evaluated for their vector competence for WEE virus. The purpose was to determine if the vector competence of this population for WEE virus changed during the summer. There was no change in ability to become infected or to transmit virus during June, July and August; but both infection and transmission decreased in September and October. This change probably reflected the influence of one or more non-genetic factors (e.g., temperature, nutrition, photoperiod, etc.) that changed as the summer progressed. The change did not reflect the selection of more refractory mosquitoes as the eggs utilized for introduction in each month's experiment came from a colony that was reared indoors under constant conditions, and the vector competence of this population did not change.

We continued a collaborative study with Dr. A. Ralph Barr to measure the susceptibility to SLE virus of genetically autogeneous strains of Culex pipiens he had established that had well identified phenotypic markers. We evaluated these populations for their susceptibility to infection by plectet feeding on 1 to 4 geographically representative strains of SLE virus, 2 from California and 1 each from Texas and Illinois. Less than 10 fold differences in ID₅₀'s were observed between 8 C. pipiens strains that ingested the Texas strain of SLE virus. Differences in the infectivities of different viral strains were noted but they were not consistent from one experiment to another. Since all genetic strains of C. pipiens contained rickettsial-like symbionts, 1 strain was divided and reared in the presence and absence of antibiotics and they were then fed on virus to determine if symbionts affected susceptibility to SLE virus. No differences were observed between the 2 populations.

Newly colonized strains of C. pipiens and C. quinquefasciatus that were anautogenous were found to be relatively resistant (i.e., ID₅₀'s of 10^{4.0} PFU or greater) to infection when plectet fed 2 California strains of SLE virus.

Culex peus (Grasshopper Slough) was found to be more susceptible to infection with TUR virus than C. pipiens (Poldervaart) or C. tarsalis (Poso Creek) when fed on House Finches with similar viremia titers. However, C. tarsalis was a more efficient viral transmitter than was C. peus after 14 days extrinsic incubation even though there were no differences in mean viral titers in infected females of the 2 species. Mean viral titers in individual female C. pipiens were low (10^{2.5} PFU) even after 14 days incubation. Thus, C. pipiens were inefficient transmitters of TUR virus. Viral growth curves for TUR virus in C.

tarsalis that had fed on House Finches with viremia titers of $10^{3.3}$ to $10^{4.3}$ PFU/0.1 ml of blood indicated that in some females peak titers of nearly $10^{5.0}$ PFU occurred as early as 4 days after infection. However, there was widespread variation in titers between individual females for up to 21 days after infection.

The infectivity, growth rate and transmission rates of large plaque (LP) and small plaque (SP) variants of WEE virus were measured in C. tarsalis (Poso Creek). The infectivity of the LP variant was 100 fold or greater than for the SP variant if infection was by plegget feeding. However, both variants were equally infective when introduced intrathoracically. Growth curves of LP and SP variants in C. tarsalis that were infected by plegget feeding were similar but females infected with the SP variant were poor viral transmitters. These results may explain why only LP variants are isolated from pools of naturally infected C. tarsalis.

Others have reported that the concentration of an arbovirus that is ingested by mosquitoes affects subsequent transmission rates by infected females. Thus, an experiment was done to determine if this applied to C. tarsalis that were fed on chicks with different viremia levels. Only 58-63 percent of infected females from the Manitoba colony transmitted virus by bite to chicks 13 days after ingestion of $10^{2.9}$ to $10^{3.9}$ PFU of virus. This contrasted with transmission rates of 95-100 percent when females were infected by ingestion of $10^{6.6}$ to $10^{7.3}$ PFU of virus. Tests done simultaneously with the Presidio strain of C. tarsalis were inconclusive because they fed poorly on normal chicks, however, the results tended to confirm those obtained with the Manitoba colony.

An interspecies mosquito model has been identified to study the mechanism(s) of the mesenteron barrier to infection to WEE virus. The model consists of C. tarsalis (Knights Landing) and C. pipiens (Poldervaart) which are highly susceptible and highly refractory respectively to infection after ingestion of WEE virus. Both species are equally susceptible to WEE virus when inoculated intrathoracically.

We thought initially that the disappearance of WEE virus from the Central Valley of California in 1969 and 1970 might be related to the increased resistance of C. tarsalis to organophosphorous (OP) insecticides, thus making them less competent vectors. Even though large scale field studies on vector competence failed to support this hypothesis, none of these studies utilized C. tarsalis that were recently stressed with OP compounds. An OP resistant strain of C. tarsalis became available in 1976 from Dr. George P. Georghious and we set up an experiment to test the vector competences of fenthion stressed and non-stressed subpopulations. This experiment demonstrated definitively that fenthion stressing of C. tarsalis did not effect their ability to become infected, to support viral multiplication or to transmit virus by bite.

Studies have been continued to determine if several nongenetic variables affect the vector competence of C. tarsalis for WEE and/or SLE viruses. It was found that when final concentrations of 5 to 10 percent sucrose in virus-defibrinated rabbit blood mixtures were used to soak pleggets it decreased the proportion of C. tarsalis that became infected with WEE virus but not with SLE virus. The susceptibility of C. tarsalis to WEE virus was not influenced if:

females fed on sucrose soaked pledgets before exposure to virus soaked pledgets, virus was mixed with defibrinated blood from fasted versus nonfasted rabbits, or larvae were reared on different diets.

Electron microscopic studies revealed that the basic ultrastructure of the mesenteron of C. tarsalis was similar to that reported for other mosquito species. It was confirmed that initial secretory vesicles found in mesenteron epithelial cells of non-blood fed females became a rough endoplasmic vesicle following ingestion of a blood meal. Electron dense material, possibly representing hemoglobin or unsaturated fatty acids from the blood meal, was found between adjacent epithelial cells of the mesenteron. The significance of this observation is that ingested virus might have access to the basal lamina without multiplication of virus in the epithelial cells and this could explain the "leaky gut" concept proposed by other investigators.

The kinetic parameters of nonspecific esterases and phosphatases found in "normal" C. tarsalis have been established. Also, the electrophoretic patterns of nonspecific esterases have been determined by conventional and acrylamide gel and isoelectric focusing methods, utilizing whole body extracts of C. tarsalis. Only 5 esterolytic isozymes could be resolved by conventional acrylamide gel electrophoreses as compared to 16 to 18 by isoelectric focusing. Variations in esterolytic isozymes were demonstrated in different aged females and between different geographic strains of C. tarsalis.

No evidence was found for transovarial transmissions of WEE virus in experimentally infected Aedes sierrensis.

We have continued our interests in the potential importance of chronically infected vertebrate hosts as long-term reservoirs for arboviruses. We are using TUR viral infection in House Finches as a model. In the past 7 years, we have demonstrated persistent TUR viral infection in the spleens or kidneys of 4 of 61 naturally infected House Finches over a period of months. The most successful procedures for viral isolation were in vitro fragment cultures of organs and culture on duck embryo cell feeder cultures. Culex tarsalis were fed on seropositive (several that also had positive organs) and seronegative birds over as long as 9-week periods in xenodiagnostic tests. Tests on the resulting 5,214 mosquitoes did not yield any viral recoveries.

The studies on TUR virus were extended by inoculation of House Finches and tests of organs from birds at various intervals. Virus was isolated from spleen, kidneys or ovaries of birds sacrificed 14, 28, 55 and 83 days after infection, but not at 180 and 341 days. Virus was isolated from the ovaries of several birds that had no detectable neutralizing antibodies at the time of sacrifice. Xenodiagnostic tests were done by feeding C. tarsalis on 12 infected and 4 control birds for a period of 9 weeks beginning 4 months after the birds were inoculated. No virus was recovered from organs of these birds or the 2,274 mosquitoes that had fed upon them.

Tests of 18 House Sparrows, 14 to 84 days after infection with TUR virus, gave no evidence of persistent infection.

A variety of strains of TUR virus were evaluated for their capacity to grow in cell cultures at 33, 36, 39, 40 and 42°C. All strains multiplied best at 33°C and not at all at 42°C. There was no evidence that TUR virus persisted as a TS mutant in chronically infected birds.

In the course of tests on organ cultures from House Finches several non-enveloped viruses were isolated. One was resistant to sodium deoxycholate, sensitive to BUDR and would multiply in Ae. dorsalis or C. tarsalis cell cultures. We suspect this is a DNA virus but have not identified it further. The second virus appeared to be an RNA virus but was not one of the expected endemic agents.

We reported previously the isolation of several new viruses from pools of C. tarsalis collected in Butte and Glenn Counties during 1970-1973. We have now characterized these agents further and will register them in the Arbovirus Catalogue. Llano Seco virus (BFN 3112) is our designation for a new orbivirus isolated in 1971. This virus is related antigenically to Umatilla, but distinct from this and other indigenous viral agents. Culex tarsalis were infected by feeding on virus but they failed to transmit by bite. Females infected by inoculation transmitted virus to blood droplets after 7, 14 and 21 days incubation. Culicoides variipennis could not be infected. We have been unsuccessful in efforts to infect chicks, mice, hamsters, guinea pigs and rabbits but found neutralizing substances in the sera of field collected horses, pigs, jackrabbits, pheasants and chickens. The neutralizing substances in pig sera were removed by treatment with $(\text{NH}_4)_2\text{SO}_4$ so we may not be detecting antibodies in the field collected sera.

A second virus is designated as Gray Lodge virus (BFN 3187) and this is a new rhabdovirus isolated from C. tarsalis. The first indication this was a rhabdovirus was through EM studies. Antigenically this agent is distinct from Hart Park and Flanders viruses. The virus multiplied after inoculation into C. tarsalis and virus was transmitted to droplets by feeding but not to chicks 14 days after the mosquitoes were infected. We have been unable to infect C. tarsalis by feeding on viral suspensions. Efforts to infect the usual laboratory animals were unsuccessful but we have detected neutralizing substances in the sera of field collected chickens and dogs.

A number of viruses that were isolated from C. tarsalis are now known to be Hart Park (HP) virus. Antibodies to HP virus have been detected in sera from grey squirrels and pigs but not in chickens. One of the field isolates multiplies slowly after inoculation into C. tarsalis. We have been unable to transmit virus by bite.

We have produced 20 TS mutants of WEE virus. Our thought has been that such adaptations might be important in the long term persistence of virus in chronically infected avian hosts and at the same time might effectively reproduce in nestling birds with their reduced temperatures. Growth curves revealed that 6 of the TS mutants were inactivated more readily than wild strains at 56°C but multiplied readily at 36 and 42°C. Temperature step-up step-down experiments indicated the RNA of the TS mutants replicated at nonpermissive temperatures and this would favor viral persistence in avian hosts.

Cloned large plaque (LP) variants of WEE virus grew poorly in C. tarsalis cell lines. These studies were extended with LP and small plaque (SP) clones and the parental strains. All viruses adsorbed to the C. tarsalis cells but varied in their growth patterns at 25, 32 and 36°C. Particularly noticeable was the failure of the LP clone to multiply at 25°C. The SP variant and its parent grew well at 32°C whereas the LP clone and an SP clone derived from a virus isolated from an antelope squirrel in the winter did not. All viruses grew well at 24, 32 and 36°C in duck embryo and Ae. dorsalis cell cultures. We are now cloning C. tarsalis cells to determine if viral growth is related to cell type.

Considerable attention has been given to the effect of insecticide resistance on the susceptibility of C. tarsalis to WEE virus. We realized that vertebrate hosts also live in an environment where they are exposed frequently to organophosphorous insecticides. A series of chicks were stressed sub-lethally with fenthion or ethyl parathion, and inoculated with WEE virus. None of the stressed chicks had viremia profiles or antibody responses different from the control chicks.

A high proportion of Blacktailed Jackrabbits collected in the Sacramento Valley have antibodies to WEE, SLE and CE viruses. A study was done to evaluate viremia and antibody responses of the jackrabbit to experimental infection with WEE, SLE, CE and TUR viruses. All inoculated animals became infected with WEE, SLE and CE virus and none with TUR virus. WEE and CE viremia occurred in all animals. WEE viremia peaked on day 2 at titers adequate to infect vectors. Animals developed barely detectable SLE viremia although all developed antibodies. Antibodies were not detectable in some animals by 8 weeks post infection. HI and SDN antibodies to WEE and CE viruses developed in all animals and persisted at least 8 weeks. We believe the jackrabbit is an effective host for WEE and CE but not for SLE or TUR viruses.

In a follow-up study a series of pregnant jackrabbits that had detectable levels of WEE or CE viral antibodies were studied to determine the levels and duration of maternal antibodies passed to their offspring. Antibody titers in leverets were generally lower than in does and generally were undetectable by 21 days of age. There was no direct evidence that parturition activated latent infection in does as viral tests were negative on post parturition bloods. Some antibody carrying does had 2 to 8 fold rises in HI antibodies to WEE or CE virus coincidental to parturition and some does converted from negative to positive for HI antibodies to CE and SLE viruses coincidental to parturition.

Sylvilagus audubonii were successfully infected with MD virus but not with LOK virus. The MD infected animals developed viremias and antibodies and had only a slight antibody increase when challenged with the homologous virus 28 days later.

A comparison has been made of the sensitivity of HI and neutralization tests to detect arboviral antibodies in equine sera. Correlations were 95 percent for SLE but neutralization tests detected many positive reactors for MD, CE, Jamestown Canyon, TUR and LOK antibodies that were negative by HI tests.

SLE, MOD, RB and POW viruses are difficult to plaque on Vero cells. Addition of MgCl₂, MgSO₄, CaCl₂, NaCl or DEAE dextran in various concentrations and at various times generally did not improve the system.

Studies of autogeny rates in C. tarsalis under controlled conditions of temperature failed to reveal a relationship between variations in light intensity and autogeny rates. Autogeny rates generally were higher in females reared from autogenous rafts than from rafts that originated from blood-fed females. The findings confirmed that dissection of females 7 to 10 days after emergence is a satisfactory time to assess autogeny status.

The range and prevalence of C. tarsalis feeding on birds versus mammals is a major factor controlling viral transmission. Shifts in the proportion of feedings on these 2 groups of hosts has been related to the abundance of the vectors as avian hosts tend to reject excessive numbers of vectors that attempt to feed. A field trial was run in stable traps that contained pairs of a jack-rabbit and chicken or pheasant. Feeding success overall was least and feeding rates on the jackrabbits greatest when large numbers of C. tarsalis entered the trap. The findings confirmed the earlier observations that seasonal shifts in host feedings reflected vector abundance. Aedes melanimon that entered the stable traps preferred to feed on jackrabbits rather than on chickens or pheasants.

A series of 4,220 blood meals from engorged C. tarsalis, that had been identified as having fed on passeriform birds in Kern County in the 1960-1965 period, were taken from storage and retested with improved reagents by the passive hemmagglutination inhibition test. The majority of feedings had been on House Finches and House Sparrows.

Tests were completed on blood meals from 538 mosquitoes collected in Butte County in 1973. The proportion that had fed on birds, 72 percent, was the same as in previous years.

Several new alphaviruses have been reported by the CDC to be isolated from and to be transmitted by Oeciacus vicarius (the swallow bed-bug) in a number of areas of the United States. We collected 12,272 specimens from Central Valley areas where WEE has been endemic for many years. Tests in Vero and duck embryo cell cultures failed to isolate any viruses. The Fort Collins Laboratory provided us with suspensions of original positive pools from Colorado and our 2 cell systems were highly susceptible to the agents they contained. We conclude that alphaviruses have a very low prevalence or do not exist in O. vicarius in our study areas.

Summaries are presented on Ph.D. theses on Bakau virus in West Malaysia and the relationships of C. tarsalis population density and environmental factors to the prevalence of encephalitis in humans and equines in California.

(William C. Reeves, James L. Hardy, S. Monica Asman)

Everglades Virus Infection In Man, 1976

Ehrenkranz et al¹ and others^{2,3} have reported 4 clinical cases of Everglades (EVE) virus (Venezuelan encephalitis, Type II) infection in humans living in or visiting south Florida. We now report an additional case with a fortuitous epidemiologic observation.

On August 31, 1976, a 43-year-old white male veterinarian from Switzerland arrived from Europe and spent the night at a motel in Miami, Florida. By 9 the following morning he had arrived in the Everglades National Park, an area of south Florida from which EVE virus has been repeatedly isolated.^{4,5} He spent the day with companions walking improved trails and board walks in the Park and camped that night at Flamingo, where there was a great number of mosquitoes. Early the next morning (Sept. 2) he awoke with fever and a feeling of "heatstroke" and by that afternoon had developed a temperature of 38°C (100.4°F). His fever persisted for 3 more days (37.5-39°C) with accompanying malaise, myalgia, severe headache, pharyngitis and enlarged, tender postcervical and postauricular lymph nodes. He attended a symposium at CDC, Atlanta, Sept. 7-9 and, because the malaise had continued, a blood sample for virus isolation was drawn on Sept. 8. Although the patient had one episode of "lack of orientation" (Sept. 10) and the malaise and asthenia continued until "nearly the end of the month," he recovered completely with no sequelae noted.

By intracranial inoculation of suckling mice (SM), a virus was isolated from the serum collected Sept. 8. Upon further passage the virus killed SM within 24-36 hours and was preliminarily identified as a strain of EVE virus by complement-fixation (CF) tests using crude alkaline extracted, infected SM brains as antigen.⁶ Short-incubation hemagglutination-inhibition (HI) tests⁷ of antigen produced in BHK-21 cells with rabbit antisera to the gp-57 envelope glycoproteins⁸ of prototype strains of VEE subtypes gave clear-cut evidence that this virus was indeed EVE virus.

Sera drawn Sept. 8 and Oct. 22, 1976 and April 18 and Aug. 26, 1977 were tested by HI, CF and plaque reduction neutralization (N) tests in primary Pekin duck embryo cells. The results, presented in Table 1, indicate diagnostically significant changes in titers by all tests, confirming a recent infection with EVE, the virus isolated from the patient's blood in the acute phase of illness.

This then is the fifth known clinical case of EVE virus infection of man. Although all 5 patients had moderate to severe illnesses, they recovered without residua. The patient whose illness is recounted here appears to have had a less serious disease progression and outcome.

Perhaps this is due to a lack of predisposition caused by age or lack of preexisting condition underlying the current illness, since 3 of the previous patients were 53, 71 and 75 years old and had cardiovascular abnormalities consistent with aging; the fourth, with a moderate illness, was 37 years old. In any event, although the virus is quite prevalent, antibody in residents of the area is high (15²-27%⁹) and antibody in male visitors is remarkable (5.8%²), frank disease appears to be uncommon. This may be due to a number of factors but probably relates to vector-host interactions or lack of virulence of the virus.

Probably the most significant finding was definition of a maximum incubation period (24 hours) in this patient infected with EVE virus; this is compatible with the findings of Bowen et al¹⁰ who reported a maximum incubation period of 24 hours for Venezuelan encephalitis subtype IB.

These data provide further evidence that sporadic instances of febrile illness in humans, particularly those intruding on an econiche new for them, warrant close clinical and laboratory investigations in order to not only provide information to resolve the final diagnosis for the patient but for epidemiologic surveillance as well.

C.H. Calisher, F.A. Murphy, H. Steck, H.S. Lindsey, J.K. France,
D.J. Muth, J.S. Lazuick and S.P. Bauer.

TABLE 1. Results of HI, CF and N tests^{a)} with sera from a citizen of Switzerland visiting the Everglades National Park, Florida, Sept. 1-2, 1976

Specimen No.	Date Bled	Antibody Titer ^{b)} to EVE Virus ^{c)}		
		HI	CF	N
1	9-8-76	10	8	<10
2	10-22-76	80	8	1280
3	4-18-77	80	16	2560
4	8-26-77	160	32	2560

a) No antibody to Eastern or Western encephalitis viruses were detected by HI or CF tests.

b) Titers given as reciprocals.

c) Prototype strain FE3-7c.

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Fort Morgan and Bijou Bridge Viruses in Mosquitoes

Studies are in progress on the arthropod host range of two alphaviruses, Fort Morgan (FM) and Bijou Bridge (BB), which are antigenically related to WEE and VEE viruses, respectively. Reported here are results thus far obtained with the mosquito species Culex tarsalis, Culex pipiens pipiens, Culiseta melanura, and Culiseta inornata.

The first FM strains used to experimentally infect arthropods were from nestling house sparrows and were passed once in Vero cell culture. Later a serially plaque-purified clone became available, and it was used in some of the experiments. The BB virus was originally obtained from a pool of swallow bugs (Oeciacus vicarius) collected in Morgan County, Colorado, and has undergone 5 passages in Vero cell culture. Mosquitoes were allowed to feed on virus suspensions or viremic hosts, or were inoculated intrathoracically. With FM virus the ingested materials contained from $10^{3.4}$ to $10^{7.3}$ PFU/ml and inocula introduced intrathoracically contained from $10^{2.8}$ to $10^{4.4}$ PFU/mosquito. The corresponding values for BB virus were $10^{4.7}$ to $10^{7.9}$ PFU/ml and $10^{4.2}$ PFU/mosquito. After incubation of 9 to 39 days at 24°C, mosquitoes were tested for virus presence. All assays were in Vero cell culture. Results are given in tables 1 and 2.

In general, mosquitoes have been refractory to FM virus. Culex tarsalis did not, with minor exceptions, contain detectable amounts of virus after incubation following feeding or inoculation of the cloned or uncloned strain. (Very small amounts of virus were detected in 2 inoculated specimens thought to have retained some of the injected virus.) Culex pipiens pipiens also did not become infected by feeding

on either virus strain, but, following 16 to 39 days extrinsic incubation, 14 of 39 and 1 of 35 yielded small amounts of virus after inoculation, respectively, with uncloned and cloned virus. Culiseta melanura did not give evidence of infection after ingesting cloned virus, but, following 20 days extrinsic incubation, 7 of 13 inoculated individuals contained relatively small amounts of virus and an additional specimen yielded a larger amount. Some or all of this virus is thought to have been residual from the inoculum. As with Cs. melanura, Cs. inornata could not be infected by feeding, but small amounts of virus were recovered from some specimens (9 of 24) following incubation after inoculation (cloned virus). Inasmuch as these species could not be infected orally, they do not appear to be important as vectors of FM virus in nature.

The response to BB virus has thus far been markedly different than to FM. Five of 11 Cx. tarsalis yielded large amounts of virus after ingesting virus, as did 20 of 20 after inoculation and 8 of 8 Cx. p. pipiens after inoculation. Large concentrations of virus were detected in 1 of 12 C. melanura and 7 of 22 Cs. inornata after feeding on virus. The single infected Cs. melanura transmitted to a host by bite. These species must be considered potential vectors of BB virus, although as yet BB virus has not been recovered from mosquitoes collected in the field.

William A. Rush, D. Bruce Franczy

TABLE 1. Experimental Infection Studies with FM Virus and Mosquitoes

Mosquito Species and Virus Strain	Method of Infection- \log_{10}/ml		Days inc. at 24C	Assay of Mosq. \log_{10}/ml^4
	Feeding ^{1,2}	Intrathoracic Inoculation ³		
<u>Culex tarsalis</u>				
CMA4-368V1	6.6	VH	9	11 with <1.0
<u>P. domesticus</u> nestling strain	7.3	VH	11	6 with <1.0
			21	20 with <1.0
			16	12 with <1.0 2 with 1.0
75-5-582V1	3.4-4.6	VH	13	15 with <1.0
<u>P. domesticus</u> nestling strain			20	12 with <1.0
Fort Morgan (CM4-146, cloned)	6.3	S	10	13 with <0.7
			16	21 with <0.7
<u>Culex pipiens pipiens</u>				
CMA4-368V1	6.5	S	23	20 with <1.0
			16	25 with <1.0 14 with 1.0-2.5
Fort Morgan (CM4-146, cloned)			21	19 with <0.7 1 with 0.7
<u>Culiseta inornata</u>			39	15 with <0.7
Fort Morgan (CM4-146, cloned)	6.3	S	9	26 with <0.7
			18	15 with <0.7 9 with 1.0-2.6
				3.5 (est.)

(Table 1)

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Mosquito Species and Virus Strain	Method of Infection-log ₁₀ /ml		Days inc. at 24C	Assay of Mosq. log ₁₀ /ml ⁴
	Feeding ^{1,2}	Intrathoracic Inoculation ³		
<u>Culiseta melanura</u>				
Fort Morgan (CM4-146, cloned)	6.3	S	23 20	11 with <0.7 5 with <0.7 7 with 1.2-2.9 1 with >2.9

1 - Assay is of ingested blood or virus suspension. Vero cell culture.

2 - VH = viremic host(s); S = suspension, from pledget.

3 - Assay is of 1 freshly inoculated mosquito ground in 1.0 ml of diluent. Vero cell culture.

4 - Each incubated mosquito was ground in 1.0 ml of diluent.

TABLE 2. Experimental Infection Studies with BB Virus and Mosquitoes

Mosquito Species	Method of Infection- \log_{10}/ml		Days inc. at 24C	Assay of Mosq. \log_{10}/ml^4
	Feeding ^{1,2}	Intrathoracic Inoculation ³		
<u>Culex tarsalis</u>	7.9	S	10	6 with <0.7
				5 with >2.7
			19	20 with >2.7
<u>Culex pipiens pipiens</u>			19	8 with >2.7
<u>Culiseta melanura</u>	7.9	S	23	11 with <0.7
				1 with >2.7 ⁵
<u>Culiseta inornata</u>	4.7-7.1	VH	9	15 with <0.7
				7 with >2.7

1, 2, 3, 4 - Refer to Table 1.

5 - Mosquito transmitted by bite.

St. Louis Encephalitis (SLE), Memphis Tennessee

In late September 1976, a concerted effort was made to determine the incidence of SLE cases in Memphis-Shelby County, Tenn. The search was prompted because, although virus activity, indicated by infected mosquitoes, seropositive wild birds, and seroconversion in sentinel chicken flocks was at a level comparable to that observed during the SLE epidemic years of 1974 and 1975 (when 50 and 63 cases of SLE were identified respectively), few cases had been reported in 1976.

In 1976 missed human cases were searched for by reviewing records on admissions to the three Memphis hospitals most likely to accommodate cases from the areas affected in 1974 and 1975. Available information varied among hospitals, but included cerebrospinal fluid examination reports, a chief resident's log of admissions to the medical service of one hospital, and, in another, forms completed and sent to the infection control nurse when infectious disease was suspected. Thirty-two cases of central nervous system (CNS) disease compatible with SLE infection were found. Although a few previously undetected cases were discovered, we concluded that cases detected through the existing surveillance system satisfactorily represented the level of CNS illnesses being seen at these three hospitals.

Serum specimens were collected from recorded cases or were obtained from the Tennessee State Health Department which had received samples during routine surveillance activities; 10 SLE cases were thereby identified (5 confirmed and 5 presumptive) from Shelby County by serologic tests performed at VBDD or the Tennessee State Department of Health Laboratory.

In an effort to elucidate factors which may have resulted in the marked reduction of human cases in 1976 compared to 1974-1975, we administered a questionnaire to and conducted serological survey of a randomized segment of the population of Memphis in February 1977. Approximately 1,500 sera were obtained and 500 household questionnaires were completed.

The prevalence of neutralizing (N) antibody (titer >10) was 5.6 percent in the 1489 residents sampled. Six percent of 843 females and 5.0 percent of 642 males were N positive. Seropositive individuals were scattered throughout the city. A statistically higher antibody prevalence was found in residents of northeastern Memphis, an area of high risk (area A, shaded in Figure 1) where most human cases occurred in 1974 and 1975. The prevalence in this area was 7.8 percent (N=397) vs. 4.8 percent (N=1088) in the rest of Memphis (area B). The greatest difference in percentage positive between these geographic areas was found among residents over 40 years of age (Table 1).

Seropositive individuals examined by age and sex indicated an overall preponderance in females over age 60, but this tendency did not obtain when the high-risk area (area A) was examined separately (Table 1). The prevalence of antibody was roughly similar for each age group, suggesting that SLE virus has not been endemic in Memphis; rather, immunity reflects infection acquired during the recent (1974-76) outbreaks.

Table 2 shows N antibody prevalence by the Hollingshead socioeconomic class index (classes of 1 to 5, with 1 being highest and 5 the lowest socioeconomic categories) based on years of education and occupation. The highest antibody rates were among persons in the retired/disabled/student category (9.9 percent) followed by index 5 (6.6 percent) and index 3 (5.7 percent); the lowest rate was found in index 1 (2.2 percent).

N antibody titers ranged from 10 to 160. The geometric mean (GM) titer of all seropositive participants was 26.1. The GM titer of participants from area A was 26.7 compared to 25.8 for area B participants.

Table 4 presents a comparison of estimated (based on the 1977 sero-survey) and actual SLE cases detected in Memphis Tennessee from 1974-1976. On the basis of an over-all antibody prevalence of 5.6 percent in the survey, the Memphis population of 767,000., and a ratio of inapparent to apparent infections of 338:1, 127 clinical cases of SLE would have been expected in 1974-76 (assuming antibody reflects recent infection). The actual number of cases detected in 1974-76 was 121. The expected and actual cases by age group are not so closely matched.

It is apparent from this survey that immunity to SLE, even within the area of highest human SLE activity in recent years, does not explain the paucity of human cases in 1976.

(Vector-Borne Diseases Division and Memphis-Shelby Co. Health Dept.)

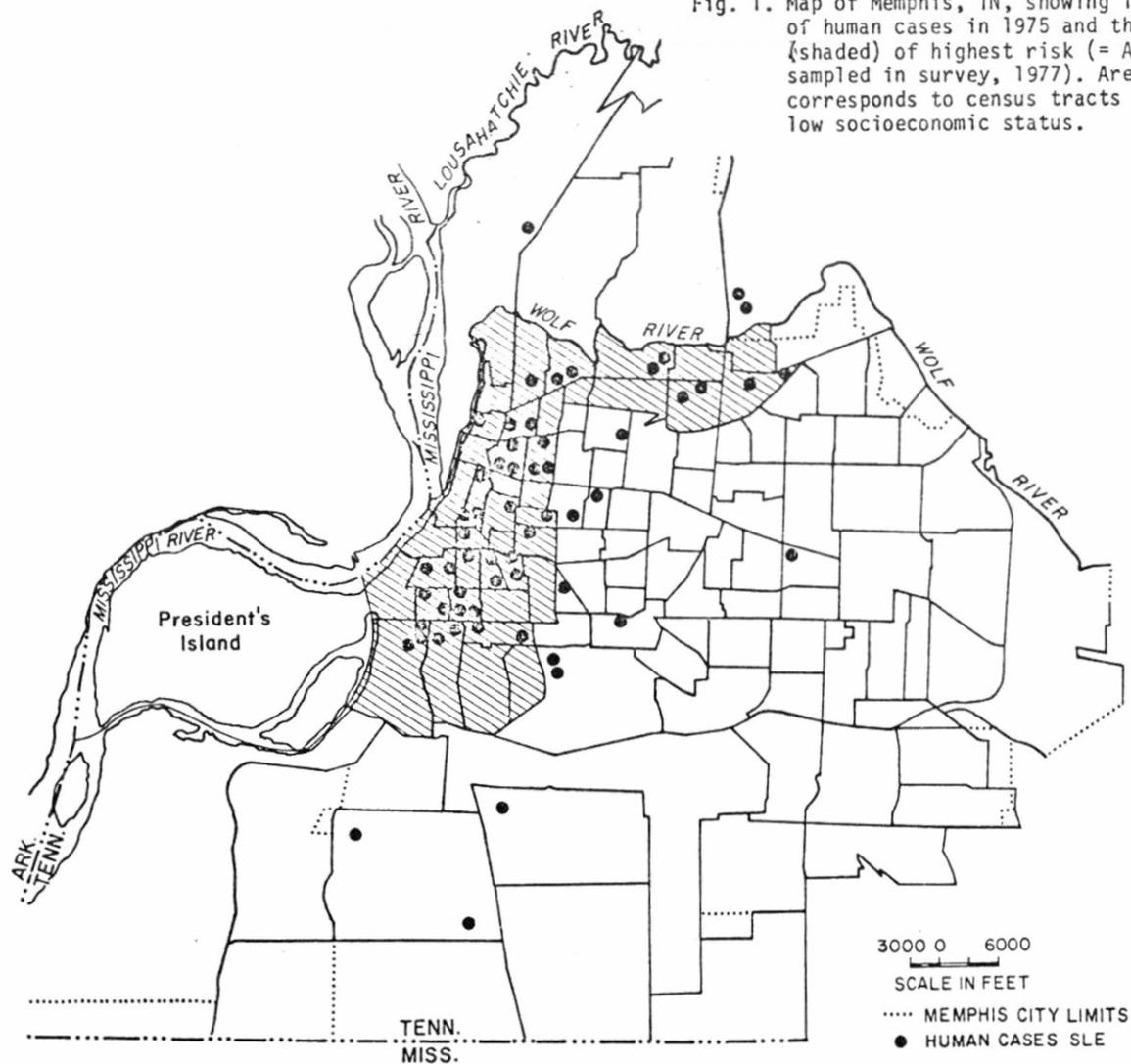


Fig. 1. Map of Memphis, TN, showing location of human cases in 1975 and the area (shaded) of highest risk (= Area A sampled in survey, 1977). Area A corresponds to census tracts with low socioeconomic status.

TABLE 1. Neutralizing Antibody Prevalence by Age and Sex, Memphis, Tn., 1977.

Age Group	Total Percent Positive	<u>Area A</u>				<u>Area B</u>			
		<u>N</u>	<u>Percent Total Positive</u>	<u>Percent Male Positive</u>	<u>Percent Female Positive</u>	<u>N</u>	<u>Percent Total Positive</u>	<u>Percent Male Positive</u>	<u>Percent Female Positive</u>
5-9	4.2	26	0	0	0	45	6.7	3.3	13.3
10-19	5.5	89	7.9	5.3	9.8	218	4.6	5.1	4.2
20-29	1.7	96	3.1	2.6	3.4	251	1.2	0.9	1.5
30-39	9.1	37	8.1	8.3	8.0	161	9.3	8.3	9.9
40-49	6.8	36	13.9	10.5	17.6	125	4.8	8.8	1.5
50-57	3.8	38	10.5	20.0	7.1	122	1.6	1.9	1.5
60-69	8.5	43	11.6	17.6	7.7	110	7.3	0	11.3
70+	10.2	<u>32</u>	<u>12.5</u>	<u>12.5</u>	<u>12.5</u>	<u>56</u>	<u>8.9</u>	<u>3.8</u>	<u>13.3</u>
TOTAL		397	7.8	7.9	7.7	1088	4.8	4.0	5.4

TABLE 2

Neutralizing Antibody Prevalence by Hollingshead
Index, Memphis Tennessee, February 1977.

<u>Hollingshead Index*</u>	<u>No. Tested</u>	<u>Percent with N Antibody</u>		
		<u>Male</u>	<u>Female</u>	<u>Total</u>
1	89	4.3	0	2.2
2	106	3.9	3.6	3.8
3	193	8.1	3.7	5.7
4	437	2.6	4.5	3.7
5	377	6.7	6.6	6.6
Retired, Student Disabled	243	6.1	12.4	9.9
Unknown	40	0	4.2	2.5

*Based on educational level and occupation.

TABLE 4

Expected vs. Detected SLE Cases
 Memphis Tennessee 1974-1976

<u>Age Group</u>	Memphis Tennessee				
	<u>Inapparent To Apparent Infection Ratio *</u>	<u>Pop. 1975</u>	<u>Detected Cases 1974-76</u>	<u>Percent N Positive 1977 Survey</u>	<u>Estimated No. Clinical Cases</u>
0-19	600:1	310,000	2	5.3	27
20-39	450:1	207,100	17	4.4	20
40-59	300:1	158,000	37	5.3	28
60-+	85:1	91,300	57	9.1	98
Unknown	-	-	8	-	-
TOTAL	338:1	767,000	121	5.6	127

*From Monath, T.P. Epidemiology, in Monath, T.P. (Ed.) St. Louis Encephalitis,
 Springfield, Thomas, In press, 1978.

FOUR ARBOVIRUSES OF REPTILES: GROWTH CHARACTERISTICS IN CELL LINES FROM COLD-BLOODED VERTEBRATES AND ELECTRON MICROSCOPIC STUDIES SHOWING THREE TO BE RHABDOVIRUSES.

It has been conjectured that the paucity of information about the role of cold-blooded animals in arbovirus cycles may be an artifact of the methods employed for isolation of viruses in arthropod or vertebrate field specimens. The use of mice or mammalian and avian cell cultures for primary isolation may prevent detection of viruses naturally adapted to poikilothermic species. Since our laboratory annually processes large numbers of Culex (Melanoconion) and other mosquitoes from tropical America, which are primarily reptile and amphibian feeders, we decided to investigate the usefulness of cell cultures derived from cold-blooded vertebrates for viral isolation attempts. As a first step, we investigated the growth characteristics of four arboviruses for which reptiles are believed to be the principal vertebrate hosts. These viruses are 1. Marco 2. Chaco, and 3. Timbo viruses isolated from Ameiva ameiva and Kentropyx calcaratus lizards in Brazil by Causey, Shope, and Bensabeth, and 4. Almpiwar virus from skinks (Ablepharus boutonii) in southeastern Australia. These viruses were kindly supplied by Dr. Shope at YARU, and passed in suckling mice (SM) to prepare seed stocks at the SM² (Almpiwar) or 6 (other viruses) level.

Cell cultures used to define viral growth were obtained from ATCC or from Dr. H. Fred Clark, Wistar Institute, Philadelphia. Cell lines were propagated, working cultures prepared, infected, incubated at various temperatures (22, 30, 37, and 42°C), observed for appearance of CPE, and supernatant fluid sampled at 24 hour intervals for viral assay. Results are summarized in Table 1. CPE was observed in mammalian cell cultures infected with Marco and Chaco viruses and in TH-1 cells infected with Marco virus (minimal, focal CPE). Optimal growth in all cell lines was observed at 30°C, with peak titers achieved between 4 and 6 days after inoculation. 37°C was a nonpermissive temperature for all four viruses in reptile and amphibian cells; in mammalian cells, temperature-sensitivity was also documented by a >2.0 dex reduction in yield at 37°C. Of the reptile and amphibian cells tested, VSW and A6 cells best supported viral growth, but, in general, mammalian cells were as or more susceptible. Chaco virus grew to higher titer in VSW than in other cells. No replication was observed in fish cell lines.

The absence of detectable CPE in cold-blooded cell lines limited their usefulness in isolation attempts from field material. However, we found that infected VSW, VH-2, 8625, and TH-1 cells resisted challenge with vesicular stomatitis virus (VSV-Indiana), whereas control cultures were totally destroyed by 48-72 hours. For VSV challenge, cultures were shifted up to 37°C. Approximately 100 pools of C. (Melanoconion) mos-

quitos collected in Ecuador in 1976 have been tested by inoculation of TH-1 cells followed by VSV-Ind challenge; no viruses were detected.

Infected cell cultures were examined electron microscopically (by FAM). Marco virus was visualized in TH-1 and Vero cells and Chaco virus in Vero Cells. Both agents were found to be rhabdoviruses. On the basis of the known antigenic relationship between Chaco and Timbo viruses, the latter is also a rhabdovirus. Almpiwar virus has not yet been examined.

(T.P. Monath, C.B. Cropp, Ft. Collins and F.A. Murphy, Atlanta)

Table 1. Growth of four arboviruses of reptiles in mammalian, reptilian, and amphibian cell lines.

Cell Type	Maximum growth ¹ (30°C) by virus.			
	Marco (BeAn402901)	Chaco (BeAn42219)	Timbo (BeAn41787)	Almpiwar (MRM4059)
<u>Mammalian</u>				
Vero	5.3	3.5	3.8	4.5
BHK-21	5.9	NT	5.0	NT
<u>Reptilian</u>				
VSW (Russell's viper spleen)	5.4	6.0	NT	3.2
VH-2 (Russell's viper heart)	2.3	0	0	0
8625 (Rattlesnake fibroma)	2.3	trace	0	0
TH-1 (Terrapene heart)	5.1	0	0	2.2
<u>Amphibian</u>				
A6 (<u>Xenopus laevis</u> kidney)	NT	4.3	NT	3.0
<u>Fish</u>				
RTG (Rainbow trout gonad)	0	0	0	0
FHM (Fathead minnow)	0	0	0	0
<hr/>				
ICLD ₅₀ /ml, Suckling mice	7.4	7.0	3.5	7.4

1. Marco and Timbo viruses assayed by plaqueing in Vero cells, Chaco and Almpiwar by IC inoculation of SM. Titers expressed as PFU or SMICLDS0/ml.

The straw colored fruit bat (Eidolon helvum) is an exceedingly common and widespread species having a range throughout West Africa from Senegal to Cameroun. It has been observed on islands far out to sea and to the edge of the Sahel vegetation zone to the east and north. E. helvum is the second largest of the West African bats and is found in colonies numbering 100,000 or more. It is commonly used for food, and at Ile Ife, Nigeria it is estimated that in excess of 100 animals per day are taken from a single colony, by hunters for this purpose. These bats are strong fliers and range widely in search of food. Long migrations occur but are not well understood.

During 1971 Eidolon bats were sampled at two large daytime roosting sites in Nigeria, the first at Ile Ife in the high forest zone in Nigeria and the second at Abuja in northern Nigeria in the savannah vegetative zone. Viral isolations from a variety of tissues were made from both sites. Two of these agents, one from each site are currently being studied. IbAn 57245 was isolated in infant mice from a liver-spleen pool collected at Ile Ife and IbAn 57892 was isolated similarly from salivary gland tissue of a bat taken at Abuja.

Chloroform and pH sensitivity: Both agents have a low sensitivity to chloroform with a reduction in titer of about 1 log. IbAn 57245 is extremely sensitive to acid pH and no replication could be shown after treatment at pH 3 for 3 hours at 4°C. The pH 7.8 control killed following ic inoculation at dilutions to approximately 10^{-6} in infant mice.

Serology: A sucrose-acetone brain antigen of IbAn 57245 did not manifest HA activity when tested with goose erythrocytes at pH 5.8 through 7.0 and at room temperature. In CF this antigen reacted with homologous mouse hyper-immune ascitic fluid to a titer of 256/64. On further testing IbAn 57245 antigen and antibody reacted indistinguishably with a crude MB antigen and antibody to IbAn 57892 (128/8). By CF the two agents appear to be identical. Antigens for IbAr 39621, IbAr 39626, IbAn 28946 and IbAn 57204, other presently unidentified Nigerian isolates, failed to react with IbAn 57245 and IbAn 57892 immune fluids. Antigens were tested at a 1:4 dilution against serial dilutions of the immune fluids beginning at 1:8. Antigen and antibody for IbAn 57245 did not react with an Eych system (256/ \geq 128) and systems for 5 Orungo strains (32/16 to 128/8) including the prototype UGMP 359 (32/8). Two and eight CF units of IbAn 57245 antigen failed to react with NIH Kemerovo grouping fluid, polyvalent Palyam (PAL, VEL, KAS, COR, ACD, EUB, PATA, DAG) and Polyvalent #8 (BLU, EHD, CGL, IRI, CTF, IbAr 22619). Thus far IbAn 57245 has not reacted with any of the known orbiviruses against which it has been tested. A few more orbiviruses remain to be tested.

Electronmicroscopic: IbAn 57245 was passed three times in BHK 15 cells in preparation for EM examination. Virions having a morphology typical of the

orbivirus taxon were seen singly and in clusters in cytoplasm of infected cells. No virions were seen in nuclei. Filamentous structures were seen commonly in masses, usually in close association with clusters of virions. Mature virions have an electron dense core and a less dense capsid. Preliminary measurements indicate capsid and cores fall within the range of other recognized orbiviruses. On one occasion virus particles were seen surrounded by a membrane, and apparently budding from the cytoplasmic membrane adjacent to the cell nucleus.

This report is thought to represent the first known isolation of orbiviruses from bats.

(Graham E. Kemp, Nick Karabatsos and C. Bruce Cropp)

Rates of venereal infection obtained during studies with prototype LAC virus in F3 generation female Aedes triseriatus during 5 induced mating trials are summarized in Table 1. Results are compared according to the time of first blood-meals as related to day of mating with infected males. Trials in which females were given blood meals 6 days before mating had higher rates of venereal infection than those with first blood meals after mating and during first studies of venereal transmission in mosquitoes in which females were not given prior blood meals (Science 196: 530, 1977 and Amer. J. Trop. Med. Hyg. 27: 187, 1978).

Salivary transmission from venereally infected females was higher in 3 trials with blood meals before mating with infected males, 7/15 (47%), than in the 2 trials in which females did not receive a blood meal until after mating. 1/37 (3%). Transmission rates were obtained from females held in individual cartons and reared on suckling mice at 7 to 14 day intervals. Survivors were later dissected and processed for detection of FA antigen in non-lower genital tract tissues.

Transovarial transmission rates were also studied in eggs and progeny of venereally infected females producing 2nd or later ovarian cycle eggs in trial IM-6. LAC was found in 80/121 (66%) of male and in 99/158 (63%) of female progeny.

Similar rates of venereal infection have so far been observed during recent trials with F1 generation female Aedes triseriatus mated with males transovarially infected with a field strain of LAC virus.

Quantitation of LaCrosse virus in males and venereally infected females.

Male Aedes triseriatus were inoculated with prototype LaCrosse (LAC) virus 14 days before being mated to female mosquitoes. From day 4 to 20 post-inoculation, male reproductive tracts (RT) and salivary glands (SG) were dissected and titrated in Vero cell culture for virus content. The male RT contained consistently greater quantities of virus than did the SG, with fewer than 50% of the SG containing detectable virus. The mean virus titers in the male organs dissected immediately after mating were $3.5 \log_{10}$ pfu/RT, and in the virus-infected SG, $1.5 \log_{10}$. The mean \log_{10} pfu in random female bursas dissected immediately after induced mating was 3.0.

Of females successfully inseminated, 14% had disseminated virus infections 3-20 days following mating. Virus was demonstrable in nearly all organs of the infected females by 10 days following mating, the highest titers being found in the salivary glands. On day 3 following mating, virus was evident only in the RT and the leg, with the titer of virus in the leg greater than at later times tested. Plaque-forming virus was first detected in the heart and pericardial cells (H/PC) on day 17. The mean measurable \log_{10} pfu/organ system of these venereally-infected females were: 4.0, SG; 3.0, RT; 2.9, digestive tract; 2.5, thoracic and abdominal ganglia; 1.6 (H/PC); 1.6, leg. This study is currently in progress using mosquitoes infected with an unpassaged field isolate of LAC virus.

(Wayne Thompson and Laura Kramer)

Table 1.

Salivary transmission and infection rates with LAC in venereally infected Aedes triseriatus females given first blood meals before or after induced-mating with infected males

Trial number	With first blood meals provided ♀♀	♀♀ transmitting LAC by bite to SM/ ♀♀ with satisfactory transmission trials; post-mating days:				Venereal infection rates:	
		0-7	8-17	18-29	30-40	Number of ♀♀ with LAC antigen in FA/	# of surviving ♀♀ tested
IM-6	6 days BEFORE MATING	---	---	5/9	6/9	6/10	(60%)
IM-7	6 days "	2/35	11/29	6/17	1/6	8/17	(47%)
IM-8	6 days "	0/7	1/8	1/8	---	1/7	(14%)
		<hr/> 2/42 (5%)	<hr/> 12/37 (32%)	<hr/> 12/34 (35%)	<hr/> 7/15 (47%)	<hr/> 15/34	<hr/> (44%)
IM-9	2 days AFTER MATING	---	1/27	1/23	1/22	1/27	(4%)
IM-10	6 days "	---	0/26	0/26	0/15	0/26	(0%)
		<hr/> ---	<hr/> 1/53 (2%)	<hr/> 1/49 (2%)	<hr/> 1/37 (3%)	<hr/> 1/53	<hr/> (2%)

REPORT FROM THE DEPARTMENT OF VETERINARY SCIENCE
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Boreal Wildlife Virus Studies

During the summer of 1976 a small mammal study was conducted in the Athabasca tar sands region, approximately 45 km north of Fort MacMurray, Alberta. The objective of the study was to determine changes in mammal populations and their associated viruses related to clearing of the boreal forest for mining purposes. Blood from two of 109 Peromyscus maniculatus deer mice yielded virus when inoculated intracerebrally into suckling mice. The incubation periods in mice for both isolates were long, 8 to 12 days. Both isolates were reisolated and were unlike any viruses that we have in the laboratory. These viruses proved to be identical by neutralization tests. One of these isolates, PM00160, was selected for identification. Sucrose-acetone extracted antigen hemagglutinated goose erythrocytes at a pH of 6.0-6.2. Hemagglutination was inhibited by group B (flavivirus) typing serum provided by the National Institutes of Health. There was no inhibition of hemagglutination with following virus grouping reference sera:

Group C	Polyvalent Anopheles A, B, Turlock
Guama Group	Polyvalent Bwamba
Simbu Group	Polyvalent Patois
Vesicular Stomatitis Group	Polyvalent Rabies
Bunyamwera Group	Capim Group
California Group	Polyvalent Palyam
Tacaribe Group	Kemerovo Group
Phlebotomus Fever Group	Polyvalent Congo
	Polyvalent #1-#10
Group A	Polyvalent #12
Polyvalent Quarantil	

Other characteristics were compatible with the identification of PM00160 as a flavivirus. The agent was chloroform sensitive (complete loss of titer following treatment) and passed 450 and 220 nm filters. PM00160 was inoculated onto monolayers of cell cultures including: BHK-21, VERO, primary albino swiss mouse embryo, primary neonatal Peromyscus maniculatus kidney, bovine turbinate line, bovine embryonic kidney line, human fetal tonsil, primary mallard duck embryo, primary chick embryo, mouse L line. Cultures were observed for a minimum of 14 days for signs of cell change or death. No evidence of virus-induced change was observed. Titration of supernatant fluids intracerebrally into suckling mice indicated that the virus did replicate in BHK-21 cells, but without CPE. The inoculation of the cells with PM00160 did not render them resistant to challenge with vesicular stomatitis virus, however. Peromyscus maniculatus were inoculated with PM00160 in dilutions from $10^{0.6}$ through $10^{5.6}$ suckling mouse intracerebral LD₅₀ (SMICLD₅₀) by the intramuscular, intraperitoneal and oral routes. Three additional mice were inoculated intracerebrally with $10^{6.2}$ suckling mouse SMICLD₅₀ of the virus. Neither death or clinical illness occurred in any of the mice. However, all responded with neutralizing antibodies indicating that infection had taken place. Viremia levels were not determined.

PM00160 virus has been tested with antisera against several flaviviruses from small mammals (Table 1). These agents were selected because of association with small mammals, hemagglutination pH requirements and failure to induce CPE in many types of cell cultures. Log neutralization indices were calculated.

Table 1. Neutralization of the Alberta mouse agent, PM00160, by reference immune sera to several small mammal-associated flaviviruses.

Virus	Geographic Origin	Log Neutralization Index
Modoc (MOD)	California	0.5
Montana Myotis Leukoencephalitis (MML)	Montana	0.0
Cowbone Ridge (CBR)	Florida	1.0
Jutiapa (JUT)	Guatemala	1.4
R1972	Texas	2.5
72-V-1251	Texas	1.4

One-way neutralization tests suggests that PM00160 is related to but not identical with R1972-72, V-1251 and Jutiapa.

Blood was drawn from humans and from small mammals of the area. Neutralization tests with these sera versus PM00160 were done in one to three-day-old suckling mice (Table 2).

Table 2. Antibody prevalence to the Alberta mouse against PM00160, in mammals captured in the area where the virus was detected.

Common Name	Scientific Name	Number Positive Number Tested	Percent
White-footed deer mouse	(<u>Peromyscus maniculatus</u>)	13/109	12
Least chipmunk	(<u>Eutamias minimus</u>)	3/35	9
Red squirrel	(<u>Tamiasciurus hudsonicus</u>)	1/38	3
Snowshoe hare	(<u>Lepus americanus</u>)	0/11	0
Red-backed vole	(<u>Clethrionomys gapperi</u>)	0/11	0
Flying squirrel	(<u>Glaucomys sabrinus</u>)	0/2	0
Human	(<u>Homo sapiens</u>)	3/50	6

Antibody prevalence was highest in Peromyscus maniculatus, further corroborating natural infection of this species. Interestingly, neutralizing antibodies were also found in sera from three humans in the area. Sera were also tested in tissue culture micro-neutralization tests against St. Louis encephalitis virus and Powassan virus. None of the sera neutralizing PM00160 neutralized either of the other two flaviviruses, suggesting that neutralization reactions observed were specific. However, human and P. maniculatus sera neutralizing PM00160 have not been tested against antigens for the more closely related R1972, 72V1251, nor Jutiapa viruses.

Population estimates were calculated for small mammal species of the area. Clearing of a jack pine ridge for mining purposes resulted in an 8-fold increase in the P. maniculatus population, as compared to undisturbed jack pine habitat. Thus, if transmission of PM00160 is density-dependent, industrial activities may produce population densities which are favorable for transmission. If human infections result in disease, the public health ramifications of ecological changes associated with the tar sands mining operations should be assessed. In addition to human clinical information, further data on the natural history of this virus (as mode of transmission) will be required before the assessment of public or animal health consequences can be made.

Eastern equine encephalitis virus (EEEV) in birds

Red-winged blackbirds (Agelaius phoeniceus) were infected with eastern equine encephalitis virus to test the hypothesis that passerines may be persistently infected and that springtime physiologic changes may induce recurrent viremia. Such recrudescence infection would be a possible overwintering mechanism for EEEV.

Two groups of red-winged blackbirds caught October 1977 near Madison, Wisconsin were inoculated with 5 and 500 SMICLD₅₀ EEEV respectively. To induce rapid gonadal development by photo-stimulation, surviving infected birds were maintained on 20-hour day lengths. Beginning at 28 days of photo-stimulation, birds were bled and swabbed twice a week. At 45 and 67 days of photo-stimulation, colonized Aedes triseriatus mosquitoes fed on all birds. The mosquitoes were subsequently incubated for 21 days at 25°C.

Attempts to detect recurrent viremia by mosquito and blood inoculations into suckling mice have been negative for the few samples tested so far. No persistent virus has been detected in tissues either explanted alone or co-cultivated with VERO cells.

Red Foxes as amplifying hosts of La Crosse virus (LACV)

The role of many medium-sized forest dwelling mammals as amplifiers and possible disseminators in the LACV cycle has yet to be studied. The habits of the red fox, Vulpes fulva, and feeding behavior of Aedes triseriatus, the main vector mosquito, suggested that this vertebrate species merited study.

The LACV used to infect the foxes was a pool of three unpassaged field mosquito isolates inoculated intrathoracically into Ae. triseriatus (Approximately 10 SMICLD₅₀ per mosquito).

Five red foxes without detectable LACV serum neutralizing (SN) antibodies were each infected by the bite of a single LACV-infected Ae. triseriatus. All five red foxes developed detectable viremia of at least four days duration, with peak viremia titers as high as 10^{3.7} SMICLD₅₀/ml of whole blood. Ae. triseriatus that had fed on four viremic foxes were then allowed to feed 21-25 days later on chipmunks Tamias striatus which had no detectable SN antibody. Mosquitoes feeding on a single fox were grouped to feed on a single chipmunk. Two of four chipmunks developed antibody titers to LACV.

If the laboratory results reflect what occurs in the field, our results suggest that red foxes can contribute to maintenance of LACV. Because of the long distances (110 km in one case) traveled by foxes, this species could also serve to disseminate the virus.

La Crosse virus (LACV) in chipmunks

To study possible virus variant selection by passage in natural hosts, five unpassaged LACV mosquito isolates have been assayed in suckling mice and VERO cells, and compared with mouse- and cell-passaged prototype LACV. Results to date indicate that

- 1) chipmunk blood LACV has a 4- to 160-fold lower plaquing efficiency than prototype stock, and
- 2) chipmunk blood plaques are smaller than prototype plaques.

Future work will study, 1) reasons for low plaquing efficiency, 2) biologic correlates of plaque size, and 3) variant selection by mosquito passage.

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REPORT FROM THE UNIVERSIDAD DE ANTIOQUIA - UNIVERSITY OF WISCONSIN PROGRAM

FACULTY OF VETERINARY MEDICINE AND ANIMAL SCIENCE
UNIVERSIDAD DE ANTIOQUIA, MEDELLIN, COLOMBIA

DEPARTMENT OF VETERINARY SCIENCE
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Assessment of the vector capability of Simulium mexicanum for Venezuelan equine encephalitis (VEE) virus continues. Experiments to test the ability of S. mexicanum to mechanically transmit VEE virus were begun. The black flies were permitted to bite viremic guinea pigs but not engorge. Guinea pig viremias ranged from $10^{5.4}$ - $10^{6.5}$ per ml of whole blood. The flies were permitted to engorge fully on suckling mice 30-45 min., 1 hr. - 4 hr., 24 hr. and 48 hr. after biting the guinea pigs. Reluctance of the flies to refeed has resulted in the testing of relatively few individuals thus far. Over 10 individuals have been tested. No VEE virus transmission has been detected.

Work has begun to develop methods to maintain adult S. metallicum in the laboratory for VEE virus transmission experiments. We have had up to 75% (but highly variable) success in inducing the flies to feed on guinea pigs and hamsters. Survival of a few individuals up to 8 days post feeding has been achieved, but these black flies do not yet live long enough to begin attempts to transmit VEE virus.

F. N. Zuluaga, T. M. Yuill

Model Development for Aedes triseriatus and La Crosse (LAC) Virus

Studies have previously indicated that the "within generation" vertical transmission rate of virus in Aedes triseriatus is considerably less than 100%. This being the case, it is apparent that the number of annual generations of A. triseriatus has an important influence on the seasonal (summer) vertical dilution rate of transovarially passed virus. A. triseriatus was formerly presumed to have 2-4 generations per year in southern Wisconsin, but work completed during 1977 yielded an estimate of 1.2 generations. It was found that the sex ratio of emerging A. triseriatus strongly favored males during the early part of the season and that this was partly responsible for delaying the beginning of oviposition until late June. This, in turn, left open only a narrow time gate for development of a partial 2nd generation before onset of egg diapause in late July.

P. Scholl, B. Miller, C. Porter, M. Lisitza, G. DeFoliart

STATE OF ILLINOIS
DEPARTMENT OF PUBLIC HEALTH
DIVISION OF LABORATORIES
2121 WEST TAYLOR STREET
CHICAGO, ILLINOIS 60612

Activity of three arboviruses was detected in Illinois during 1977. Principal emphasis continued to be on the surveillance for St. Louis Encephalitis (SLE) virus, as evidenced by serologic testing of 4881 birds in 26 species for HI antibodies to SLE, Western Equine Encephalitis (WEE), and Eastern Equine Encephalitis (EEE). House sparrows comprised 90 percent of all birds and 3102 (72%) were immature. Antibodies to SLE virus were detected in 108 house sparrows (53 adults and 55 immatures), 1 nestling starling, and 1 adult in each of the following species: blue jay, catbird, European tree sparrow, mockingbird, mourning dove, robin, and rose breasted grosbeak. One adult house sparrow had antibodies to WEE while none were found to EEE.

Over 1600 birds, predominantly nestling house sparrows were tested in suckling mice. Four SLE strains and one WEE strain were made from nestling house sparrows. The WEE isolate, identified by HA technique, was apparently the first made from an avian host in Illinois. Mosquitoes (48,561 in 1448 pools) have yielded 4 strains of SLE. The first isolation, the earliest by this program in 2 years, was from a pool of Culex salinarius on June 3. The other strains were from Culex pipiens.

Twelve human SLE cases (Fig. 1) were confirmed during 1977. The first had an onset date of June 16, approximately 5 weeks earlier than her counterpart in 1976. Fifteen LaCrosse (LAC) Encephalitis infections were confirmed in children ages 7 months to 11 year*. Seven were from Peoria County (Fig. 1), a recurring focus of LAC activity.

Considerable WEE activity was recorded in the northern half of Illinois. Information on equines tested at the USDA Laboratory in Ames, Iowa for the Illinois Department of Agriculture indicated that 92 horses were positive for WEE. No human cases were confirmed.

*Definitive California group confirmation made by Dr. C.H. Calisher, Vector Borne Diseases Division, CDC, Ft. Collins, Colorado.

(Dr. Gary G. Clark and Harvey L. Pretula)

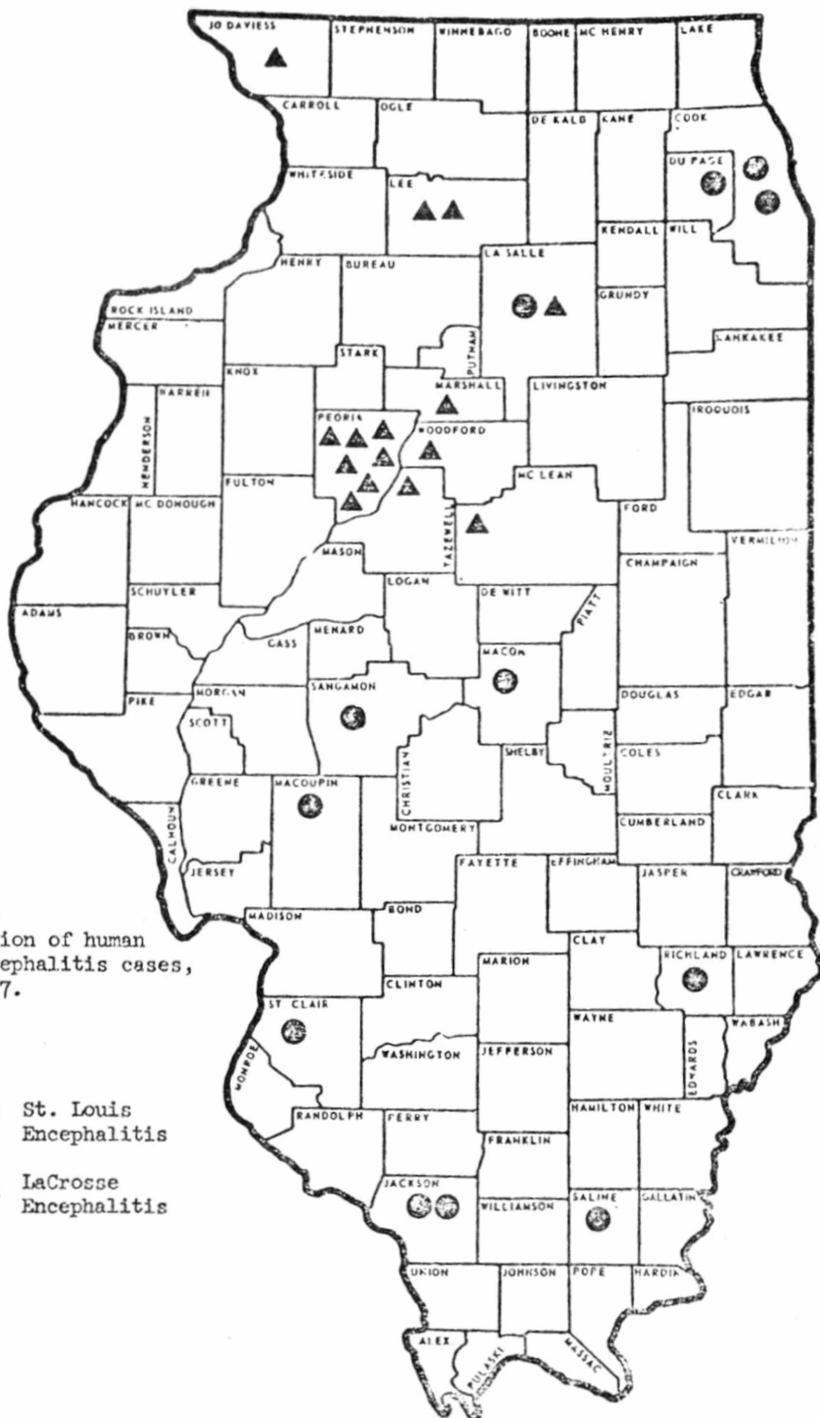


Fig. 1. Location of human arboviral encephalitis cases, Illinois, 1977.

- St. Louis Encephalitis
- ▲ LaCrosse Encephalitis

REPORT FROM THE LABORATORY FOR ARBOVIRUS RESEARCH AND SURVEILLANCE
THE UNIVERSITY OF NOTRE DAME (UNDLARS), NOTRE DAME, INDIANA

An arbovirus surveillance program was initiated in late June of 1977 and continued through September of that year. Joint participation with and funding by the Indiana State Board of Health (ISBH) has previously been described (Arthropod-Borne Virus Information Exchange 33: 58-60).

Four 2-man teams continued mist netting house (English) sparrows until October on a regular basis. A total of 2443 individual birds were collected for antibody screening. All captured birds were banded, bled (< 1.0ml drawn) and released. Whole blood samples were sent to UNDLARS within 48 hr of drawing for serological screening. All serum samples were screened for SLE, EEE, and WEE by hemagglutination-inhibition. Positive HI samples were confirmed using serum dilution neutralization tests.

Through September a total of 59 HI positive birds, all house sparrows, were found (Table 1). All HI positive samples were to SLE only. Included in this number were several recaptured birds which showed seroconversion based on HI results.

A low level of virus transmission was evident throughout much of Indiana this past summer; however, in three counties rates were considerably higher. These three counties, Bartholomew, Jackson, and Vanderburgh, had the highest SLE antibody rates noted all summer. In some collections, rates exceeded 10%. Interestingly, the 1975 SLE human case record indicated that the attack rate per 100,000 population for these three counties was 71.9, 24.2, and 10.1 respectively. The Indiana total for 1975 was 6.1. Fourteen SLE positive juveniles and 1 positive adult were detected in Bartholomew Co., 10 positive juveniles and 3 positive adults were detected in Jackson Co., and 8 positive juveniles in Vanderburgh, or 36 out of a total of 59. Positive birds were noted in 13 of the 20 counties under surveillance.

Transmission apparently peaked early in the summer in the northern portion of the state (Table 1) and declined to undetectable levels by September. In the central portion of the state, activity peaked in July and also declined to undetectable levels by September. In the southern portion of the state, however, early activity in June preceded a decline in July, as was noted in the north. However, there was a marked increase in August and especially September. It was at this time in late summer and early fall that the first human cases apparently were contracted. Most of the late season activity in the south occurred in the three counties mentioned earlier.

Of four birds recaptured, one was recaptured twice. The HI antibody titer demonstrated at the time of both recaptures was essentially similar. Three of these birds showed apparent seroconversion between the initial capture and banding and the subsequent recapture and bleeding based on HI results. However, confirmation NT tests indicated that only one bird seroconverted between initial capture and recapture (Table 2). In addition, four avian serum samples that were HI positive proved to be NT negative. This was not unexpected and indicated to us the need for continued NT test confirmation of HI results.

Surveillance activity will begin in April 1978. This year in addition to avian serological screening, surveillance will include virus isolation attempts from mosquitoes and nestling birds. Attempts will also be made to detect California group virus foci in the state, with emphasis on La Crosse virus.

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TABLE 1. ST. LOUIS ENCEPHALITIS VIRUS ACTIVITY (ANTIBODY RATES) IN THE WILD HOUSE SPARROW POPULATION IN THREE REGIONS OF INDIANA DURING THE SUMMER OF 1977.

REGION	MONTH OF COLLECTION				TOTAL
	JUNE	JULY	AUGUST	SEPTEMBER	
North	1/20 ^a (5.0%)	3/134 (2.2%)	2/139 (1.4%)	0/121 (0)	6/414 (1.4%)
Central	1/47 (2.1%)	6/215 (2.8%)	1/268 (0.4%)	0/92 (0)	8/622 (1.3%)
South	3/97 (3.1%)	5/465 (1.1%)	19/542 (3.5%)	18/303 (5.9%)	45/1407 (3.2%)
Total for State	5/164 (3.0%)	14/814 (1.7%)	22/949 (2.3%)	18/516 (3.5%)	59/2443 (2.4%)

^aNo. positives/No. tested
(percent positive)

TABLE 2. SEROCONVERSION RECORD FOR THREE RECAPTURED SPARROWS

Bird Number	Date Captured	Reciprocal HI Titer	Reciprocal NT Titer
377071205	7/12	< 10	256
	7/26	160-320	256
277081815	8/18	< 10	< 4
	8/22	20-40	8
277090805	9/08	< 10	8
	9/19	≤ 80	8

Note: the fourth of the four recaptured birds had a positive HI titer on initial capture; this titer varied only slightly on two subsequent recaptures and testing

Arbovirus Surveillance 1977

During the summer of 1977 a total of 3,550 pools of 226,489 mosquitoes were collected in 16 counties of New York State for virologic studies. From 8 of the 16 counties, 20 isolates of Eastern equine encephalomyelitis (EEE) and 24 isolates of California group (CAL) viruses were obtained (Table 1). EEE virus was only detected in Culiseta species from Oswego county in upstate New York. There and in the neighboring counties of Oneida and Madison, EEE was serologically indicated in 10 sick horses. Oswego county was also the site of an extensive outbreak among equines in 1976. Most of the CAL isolates were obtained from mosquitoes collected in the Eastern part of the state, while the remaining isolates were from 6 counties in central New York and on Long Island.

In 1977, a total of 509 patients were tested for evidence of infections with EEE, Western equine encephalomyelitis, St. Louis encephalitis (SLE), Powassan (POW) and CAL viruses. California encephalitis (CE) was serologically confirmed in 2 children by seroconversion and antibody findings permitted a presumptive diagnosis of CE in an additional 5 children or adolescents and 1 adult. The POW virus encephalitis was confirmed in a 10-year-old girl with encephalitis who developed a psychosis 4 weeks after onset of illness.

Serologic findings in 13 patients with febrile illness suggested a group B arbovirus infection, i.e., their sera reacted strongly in HI or HI and CF tests with SLE or SLE and POW antigens without neutralizing activity against these viruses. All but one of these patients had a history of recent exposure to dengue fever in Jamaica or Puerto Rico. In 2 of the 13 patients, seroconversions in the HI test indicated a current infection; stable reactions by HI and CF in another 9 patients suggested a recent infection while the remaining 2 patients with stable HI reactions only are presumed to have had a group B arbovirus infection in the past.

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Table 1

Arboviruses Isolated from Mosquitoes Collected in New York State
June - September 1977

Species	No. Tested		Isolates		
	Pools	Specimens	No.		County
			EEE	CAL	
<u>Coquillettidia perturbans</u>	839	74,310		2	Warren
<u>Aedes vexans</u>	665	44,622		1	Suffolk
<u>Anopheles</u> spp.	300	15,216		3	Chemung
<u>Aedes canadensis</u>	217	14,404		3	Saratoga, Warren
<u>Aedes aurifer</u>	134	13,204		1	Schuyler
Other <u>Aedes</u> spp.	184	9,348			
Other <u>Culiseta</u> spp.	115	9,138	1		Oswego
<u>Culiseta melanura</u>	129	8,689	19		Oswego
<u>Aedes stimulans</u>	159	8,500		2	Saratoga, Schuyler
Other <u>Culex</u> spp.	235	7,597			
<u>Aedes communis</u>	158	6,996		6	Warren, Cattaraugus
<u>Aedes cinereus</u>	64	4,469		4	Warren
Mixed <u>Aedes</u> spp.	85	3,150		1	Warren
<u>Aedes cantator</u>	32	2,786			
<u>Culex pipiens</u>	80	2,592			
<u>Aedes triseriatus</u>	139	1,404		1	Greene
Others	15	64			
Total	3,550	226,489	20	24	

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
CORNELL MEDICAL COLLEGE, NEW YORK, N.Y. 10021

Mosquito vector studies in Guatemala during 1977 showed Culex (Melanoconion) opisthopus to be the predominant mosquito where sentinel VE-susceptible, Nepuyo/Patois-immune hamsters were dying. Collections of these mosquitoes (human biting collections) with subsequent identification and exposure to hamsters resulted in deaths of two hamsters. The mosquitoes and the experimental and sentinel hamsters are currently being tested for VE virus.

Surveillance for VE virus infection of sentinel horses in Nicaragua was begun in July and August 1977 by obtaining sera from over 90 young, unvaccinated horses on ranches at the western and eastern extremes of the Pacific Coast. The sera will be tested for antibodies to VE virus. Hemagglutination-inhibition and neutralization antibody tests of horse sera from Nicaragua obtained during 1972-1975 revealed no definite antibody evidence of eastern encephalitis virus. A few sera were positive to western encephalitis virus without a consistent correlation with VE antibodies.

Since English shorthair guinea pigs died after subcutaneous inoculation of equine-virulent strains of VE virus, but survived infections with equine-benign strains (with the possible exception of Panamanian and Colombian strains of HI antigenic subtype I-D), the possibility arose that these guinea pigs might function as sentinel animals to detect equine-virulent VE virions in nature. These guinea pigs were found to be infected but not killed by Nepuyo virus which also exists in some Guatemalan habitats with VE virus; thus Nepuyo virus should not cause confusing results with sentinel guinea pigs. The feasibility of using guinea pigs in the field was explored during 1977, and methods for caging and survival were successfully developed.

Studies of virulence of Venezuelan encephalitis virus were extended to blood mononuclear cells of the hemopoietic system. Cultures of hamster blood mononuclear cells inoculated with several strains of VE virus in vitro failed to support viral replication whether cells were obtained from normal hamsters or hamsters previously immunized with benign strains. Viral growth occurred when hamsters were infected by intraperitoneal inoculation of VE virus and cultures of blood mononuclear cells prepared 15 hours later.

Hamster peritoneal macrophages left in cultures for up to a week change shape, lose contact inhibition and form solid sheets of clear, elongated cells. Hamster-virulent and -benign strains replicated in these cells as in freshly prepared cultures of hamster peritoneal macrophages. However strain BeAr35645 (Pixuna) failed to produce complete cytopathic effects in cultures of modulated macrophages.

Aedes albopictus cell cultures supported growth of virulent and benign strains of VE virus without showing cytopathology. Strain BeAr35645 (Pixuna) grew more slowly than vaccine strain TC83 or virulent, epizootic

strain 6921. Comparisons of plaque forming units in primary chicken embryonic cell cultures and 50% infectious doses in Aedes albopictus cell cultures indicated that ratios of pfu to ID_{50} were 5:1 for strain 6921, 10:1 for strain TC83 and 25:1 for strain BeAr35645. Infectious center assays with strain 6921 showed that one ID_{50} for primary chicken embryonic cell cultures was associated with 3.8 viable cells after 5 hours incubation at 30°C and with one viable Aedes albopictus cell after 16 hours incubation at 30°C. VE virus strain 6921 produced very little inhibition of cellular, high molecular weight protein synthesis in Aedes albopictus cells. By discontinuous slab polyacrylamide gel electrophoresis, envelope and core virion proteins of VE strain TC83 from Aedes albopictus cells appeared similar in number to those of purified virus from chicken embryonic cells in culture.

A temperature-sensitive mutant (ts 126) derived by nitrosoguanidine treatment of hamster-virulent VE strain 68U201 is attenuated for adult hamsters inoculated subcutaneously. After intracranial inoculation it killed less than half of the hamsters inoculated. No structural differences between these virions and parental virus were found by electron microscopy and negative staining. No structural alterations occurred after exposure of ts 126 virus to 40°C for one hour even though this treatment produced significant loss of infectivity and hemagglutinating activity. Coinfection of chicken embryonic cells with ts mutant 126 and parental virus significantly decreased yields of parental virus at the nonpermissive temperature of the ts mutant. Clones of the virus produced were genotypically parental, but when observed with the electron microscope, there were giant and aberrantly tailed forms not found in yields from single virus infections.

By electrophoresis of purified virions of 27 strains of VE virus in discontinuous slab gels of SDS-polyacrylamide, 11 strains contained four detectable polypeptides and 16 strains, three polypeptides. All strains had three polypeptides of similar weights, 53,500-55,000, 52,000 and 33,000 daltons. Some strains had a fourth polypeptide of either 57,000-58,000 or 48,000 daltons. No differences in numbers or molecular weights of virion polypeptides were detected when virus was grown in chicken, hamster or monkey cells in culture. All proteins were glycosylated except the one of 33,000 daltons. Results of pulse-chase experiments indicated that VE virion glycoproteins other than the common 52,000 daltons glycoprotein are derived by post-translational cleavage from a precursor protein. Since the heterogeneity of VE virion polypeptides is manifested only in the polypeptides which are products of such a cleavage, it may be that the biologic diversity is determined by post-translational cleavage.

Data concerning 28 TC83 vaccinations of 75 persons done between 1970-1976 were analyzed. There were 22 mild, early, systemic reactions and no local or late (beyond one week) reactions. Urine tests of a few vaccinees revealed no glycosuria or other abnormalities.

Three unidentified viruses, two from Mexico (from mosquitoes or a bird) and one from Peru (from mosquitoes) were found not to react by complement fixation tests to antibodies representing over 200 arboviruses and five other viruses.

(William F. Scherer)

I. Vector Competence Studies

Studies were conducted to assess vector competence of three geographic strains of Aedes aegypti for yellow fever virus; an oriental strain - Amphur from Thailand, an African strain - Kampala from Uganda, and a Caribbean strain - Santo Domingo from the West Indies. An in vitro feeding technique for mosquitoes developed in this laboratory (see Info. Exchange No. 28, March 1975, p. 139) was used in conjunction with an immunofluorescent technique to determine transmission and infection rates. Mosquitoes were infected by intrathoracic inoculation of virus.

The oriental strain of Aedes aegypti was shown to be a less efficient transmitter of yellow fever virus than both the Caribbean and African strains of Aedes aegypti (Table 1), thus confirming earlier in vivo studies (Am. J. Trop. Med. Hyg., 26: 985, 1977).

(B.J.Beaty, T.H.G.Aitken, and R. Kowalski).

II. Transovarial Transmission of Yellow fever Virus in Aedes aegypti

Studies were conducted to determine if yellow fever virus could be transmitted transovarially by Aedes aegypti as well as other species of mosquitoes. Following infection by intrathoracic inoculation, parent mosquitoes were incubated for 5 days at 27°C. They were then permitted to engorge a blood meal at approximately weekly intervals. A portion of the egg progeny from these females was surface sterilized. After development and emergence of adults, these were sexed, pooled, (100-200/pool) and triturated. Suspensions of these mosquitoes were inoculated intrathoracically into recipient uninfected mosquitoes. The recipients were incubated for 10-12 days at 27°C, killed by freezing, and headsquashed. These headsmears were stained with anti-yellow fever conjugated antibody and examined for the presence of viral antigen with a fluorescence microscope.

Only a portion of the 1st ovarian cycle of the parent Aedes aegypti has so far been examined. Transovarial passage of virus has been demonstrated for all strains of A. aegypti reared from both surface-sterilized and untreated eggs.

(T.H.G.Aitken, B.J.Beaty, R. Tesh, and R. Kowalski).

Table 1

Transmission rates of Aedes aegypti mosquitoes intrathoracically inoculated with yellow fever virus

Mosquito strain	Incubation (inclusive days post infection)			
	26	33	75	Total
Amphur	2/11 (18%)**	-		2/11 (18%)
Kampala	5/8 (62%)	6/12 (50%)	7/25 (28%)	18/45 (40%)
Santo Domingo	8/9 (88%)	10/13 (77%)		18/22 (82%)

* $\chi^2 = 10.31, P < .01$

** Numerator - Number females transmitting virus.

Denominator - Number females infected.

III. Senegal studies: Serology

In November 1976 a few ticks were collected in the lower Senegal River basin region (between Richard-Toll and St. Louis) and in January-February 1977 over 1000 serum specimens were collected from inhabitants of small villages (Savoigne, Lampsar, Kassack-Sud, Kassack-Nord and Ronkh) of the region, as well as biting arthropods, and materials from bird and mammal collections.

One hundred serum specimens were selected as an intentionally biased sample, equal numbers of males and females and equal numbers from the 5 villages, limited to those between 10 and 25 years of age. The age selection bias is intentional. Individuals are old enough to have acquired infections, yet young enough possibly to avoid being saturated by infections with several related viruses in the alphavirus and flavivirus groups, particularly. It is in this group that one may hope to encounter individuals (in hemagglutination-inhibition testing) whose serological pattern may permit a guess as to causative virus. Specific virus neutralization tests to define further the virus specific reactor have not yet been carried out.

In the alphavirus group, there were reactions as follows:

Virus	Number positive/Number tested	Range of titer levels
chikungunya	2/100	1:80 and 1:640
Middelburg	0/100	-
Ndumu	1/100	1:20
Semliki Forest	2/100	1:10 and 1:40
Sindbis	29/100	1:10 to 1:160

The Sindbis positive reactors have 15 reacting at 1:10, 7 at 1:20; 6 at 1:40 and 1 at 1:160. There is no reason to believe that these do not represent immunity conferred by Sindbis virus infection.

The Semliki Forest reactor at 1:40 reacts with chikungunya at 1:640 and the reactor at 1:10 reacts with chikungunya at 1:80. Both reactors almost certainly reflect chikungunya virus infections (possibly not acquired in the immediate region). The virus has been isolated in Senegal by personnel of the Institute Pasteur of Dakar, working south and east of Dakar.

The single Ndumu reactor, at 1:20, was reactive to all the other alphavirus hemagglutinins, including chikungunya at 1:640, and almost certainly represents a non-specific alphavirus serological cross-reaction, possibly induced by infection with more than one alphavirus.

In the flavivirus group, the picture is much more complicated.

Virus	Number positive/Number tested	Range of antibody levels
Banzi	68/100	1:10 - 1:640
Dengue I	35/100	1:10 - 1:80
Dengue II	38/100	1:10 - 1:80
Wesselsbron	73/100	1:10 - 1:640
West Nile	54/100	1:10 - 1:160
Yellow Fever	48/100	1:10 - 1:160
Zika	31/100	1:10 - 1:320
Kadam	47/100	1:10 - 1:160
Dakar Bat	31/100	1:10 - 1:80
Israel Turkey Meningoencephalitis	52/100	1:10 - 1:640

At casual glance, there appears to be the expected finding of a hopelessly entangled flavivirus HI cross-reactivity. This would indicate infection with one or more flaviviruses, with the specific viruses not determinable, probably not even by neutralization test.

Sera which reacted to only one or two antigens were selected out, and 8 Wesselsbron reactors thus located. Three of these reacted only to Wesselsbron antigen and at 1:10 dilution only. Three reacted to Wesselsbron 1:10 and Banzi: 1:10; one reacted to Wesselsbron 1:20, Banzi 1:10 and one reacted to Wesselsbron 1:10, West Nile 1:10. A Wesselsbron neutralization test will be carried out to see whether further facts can be extracted.

The remainder of the sera, as mentioned earlier, are broadly cross-reactive and interpretation to specific levels is not possible.

(W.G.Downs and G. Roze).

IV. Senegal studies: Virus isolations

The Institut Pasteur - Dakar carried out most of the arbovirus isolation work, by infant mouse inoculation technic (the Congo virus strain and several Soldado virus strains were isolated at YARU-New Haven). Isolations were as follows:

November 1976 - One Congo (presumptive) isolate from ticks.

Jan-Feb 1977 - 100 serum specimens from febrile humans seen in clinics at Richard-Toll, Dagana, and Rosso Mauretania - Negative
72 serum specimens from birds - Negative
58 serum specimens from mammals - Negative
22 mosquito pools - Negative
270 tick pools (Ixodid: hard ticks) Wad Medani 9 isolates
24 tick pools (Argasid:soft ticks) Soldado virus 14 isolates

Arbovirus isolations are of course dependent on arbovirus activity in vectors and vertebrates of a region. The months of January-February, in dry season, may well represent a period of minimal activity.

These studies are supported by U. S. Agency for International Development / Africa / C-1259 grant.

(W. G. Downs, A.J.Main, and G. Roze).

REPORT FROM THE STATE OF NEW JERSEY
DEPARTMENT OF HEALTH, TRENTON, N.J. 08625

The following tables summarize isolations of arbovirus made from mosquitoes in New Jersey during the third quarter (July 1 thru September 30) and fourth quarter (October 1 thru December 31) of 1977.

No isolations of arboviruses were made in our laboratories during the first and second quarters of 1977.

(Oscar A. Ross)

Isolations from Arthropods in New Jersey

3rd Quarter

Group	Virus and No. of Strains		Isolated from	Collected in	Month	Year
	EE	WE				
A		1	C. melanura	Woodbine	July	1977
A		1	C. melanura	Woodbine	August	1977
Totals		2				

4th Quarter

Group	Virus and No. of Strains		Isolated from	Collected in	Month	Year
	EE	WE				
A		1	C. melanura	Woodbine	Sept.	1977
Totals		1				

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

Treatment of Machupo virus infection of rhesus monkeys using triacetyl-
ribavirin

Rhesus monkeys infected with Machupo virus usually die while still viremic, with no detectable antibody prior to death. Approximately 20% of the infected monkeys show clinical improvement by day 20, but usually develop a second, late neurological syndrome by days 26 to 40, and die (1).

When triacetylribavirin was given by intramuscular (IM) injection twice daily, beginning on day 0 and continuing through day 16, viremia titers were significantly lower ($P < 0.05$) in monkeys given either 10 or 20 mg/kg/injection of drug compared with sham-treated virus control monkeys (see Fig. 1). In addition, monkeys given 20 mg/kg had somewhat lower titers than monkeys given 10 mg/kg. All sham-treated virus control monkeys died, with a mean time-to-death of 26 days (see Table 1). Drug-treated monkeys were sacrificed for complete histopathological examination when they became paralyzed and prostrate, and lost their prehensile

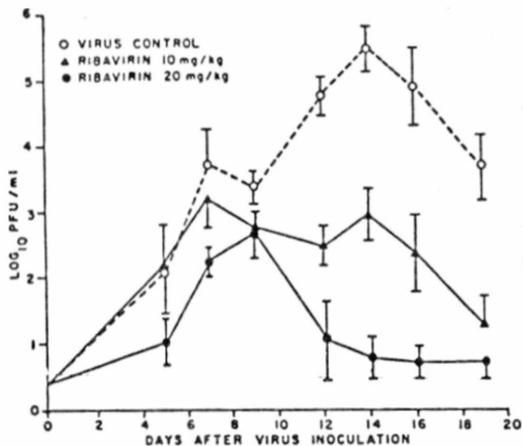


Fig. 1. Effect of triacetylribavirin on viremia titers in Machupo virus-infected rhesus monkeys

Table 1. Response of Machupo Virus Infection of Rhesus Monkeys (4/Group) Treated with Multiple IM Doses of Triacetylribavirin

Treatment (Dose)	No. Dead	% Survival	MTD (Days \pm SE)
None (saline)	4	0	26 \pm 3.3
Triacetylribavirin			
10 mg/kg/injection	1*	75	43
20 mg/kg/injection	2*	50	39 \pm 2.5

*Sacrificed when prostrate.

capability, even though they remained alert and retained their appetites. Clinical signs in the late phase of illness in drug-treated monkeys differed from the late neurological signs exhibited in previous experiments (2). Specifically, drug-treated monkeys were alert, with no signs of depression, anorexia or convulsions. The cause of this phenomenon is currently being investigated.

References

1. Eddy, G. A. et al. 1975. Bull. W.H.O. 52:517-521.
2. Eddy, G. A. et al. 1975. Bull. W.H.O. 52:723-727.

(Edward L. Stephen, Dennis E. Jones, and Gerald A. Eddy)

DEVELOPMENT OF A RADIOIMMUNE ASSAY FOR ANTIBODIES TO ALPHAVIRUSES USING STAPHYLOCOCCUS PROTEIN A

We are developing a radioimmunoassay (RIA) procedure to measure antibodies to alphaviruses in human and other mammalian sera. The test employs protein A-bearing *Staphylococcus aureus*, prepared by formaldehyde and heat treatment as described previously (1), as a solid-phase immuno-adsorbent. Protein A rapidly binds to most mammalian IgG molecules (2) through an interaction with the Fc region. In our test, protein A-bearing staphylococci are substituted for the species-specific anti-IgG antibodies employed in a conventional RIA. Briefly, sera are diluted in Hanks' balanced salts solution (HBSS) containing 0.5% bovine serum albumin, and HEPES buffer (10 mM, pH 7.6). ³H-labeled alphaviruses (VEE, WEE and EEE) are mixed with serum dilutions (0.45 ml serum dilution plus 0.05 ml [³H]virus) and incubated for 1 hr at 37°. Following incubation, 0.1 ml of *S. aureus* (10% w/v) is added, mixed by vortex and then centrifuged at 2500 RPM for 10 min. Following centrifugation, 0.1 ml of the supernatant is withdrawn and residual unbound radioactivity in the supernatant is determined. The proportion of virus bound to the immuno-adsorbent is then calculated. Under the conditions stated, less than 10% of the [³H]virus adsorbs nonspecifically to the *S. aureus* reagent.

Figure 1 illustrates a typical titration of hyperimmune anti-VEE serum prepared in guinea pigs, against [³H]VEE. The dilution of anti-serum which effected 50% binding was determined by probit analysis to be 1:44565. The 95% confidence interval for this point ranged from 1:388862 to 1:50268 (i.e., less than one 2-fold dilution). By a conventional 80% plaque reduction neutralizing antibody test (PRNT), this serum titered 1:5120. Figure 2 illustrates the dependence of RIA titers on the concentration of radiolabeled antigen employed.

Fig. 1

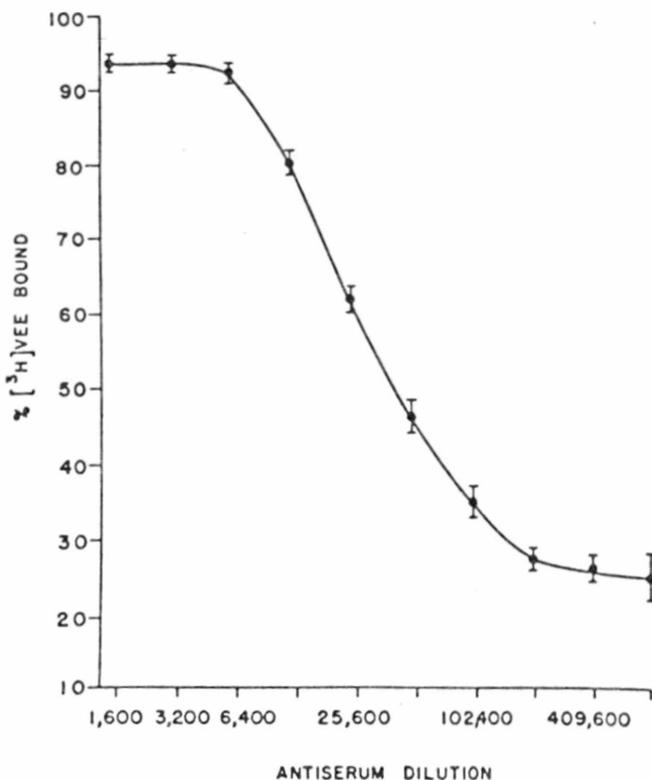


Fig. 2

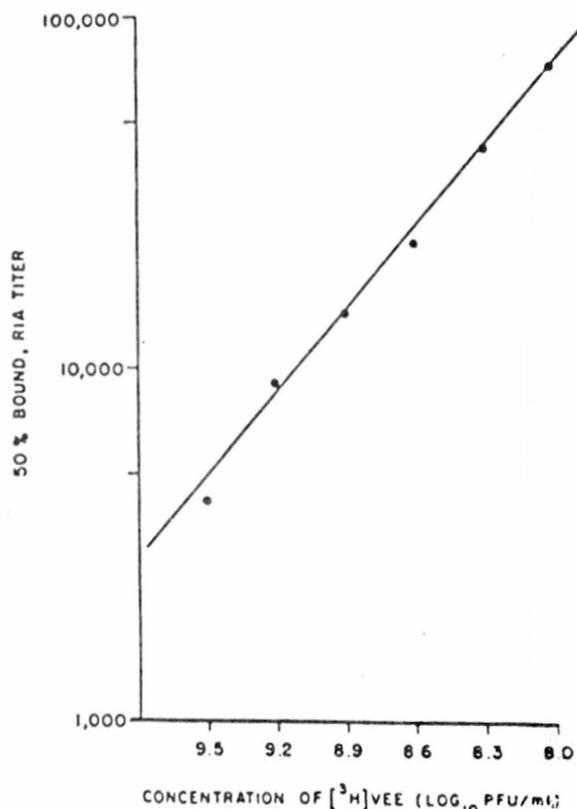


Table 1 compares RIA and PRNT titers of hyperimmune anti-VEE sera obtained from a number of mammalian species. Although this list omits many species of epidemiologic importance, the data suggest that the protein A-mediated RIA may be useful for detecting VEE antibodies in a variety of mammalian sera.

TABLE 1. Comparative Sensitivities of PRNT vs. Protein A-mediated RIA Tests for Measuring VEE Antibodies in Selected Mammalian Sera

Animal Species	Titer		Ratio, RIA/PRNT
	RIA*	PRNT	
Human	1071	160	6.69
Guinea pig	44565	5120	8.70
White rat	11481	1280	8.96
Rabbit	18749	2048	9.15
Burro	814	160	5.05
Dog	3698	320	11.55
Rhesus monkey	11384	1024	11.11
Sheep	1840	320	5.75
Cotton rat	1155	160	7.21

* [³H]VEE, 8.1 log₁₀ PFU/ml

Table 2 is a block titration of hyperimmune guinea pig sera against homologous and heterologous alphaviruses. The RIA procedure clearly differentiated among antibodies to VEE, WEE, and EEE. Preliminary data,

TABLE 2. Separation of Virus-antibody Complexes from Mixtures of ³H-labeled Alphaviruses and Homologous or Heterologous Hyperimmune Guinea Pig Sera

Antiserum	Dilution 1:	% ³ H-labeled virus bound*		
		VEE	WEE	EEE
VEE	100	85.9	7.0	11.2
	1000	82.6	2.4	5.1
	10000	49.1	4.4	2.1
WEE	100	6.3	87.0	9.2
	1000	6.3	77.2	2.0
	10000	5.3	39.7	0.1
EEE	100	2.1	0.4	92.5
	1000	1.4	0.1	52.8
	10000	0.9	0.3	11.8
Nonimmune	100	3.6	5.9	5.7
	1000	1.4	4.9	3.7
	10000	1.7	4.8	3.9

* ³H-labeled virus preparations adjusted to contain 8.1 log₁₀ PFU/ml

using a single injection cotton rat sera, suggest that this procedure may also be useful for differentiating among antibodies to the serologic subtypes of VEE.

The development of RIA procedures to measure alphavirus antibodies is a necessary first step in the development of a competitive binding assay for measuring antigenic potency of inactivated alphavirus vaccine preparations. Such an assay, if successfully developed, might eventually replace the mouse potency assay for assessing potential vaccine efficacy. Further, the RIA procedure might be adapted to differentiate among virion surface and nonstructural antigens.

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(Peter B. Jahrling)

REPORT FROM THE VIRAL DISEASES DIVISION (VDD), BUREAU OF EPIDEMIOLOGY
CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

Surveillance for Human Arbovirus Infection, United States, 1977

The 1977 arboviral season began with scattered human WEE cases, following widespread equine disease, and early sporadic reports of SLE. Dengue outbreaks in the Caribbean, and a late fall outbreak of SLE in Florida resulted in an unusually extended season of arboviral activity. Preliminary totals for cases of laboratory diagnosed arboviral infection reported by the states to CDC are tabulated below.

The total of 164 cases of SLE is less than half the 1976 total, and less than a tenth of the 1975 total, yet larger than any other annual total since 1966. Cases were reported with onset in mid-June from Texas, and in early December from Florida; a longer season than any other we have found for the United States. SLE cases were reported from 14 states, but Florida accounted for two thirds of the total. Florida cases were scattered in 18 counties across the central and southern part of the State. SLE virus was isolated from Culex nigripalpus at numerous locations by the Tampa and Jacksonville Laboratories of the Florida Department of Health and by the Vector-Borne Diseases Unit, Bureau of Laboratories, CDC. There was serologic evidence of SLE activity in back yard chicken flocks for most of the involved counties, but little evidence of similar activity in wild birds.

The number and distribution of California Encephalitis cases was similar to that reported for most years of the preceding decade. Cases from 3 states, Wisconsin (16), Illinois (15), and Ohio (13) accounted for 72% of the total of 61 cases.

A total of 34 WEE infections were reported from 11 states, following a major epizootic of WEE in horses. With the exception of 1975, this was the largest number of WEE cases reported since 1966. The remaining reports of encephalitides included 3 of Powassan infection from Upstate New York and 1 fatal case of EEE from Louisiana.

A series of dengue outbreaks occurred in the Caribbean during 1977 (detailed elsewhere in this issue). Dengue types 2 and 3, and, for the first time type 1, were associated with both epidemic and sporadic activity. Dengue hemorrhagic fever and dengue shock syndrome were not seen. A major epidemic of dengue type 1 in Jamaica was followed by type 1 activity in the Bahamas, Cuba, Haiti, Puerto Rico, and the Virgin Islands. Dengue type 2 cases were documented in French Guiana, Puerto Rico, and the Virgin Islands, and type 3 cases in the Dominican Republic and Puerto Rico. Dengue outbreaks, not identified to type, affected Guadeloupe and Sint Maarten.

Sixty laboratory documented cases of dengue were imported into the continental United States by travelers. Persons infected in the Caribbean imported cases into 21 states and the District of Columbia. Dengue was also documented in travelers returning to California from Tahiti, and to Hawaii from Thailand. Despite active surveillance, no secondary cases were detected in the continental United States.

(Karl Kappus, David Morens, David Nelson, and Lawrence Schonberger)

Reports of Arboviral Disease in Humans, United States, 1977

STATE	SLE	CE	WEE	Other Encephalitis	Imported Dengue
Alabama	1				1
Alaska					1
Arizona					
Arkansas	1				
California	1				7
Colorado			11		1
Connecticut			1		3
Delaware					
Dist. of Col.					6
Florida	109				3
Georgia					
Hawaii					1
Idaho					
Illinois	12	15			
Indiana	8	2			1
Iowa		3	1		
Kansas	1		1		
Kentucky	2				
Louisiana	7		1	1 EEE	1
Maine					
Maryland					2
Massachusetts					2
Michigan		3			
Minnesota			1		
Mississippi	7				2
Missouri		1			
Montana			2		
Nebraska					
Nevada					
New Hampshire					
New Jersey					3
New Mexico	1		2		
Upstate New York	2	3		3 Powassan	1
New York City					9
North Carolina		4			2
North Dakota			3		
Ohio	3	13			3
Oklahoma					
Oregon					1
Pennsylvania					
Rhode Island					
South Carolina					
South Dakota			6		
Tennessee	2				1
Texas	7		5		4
Utah					
Vermont					
Virginia					5
Washington					
West Virginia					
Wisconsin		16			
Wyoming					
Totals	164	61	34	4	60
Guam					
Puerto Rico					
Virgin Islands					

REPORT FROM
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES
OFFICE OF LABORATORY SERVICES AND HEALTH PROGRAM OFFICE
JACKSONVILLE AND TALLAHASSEE, FLORIDA

Florida experienced a significant increase in SLE activity during the latter half of 1977. As of the end of the year, we have identified 110 human cases in the laboratory. These included 76 cases in which there was a 4-fold rise or fall in HI and/or CF antibody titer in paired sera and 34 cases in which single sera showed titers of HI \geq 1:40 or CF \geq 1:16. This is the largest number of human cases in Florida, since the 1962 outbreak when they were concentrated in 5 counties in the Tampa Bay area. In contrast, the 1977 cases were scattered in 19 counties as shown on the attached map (Figure 1).

As in the 1962 episode, those in the over 65 year age group were hardest hit, comprising 43.0% of the cases. Case fatalities were much reduced, there being only 8 in 1977 as compared to 43 in 1962. The initial human case had an onset date of August 12 and the latest one in 1977 occurred during the week of December 9. The outbreak peaked in mid-October.

A summary of serologic SLE conversions of mammalian and avian hosts is presented in Table I. Mammals and chicken flocks were much better temporal and quantitative indicators of SLE activity than urban pigeons and sparrows. A total of 42.3% of the chickens and 20.0% of the mammals converted as compared to only 1.2% of the urban birds. It will also be noted that conversions appeared earlier in mammals and chickens as compared to the birds.

Mosquito collections were made and tested. A total of 1,538 pools (approximately 60 mosquitoes/pool) of C. nigrapalpus yielded 36 SLE isolates. However, 33 of the isolates came from 2 counties where local funding made it possible to intensify surveillance efforts. The remaining 3 SLE isolates came from 3 other counties. To rule out the possibility of mosquito species other than Culex nigrapalpus carrying SLE virus, the CDC, Vector-Borne Diseases Division, Ft. Collins, Colorado, made field collections in 12 counties and tested 1,235 pools of other species with no isolations of SLE. However, 4 isolates of VEE, one Bunyamwera group and one California virus were made.

Acknowledgement and thanks for field and laboratory assistance are extended to CDC Epidemiology Branch, Atlanta, and CDC, Vector-Borne Diseases Division, Ft. Collins, including Dr. D. B. Franczy, Dr. C. Calisher, Dr. K. Kappus, Dr. D. Nelson and Dr. T. Monath. Also, the data includes information supplied by the Department of Health and Rehabilitative Services staff, including E. C. Prather, M. D., R. Michael Yeller, M. D., Henry Janowski, M.P.H., Juan Tomas, D.V.M., E. E. Buff, M.S., F. M. Wellings, D.Sc., A. L. Lewis, D.V.M., Betty Koehler, R.N., A. J. Rogers, Ph.D., and E. C. Beck, B.S.

TABLE I

SLE ANTIBODY CONVERSION OF SENTINEL MAMMALIAN AND AVIAN HOSTS IN SERA
COLLECTED MONTHLY IN FLORIDA, 1977

Sera from:	August	September	October	November	December	Total	Percent Positive
Pigeons and Sparrows	0/564*	0/500	21/667	0/97	-	21/1731	1.2
Chickens	0/17	10/57	286/665	205/445	-	501/1184	42.3
Mammals	0/12	3/19	7/25	5/14	2/15	17/85	20.0

* Number positive SLE titers/number of sera tested

REPORT FROM THE SAN JUAN LABORATORIES,
CENTER FOR DISEASE CONTROL, SAN JUAN, PUERTO RICO

The 1977-78 Dengue Epidemic in Puerto Rico

The course of the epidemic is shown in the figure. From June 26, 1977, through February 22, 1978, 12,531 suspect cases of dengue had been reported to the San Juan Laboratories (SJL), accompanied by blood specimens. Of these, 25% were from the San Juan metropolitan area; this percentage is approximately in proportion to the population. A total of 3,458 pairs of sera were received, and by the hemagglutination-inhibition (HI) test, 40% were diagnosed as current dengue. Antibodies were present in many more pairs, but they either did not have high titers or did not demonstrate significant seroconversion. Cases were reported from 76/78 municipalities and dengue was confirmed in 57 of these municipalities. Among the suspected cases, the ratio of females to males was 4:3, and the age group most heavily affected was 16-30 years.

House-to-house and industrial surveys carried out in October by epidemiologists from CDC, Atlanta, indicated a clinical attack rate of over 20%. However, serological tests on paired sera from 130 participants in the industrial survey, bled in October 1977 and again in January 1978, revealed only six conversions, for an attack rate of 4.6%.

More than 100 strains of virus were isolated in mosquitoes inoculated with the sera of patients. Those so far identified include three strains of type 1, sixteen of type 2, and eleven of type 3. The type 1 isolates came from patients in the southwestern part of the island who had onset of illness in December. The identities of several of the type 1 and type 3 isolates were confirmed by plaque reduction neutralization test at the Walter Reed Army Institute of Research. One patient from whom dengue type 2 virus had been isolated in 1975 became ill again in 1977, and type 3 virus was isolated.

Approximately 9% of patients had one or more hemorrhagic signs, mostly epistaxis. Age and sex distributions of the hemorrhagic cases were similar to those of all cases. An analysis of 232 hemorrhagic cases (by Dr. David Morens, Bureau of Epidemiology, CDC) showed that 40 (17%) had more than one hemorrhagic sign, and 16 had platelet counts below 100,000 (one had only 12,000). None of these patients had life-threatening hemorrhage, and all made complete recoveries. None of them fulfilled the WHO criteria for dengue hemorrhagic fever or dengue shock syndrome.

Puerto Ricans traveling to the United States mainland became ill with serologically confirmed dengue after arriving in New York in October, Florida in November, and Alaska in December 1977.

The Aedes aegypti ovitrap index in the metropolitan San Juan area rose from 22% in late March to a peak of 58% in early August 1977, based on a transect of 30-60 ovitraps exposed for 6-11 days. House indices around the island in July ranged from 2 to 88% with 52/78 municipalities reporting indices of greater than 20%, and 17 greater than 50%.

Eight truck-mounted ULV spray machines were purchased to supplement the single unit available. These went into operation in the San Juan metropolitan area on August 19, spraying 96% malathion at approximately 0.7 oz/acre. They were operated every night, except on a few occasions when it rained, and extended to cover 40,000 acres of the metropolitan area in approximately 5-day cycles, which continued until mid-January. On September 25, air ultra-low-volume spraying was begun, using 96% malathion at 3.0 oz/acre. Part of the metropolitan area and 60 other townships around the island, totalling 120,000 acres, were covered in four 5-day cycles ending on October 14. Mosquito populations declined markedly after spraying. Dengue case reports also began to decline coincidentally with the air spraying. Ground spraying continued during and after air spraying. Rainfall increased in October, and mosquito populations increased in November, but case reports continued to decline. The average house index for the island at the end of October was 12% (533 positive houses/4,429 inspected).

An extensive public health education program was conducted through the news media, encouraging householders to clean up their premises. Source-reduction campaigns were held in 75 of the 78 municipalities, and a massive island-wide source-reduction project involving several million dollars in Federal funds is due to be implemented in March 1978.

Dengue Elsewhere in the Caribbean

a. Haiti

Haiti reported 238 suspect cases for 1977, and twelve of these were confirmed serologically. Some of these were children aged 5-14 years, with onset in October 1977. Apparently there was an epidemic that peaked in November, and dengue was confirmed serologically in travelers from Haiti arriving in New Jersey, Ohio, and South Carolina, with onset in October, November, and December.

Dengue type 1 virus was isolated from both Ohio cases by the Ohio State Health Department, and identified by the San Juan Laboratories. The identification of another isolate, from a 13-year-old Haitian boy with onset of illness in November, is pending. Paired sera were recently received from five adult patients with onset of dengue-like illness in January 1978.

b. Dominican Republic

A serum survey of children from Santo Domingo in 1977, who ranged in age from a few days to 7 years, showed 64/118 (54%) with dengue HI antibodies. Type 3 dengue virus was recovered from a Puerto Rican medical student attending college in the Dominican Republic, with onset of illness in July, and another strain, identification pending, came from a 9-year-old boy who had onset in September. Out of 29 pairs of sera collected from patients with onset of illness from August 1977 through January 1978 (mostly December), 21 could be diagnosed as dengue by the HI test. Four of these were primary infections, all in children aged 7 months to 10 years. The remaining positives were secondary infections, all in older children and adults. Seven of the 21 confirmed cases had hemorrhagic manifestations of various types, including two cases of hematuria.

c. U.S. Virgin Islands

Six pairs of sera from St. Thomas and one pair from St. John showed serological confirmation of current dengue infection. Patients were aged 14 and older, with onset dates in November and December 1977. Dengue virus type 1 was isolated in January 1978 from a patient from whom another dengue virus (identification pending) was recovered 12 months earlier.

d. Netherlands Antilles

A traveler who visited St. Maarten and St. Eustatius in August, and another who visited St. Maarten and Aruba in November, developed serologically confirmed dengue when they returned to New York. Another traveler to Florida from Curaçao was diagnosed serologically as a case of dengue, with onset in January 1978. Three strains of dengue virus were isolated from patients living in St. Maarten, with onsets in November and December 1977; type identification is pending.

Dengue Imported into the United States, 1977-78

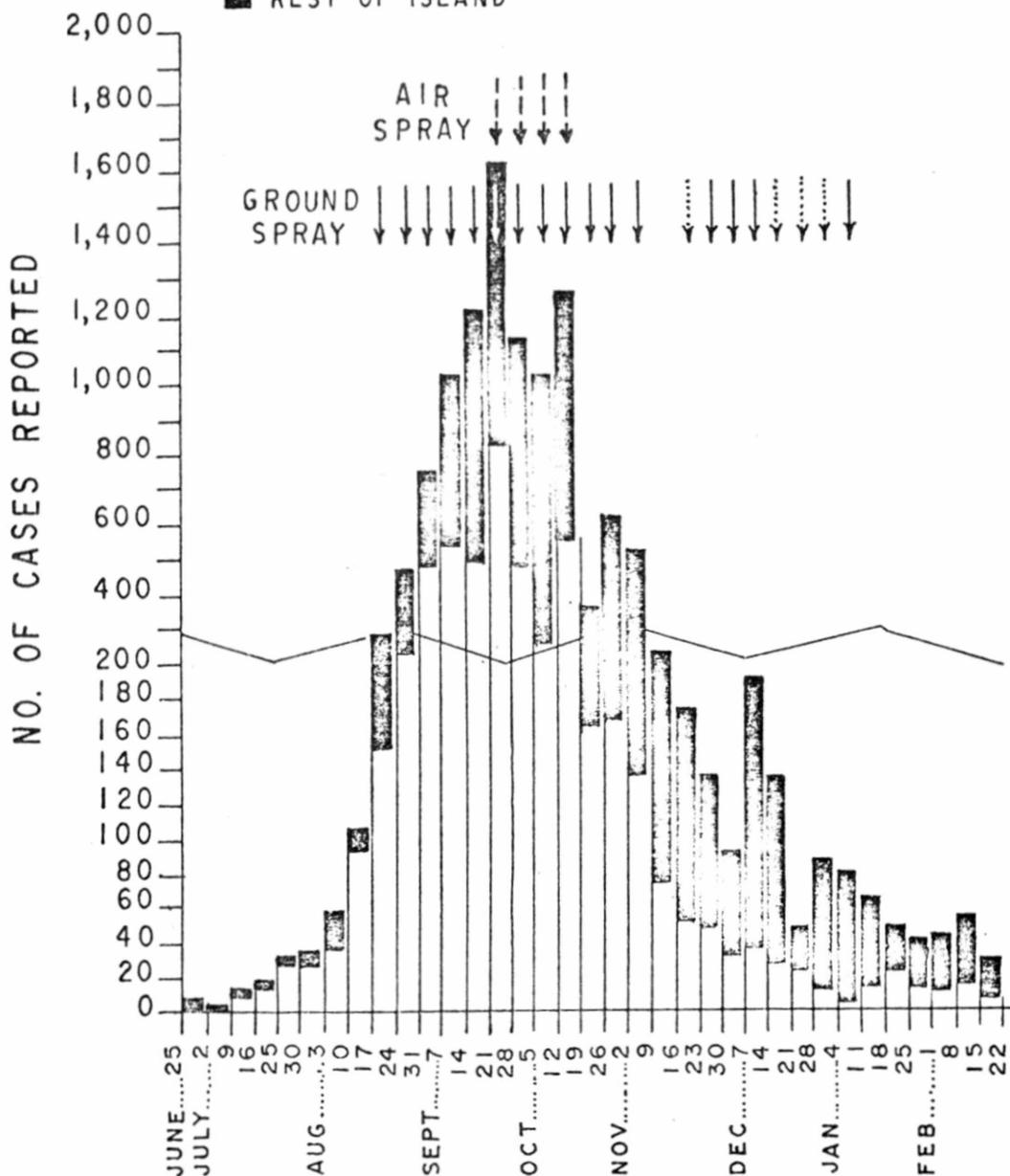
Through February 1978, 58 laboratory-confirmed imported cases of dengue had been reported in 19 states and the District of Columbia. These included 7 of the 10 southeastern states receptive to dengue because of the presence of Aedes aegypti. Fortunately, no secondary cases were found.

Most of the cases originated in Jamaica, but there were also people from Barbados, Cuba, the Dominican Republic, Haiti, the Netherlands Antilles, and Puerto Rico. Some travel histories said simply, "Caribbean," "Tropics," or "dengue endemic area." One classic response to our query as to the location where illness was contracted was, "patient became ill elsewhere." Dengue type 1 virus was isolated from two travelers returning from Jamaica: one in Baton Rouge, Louisiana, and one in Johnson City, New York. These identifications were confirmed by the Walter Reed Army Institute of Research.

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SUSPECTED CASES OF DENGUE REPORTED TO SAN JUAN LABORATORIES 1977 - 78

METRO AREA INCLUDING BAYAMON
 REST OF ISLAND



Isolation of Dengue Viruses in Mosquito Cell
Cultures under field conditions

The increase in dengue virus activity in the Caribbean has provided opportunities for the evaluation of a mosquito cell line as a means of virus isolation. The cell line LSTM-AP-61, derived from Aedes pseudoscutellaris has been shown not only to support the multiplication of several flaviviruses, but also has been used for the isolation of certain arboviruses from wild caught mosquitoes and human serum and organs^{1, 2, 3, 4}. During the investigation of an outbreak of dengue fever on the island of Dominica, mosquito cell cultures were transported from the P.A.H.O./W.H.O. Caribbean Epidemiology Centre (CAREC) in Trinidad and inoculated on the spot with sera from suspected acute cases. The cells were held at room temperature (approximately 25⁰C) for 2 days before being transported to Trinidad for incubation at the optimal 28⁰C. Cytopathic effect (CPE) was evident from 4 to 8 days after inoculation and, by complement-fixation tests on cell culture fluids using type-specific sera, it was shown by day 8 that dengue type 1 was the virus involved.

Isolations were made in mosquito cell cultures from a further 5 of 36 sera which had been stored at -10⁰C in Dominica before being transported to Trinidad. The corresponding mouse inoculation tests and LLCMK₂ cultures were negative.

During a recent trip to the Bahamas Islands acute sera from suspected dengue cases were again inoculated into mosquito cell cultures for transportation to Trinidad. Since then we have investigated outbreaks and cases in Grand Turk, Grenada, Antigua, St. Lucia, St. Kitts, Guyana and Trinidad. The results of the isolations obtained and their identities to date are shown in the Table.

Preliminary identifications were made by the complement fixation test (CFT) using control antigens and antisera kindly provided by the Centre for Disease Control, Atlanta (Dengue Type 1 - Hawaiian; type 2 - New Guinea C, type 3 - H-87 and type 4 - H241).

Grateful acknowledgement is made to the Chief Medical Officers and the health staff of the territories concerned.

P.J.S. Hamilton
Director CAREC

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DENGUE ISOLATION STUDIES WITH HUMAN SERA

Country	Collected	Mosquito Cells Isolates/ No. Inoc	Mouse Inoc Isolates/ No. Inoc	Provisional Identification by C.F.T.			
				Type 1	Type 2	N.T.	TOTAL
1. Trinidad	June 77 - February 78	117/397	53/432	31	-	86	117
2. Dominica	August	8/56	1/56	6	2	-	8
3. Grand Turk	September-October	4/6	0/6	2	-	2	4
4. Grenada	September 77-January 78	38/90	0/13	2	-	36	38
5. Bahamas	November	51/76	37/76	12	-	39	51
6. Antigua	November	3/33	0/14	-	-	3	3
7. St. Lucia	November	0/2	0/2	-	-	-	-
8. St. Kitts	November 77-January 78	7/25	0/6	1	-	6	7
9. Guyana	November 77-December 78	4/34	0/34	-	-	4	4
TOTALS		232/699	91/639	54	2	176	232

N.T. = Not tested.

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUT PASTEUR and O.R.S.T.O.M.
Groupe INSERM U.79
CAYENNE, GUYANE FRANCAISE

1. Detection of the Dengue virus with the fluorescent foci method in LLCMK2 cells inoculated with ground mosquitoes, Aedes aegypti.

Using a new technique Dengue virus was isolated from human sera and mosquitoes during recent outbreak in Cayenne, January-December 1977. The sera or ground mosquitoes were inoculated into 6 suckling mice intracerebrally, and into four batches of ten female of Aedes aegypti each by the intrathoracic route. After ten days the mosquitoes were ground, centrifuged and inoculated in LLCMK2 cells cultured on Lab Tek slides. After three days the dengue virus was visualized by indirect immunofluorescence using an anti-dengue 2 mouse ascitic fluid followed by a fluorescein-labeled anti-mouse gamma-globulin serum.

Recently we have modified the technique: the female Aedes aegypti are kept only four days after intrathoracic inoculation rather than 10 because the titer of the virus is higher at four days. Also, the Hanks medium is poured off the LLCMK2 cells before inoculation.

The results for the human sera and the mosquitoes are given in the Tables I and II.

Eighteen strains have been isolated from human sera and four from mosquitoes, three from Aedes aegypti and one from Coquillettidia venezuelensis.

The strains isolated only by visualization of fluorescent focus on LLCMK2 cells were identified by fluorescent focus reduction using two immune ascitic fluids, Dengue 2 and Dengue 3, at a dilution of 1/100. The five strains isolated by intracerebral inoculation of suckling mice were identified by complement fixation test. All strains except one were Dengue 2.

2. Strains isolated in 1976-1977.

a. All the strains from humans were Dengue 2, except one isolated in September 1977 and not yet identified.

b. From 233,673 mosquitoes tested in 4,463 batches 117 strains of virus have been isolated.

- Group A VEE complex Tonate virus
23 strains from Culex portesi.
2 strains " Culex zeteki.
1 strain " Anopheles mediopunctatus.
2 " Anopheles braziliensis.
2 " Coquillettidia albicosta.
2 " Coquillettidia venezuelensis.
1 " Mansonia pseudotitillans.
1 " Lutzomyia sp.

- VEE group. Cabassou virus.

4	strains	from	<u>Culex portesi.</u>
1	"	"	<u>Culex sp.</u>
1	"	"	<u>Culex nigripalpus.</u>
2	"	"	<u>Coquillettidia venezuelensis.</u>
1	"	"	<u>Mansonia titillans.</u>
1	"	"	<u>Wyeomyia occulta.</u>
1	"	"	<u>Limatus pseudomethysticus.</u>

- Group C. Murutucu- Oriboca.

9	strains	from	<u>Culex portesi .</u>
1	"	"	<u>Coquillettidia albicosta.</u>
2	"	"	<u>Coquillettidia venezuelensis.</u>
1	"	"	<u>Mansonia titillans.</u>
1	"	"	<u>Aedes arborealis.</u>
1	"	"	<u>Trichoprosopon digitatum.</u>
1	"	"	<u>Trichoprosopon sp.</u>

- Guama group.

27	strains	from	<u>Culex portesi.</u>
1	"	"	<u>Culex spissipes.</u>
1	"	"	<u>Anopheles braziliensis.</u>
1	"	"	<u>Anopheles darlingi.</u>
1	"	"	<u>Anopheles darlingi.</u>
11	"	"	<u>Coquillettidia venezuelensis.</u>
2	"	"	<u>Mansonia titillans.</u>
1	"	"	<u>Trichoprosopon digitatum.</u>
1	"	"	<u>Psorophora ferox.</u>
1	"	"	<u>Phonimyi splendida.</u>
1	"	"	<u>Lutzomyia sp.</u>
1	"	"	<u>Sabethes undosus.</u>

- Ilheus.

1	strain	from	<u>Coquillettidia venezuelensis.</u>
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- Una.
 - 1 strain from Anopheles nimbus.
- Itaporanga.
 - 1 strain from Culex albinensis.
- Anopheles group.
 - Ca Ar 24 626 from Anopheles aquasalis(not yet identified).
- Unidentified.
 - Ca Ar 24 246 from Anopheles aquasalis.
 - Ca Ar 24 400 from Culex portesi.
 - Ca Ar 24 598 from Coquillettidia albicosta.
 - Ca Ar 24 746 from Culex portesi.
- a) From 1.111 animals (birds, rodents and marsupials)
 - 16 strains have been isolated.
- Group VEE Tonate.
 - 1 strain from Chiroxiphia pareola.
 - 1 strain from Ardeola ibis.
 - 1 strain from Leucopternis albicollis.
 - 1 strain from Nycticorax violacea.
 - 1 strain from Ramphastos tucanus.
 - 2 strains from Glyphorynchus spirissus
- Group VEE Cabassou.
 - 1 strain from Turdus nudigenis.
 - 1 strain from Tolmomyias poliocephalus.
 - 1 strain from Metachirops opposum
 - 1 strain form Didelphis marsupialis.
- Group B.
 - Saint-Louis Encephalitis from Anhinga anhinga.

- Group C.

1 strain from Metachirops opposum.

- Group Guama.

2 strains from Didelphis marsupialis.

- Group Phlebotomus.

1 strain unidentified Ca An 4668 a from Nycticorax violacea.

J-P.DIGOUTTE, Y. LE QUEREC, G.GIRAULT INSTITUT PASTEUR.

F-X. PAJOT, N.DEGALLIER O.R.S.T.O.M.

T A B L E A U I

STRAINS ISOLATED FROM HUMAN SERA BY INTRATHORACIC INOCULATION ON
AEDES AEGYPTI AND VISUALISATION OF FLUORESCENT FOCUS OR INOCULATION TO THE SUCKLING MICE

Number	Date of the beginning of the disease	Place of contamination	Days after the beginning	Isolation L L C M K 2	Isolation on suckling mice after Aedes	Direct isolation on suckling mice
77-304	23/02/1977	Montjoly	2	+	-	-
77-375	02/03/1977	Montjoly	3	+	-	-
77-695	04/04/1977	Cayenne	4	+	-	-
77-730	12/04/1977	Montjoly	1	+	+	-
77-998	05/05/1977	Cayenne	?	+	-	-
77-1068	11/05/1977	Sinnamary	1	+	-	+
77-1070	11/05/1977	Sinnamary	1	+	-	-
77-1071	11/05/1977	Sinnamary	1	+	-	-
77-1072	11/05/1977	Sinnamary	1	+	-	-
77-1073	11/05/1977	Sinnamary	1	+	-	-
77-1074 S.M.	11/05/1977	Sinnamary	1	-	-	+
77-1095	12/05/1977	Cayenne	?	-	-	+
77-1313	07/06/1977	Cayenne	3	-	-	+
77-1563	08/07/1977	Cayenne	?	+	+	-
77-1893	24/08/1977	Cayenne	?	+	-	-
77-2087	15/09/1977	Cayenne	2	+	-	-
77-2210	27/09/1977	St-Laurent	2	+	+	-
77-2647	10/11/1977	Cayenne		+	+	+

TABLEAU II

STRAIN OF DENGUE 2 ISOLATED FROM MOSQUITOES

Number	Date of collection	Place of collection	Species	Isolation on		Direct isolation on suckling-mice
				L L C M K 2	suckling-mice after Aedes	
Ca Ar 24871	16/05/1977	Sinnamary	Aedes aegypti		+	-
Ca Ar 24874	16/05/1977	Sinnamary	Coquillettidia venezuelensis		+	-
Ca Ar 24885	17/05/1977	Sinnamary	Aedes aegypti		+	-
Ca Ar 24896	17/05/1977	Sinnamary	Aedes aegypti		+	-

Transovarian passage of Gamboa virus:

Transovarian passage of virus in mosquitoes has been demonstrated for a few viruses in the holarctic region (where the mechanism favors virus survival over the winter). We recently found this same phenomenon occurs in the tropics - with Aedeomyia squamipennis mosquitoes and Gamboa, a Bunyavirus.

A. squamipennis (Lynch-Arribalzaga) is a little known neotropical species that occurs from southern Mexico to northern Argentina and Ecuador. It breeds the year around in weed-infested, permanent swamps and lakes. Gamboa virus was originally recovered in 1962 in Panama, from A. squamipennis collected along the Chagres River near the town of Gamboa. In that study, 5 of 13 mosquito pools were positive.

In October 1976, we began regularly encountering A. squamipennis in routine collections made at our Bayano River station where a lake had begun to form behind the new hydroelectric dam. Since December 1976 Gamboa virus has been persistently isolated there from A. squamipennis pools (Table 1). The persistence of Gamboa virus in a high proportion of pools, in the same area, throughout the year led us to suspect transovarian transmission. In September 1977, we collected over 1000 mosquito larvae near our Bayano field station and maintained them in the laboratory to pupation. Individual pupae were isolated in vials and allowed to emerge as adults. We tested pools of approximately 10 adults for virus. From 73 pools (782 mosquitoes), we recovered 5 Gamboa strains - 3 from 38 male pools and 2 from 35 female pools.

We just completed a similar experiment with A. squamipennis larvae collected in Chiriquí Province, in westernmost Panama. Again, some of the emerging adults were virus-positive, thus indicating that this phenomenon is generalized.

We are currently examining the ecology of Gamboa virus in more detail to elucidate the possible adaptive advantage of transovarian passage in A. squamipennis. We particularly need to discover the natural host(s) of Gamboa virus. Because A. squamipennis mosquitoes have been previously described as feeding only on birds, we are surveying sentinel chickens and wild birds for antibody to Gamboa virus. But since we regular capture A. squamipennis on human bait, we also plan to survey wild animals for antibody.

Transovarian virus transmission seems to be an unnecessary adaptation for survival of Gamboa virus in the tropics. There is no harsh winter and A. squamipennis breeds readily throughout the year.

However, if Gamboa virus has a restricted transmission cycle, with one vector and one, or few vertebrate hosts, then transovarian transmission might be vital to insure virus survival during periods of host scarcity.

(P. H. Peralta, P. Galindo, A. J. Adames)

Table 1

Isolation of Gamboa Virus from Aedeomyia squamipennis
at Altos de Majé Station, Bayano, Panamá

Month	No. mosqs.	No. pools	No. isolates
Aug. 76	2	1	0
Sept.	0	0	
Oct.	121	4	0
Nov.	389	9	0
Dec.	41	2	1
Jan. 77	75	8	0
Feb.	605	17	3
Mar.	720	20	6
Apr.	1280	29	7
May	709	18	4
Jun.	708	17	4
Jul.	193	7	1
Aug.	223	9	1
Sep.	156	6	0
Oct.	316	8	3
Nov.	432	13	4
Dec.	348	10	3
Totals	6318	178	37

Venezuelan equine encephalitis virus infection in pregnant mares:

Up to the present there is no good explanation as to how epizootic VEE virus is preserved in nature during inter-epizootic periods. We have recently initiated studies to demonstrate whether epizootic VEE produces either latent or persistent infection in horses.

Pregnant mares were infected with MF-8 or P-676, epizootic strains of VEE virus. All horses were free of antibody to the 3880 (Panama enzootic) or TC-83 strains before inoculation with 100,000 SMLD₅₀S.C. The animals under study were kept in insect-proof facilities, and blood samples were collected daily for virus or antibody assays, until the mare died, was sacrificed or survived. Fetal or mare tissues were rushed to the Laboratory where cell cultures were attempted. Otherwise the specimens were stored in liquid N₂ until processed.

Table 1-4 summarize preliminary data. Fluid and cells from both minced organ explants and trypsinized cell cultures are collected once or twice a week for 6 weeks and assayed for virus in newborn mice. After this period, negative cultures are discarded. Those cell cultures yielding VEE virus, retained a normal morphology and appeared to have the same growth rate as those non-infected cells.

(G. Justines, G. Oro, O. Alvarez and H. Sucre)

Table 1
Pregnant Mares Infected with Epizootic VEE Virus

Mare #	Virus	Gestation Months	Viremia	Abortion	Day Died/Killed
230	MF-8	3-4	+	+(4)*	6
231	MF-8	6-7	+	-	7
232	P-676	10	+	-	5
233	P-676	5	NT	+(2)*	-

* Aborted 4 and 2 days after infection
NT Not tested yet

Table 2

VEE Virus Detected in Fetal Organ Tissues Suspensions

Organs Tested	Fetus from Mares			
	230	231	232	233
Spleen	+	0	+	0
Liver	0	0	+	0
Thymus	NT	0	NT	0
Lymph node	NT	0	+	0
Kidney	0	0	+	0
Heart	NT	0	+	0
Lung	0	NT	NT	0
Brain	0	0	NT	0
Cerebellum	0	0	NT	NT
Ovary	NT	NT	NT	0
Spinal cord	0	0	NT	NT
Pancreas	NT	0	NT	NT
Bone marrow	NT	NT	NT	0
Blood	0	0	+	0

+ VEE virus recovered

0 No virus present

Table 3

Fetus Trypsin Dispersed Primary Organ Culture and
Fluid or Cell Assayed for VEE Virus

Mare #	Organs Cultured					
	Heart	Kidney	Lung	Spleen	Liver	Thymus
230	0	0	0	NT	NT	NT
231	NT	0	NT	0	0	0
232	C	+	NT	C	C	NT
233	NT	NT	NT	NT	NT	NT

+ VEE virus recovered

0 No virus present

C Tissue contaminated with fungus

NT Not tested

Table 4

Cells or Fluid from Minced Fetal Organ Explant
Cultures Assayed for VEE

Fetal Organ Explants	Fetus from Mares			
	230	231	232	233
Spleen	0	0	+	NT
Liver	0	0	+	NT
Thymus	NT	0	NT	
Lymph node	NT	0	+	
Kidney	0	0	+	
Heart	0	0	+	
Lung	0	0	NT	
Brain	NT	0	NT	
Cerebellum		0		
Ovary		0		
Spinal cord		0		
Pancreas		0		
Bone marrow	NT	0	NT	NT

+ VEE virus recovered

0 No virus present

HOSPITAL GENERAL DE MEXICO, S.S.A.

AND

INSTITUTO DE INVESTIGACIONES BIOMEDICAS, U.N.A.M.

HUMAN AND ANIMAL SERA FROM ATOYAQUILLO, OAXACA (MEXICO), SCREENED BY THE HI TECHNIQUE WITH VENEZUELAN ENCEPHALITIS ANTIGEN.

SPECIES	NUMBER OF SERA	DATE COLLECTED	DATE TESTED	NUMBER OF POSITIVES
HUMANS	51 (1)	Feb. 73	Jan. 75	3 (2)
HORSES Vaccinated (3)	38	Feb. 73	Jan. 75	32 (4)
Non-Vaccinated	3	Feb. 73	Jan. 75	0
TOTAL	92			35

(1) Single serum of people from 8 to 67 years old, residents of Atoyacuillo, Oaxaca.

(2) HI positive titers ranged from 40 to ≥ 80 in males of 26, 42 and 61 years old.

(3) Horses were vaccinated on 22 Jan. 73 against VE.

(4) HI positive titers were ≥ 80 against 4 HA units of VE antigen.

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