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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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TABLE OF CONTENTS

	Page
Comments from the Editor	1
Monograph, "Tick-Borne Encephalitis in Croatia (Yugoslavia)"	2
1976 Annual Report on the Catalogue of Arthropod-borne and Selected Vertebrate Viruses of the World	5
Reports from:	
Virus Research Unit and the Department of Microbiology, University of Otago, Dunedin, New Zealand	55
Arbovirus Laboratory, Institut Pasteur De Noumea, New Caledonia	58
Queensland Institute of Medical Research, Brisbane, Australia	61
CSIRO Division of Animal Health Laboratories Parkville and Glebe, Australia	72
Department of Microbiology, University of Western Australia, Perth, Australia	74
Virology Department, NAMRU-2, Jakarta Detachment, Jakarta, Indonesia	76
Virus Research Centre Indian Council for Medical Research, Poona, India	81
Virology Section National Health Laboratory, Rangoon, Burma	87
Department of Microbiology, University of Hong Kong, Pathology Building, Queen Mary Hospital Compound, Hong Kong	89
Bureau of Research Laboratories, Philippine Department of Health, Manila and the US Naval Medical Research Unit No. 2, Taipi, Taiwan	91
Department of Microbiology, Kobe University School of Medicine, Kobe, Japan	97
Department of Preventive Medicine Research, Institute for Microbial Diseases, Osaka University, Japan	

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.

TABLE OF CONTENTS (continued)

Page

Reports from (continued):

Department of Virology and Rickettsiology, National Institute of Health, Tokyo, Japan	100
Institute of Poliomyelitis and Viral Encephalitis, Moscow	101
Arbovirus Department, D.I. Ivanovsky Institute of Virology, Academy of Medical Sciences, USSR, Moscow	104
Department of Virology, University of Helsinki, Finland	107
Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia	111
Department of Ecology of Arboviruses, Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia	116
National Institute of Hygiene, Budapest, Hungary	120
Arbovirus Unit, Virus Department, Istituto Superiore Di Sanita, Rome, Italy	121
Institute of Tropical Medicine "Prince Leopold" Laboratory of Tropical Bacteriology and Virology, Antwerp, Belgium	125
Arbovirus Laboratory, Viral Ecology Unit, Pasteur Institute, Paris, France	128
Microbiological Establishment, Porton Down, Salisbury, Wiltshire, England	130
Arbovirus Unit and Entomology Department, London School of Hygiene & Tropical Medicine, England	133
Arbovirus Research Unit, Egyptian Organization for Biological & Vaccine Production, Agouza, Cairo, Egypt	139
Arbovirus Laboratory, Institut Pasteur and Orstom, Dakar Senegal	146
World Health Organization, Arbovirus Vector Research Unit, Enugu, Nigeria	149
Arbovirus Unit, National Institute for Virology, Johannesburg, South Africa	150
Virus Unit, Instituto Nacional De Microbiologia "Dr. Carlos R. Malbran", Buenos Aires, Argentina	152
Department of Epidemiology, School of Public Health, University of Sao Paulo, Brazil	154
Evandro Chagas Institute, FSESP Brazilian Ministry of Health, Belem, Brazil	159
Departamento De Virus, Instituto Nacional De Higiene "Leopoldo Izquieta Perez", Guayaquil, Ecuador	169
Virology Section of the Institute De Investigacion Clinica, Facultad De Medicina, Universidad Del Zulia, Maracaibo, Venezuela	170
Ministry of Agriculture, Guyana and the Caribbean Epidemiology Centre, Carec, Trinidad	171
San Juan Laboratories, Center for Disease Control, San Juan, Puerto Rico	174
Departamento De Virologia, Hospital General De Mexico, S.S.A. and Instituto De Investigaciones Biomedicas, U.N.A.M.	177

TABLE OF CONTENTS (continued)

	Page
Reports from (continued):	
Department of Equine Encephalitis, Instituto Nacional De Investigaciones Pecuarias, Mexico, D.F.	180
Bureau of Laboratories, Texas Department of Health Resources, Austin, Texas	182
Department of Health and Rehabilitative Services, Office of Laboratory Services, Jacksonville, Florida	184
Special Pathogens Branch, Virology Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia	185
Viral Diseases Division, Bureau of Epidemiology, Center for Disease Control, Atlanta, Georgia	187
Vector Biology and Control Division, Bureau of Tropical Diseases, Center for Disease Control, Atlanta, Georgia	190
Department of Entomology, Walter Reed Army Institute of Research, Washington, D. C.	194
Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University, New Haven, Connecticut	195
State of New York Department of Health, Division of Labora- tories and Research, Albany, New York	200
Laboratory of Virology Research Group on Biting Flies, Universite Du Quebec, Trois-Rivieres, Canada	202
National Arbovirus Reference Service, Department of Medical Microbiology, University of Toronto, Canada	206
Division of Laboratories, Illinois Department of Public Health, Chicago, Illinois	210
Zoonoses Research Unit, Department of Preventive Medicine, University of Wisconsin, Madison, Wisconsin	214
Arthropod-Borne Animal Disease Research Laboratory, USDA-ARS, Denver, Colorado	216
Vector-Borne Disease Division, Center for Disease Control, Fort Collins, Colorado	221
Rocky Mountain Laboratory, National Institutes of Health, Hamilton, Montana	236
Viral and Rickettsial Disease Laboratory, California State Department of Health, Berkeley, California	238
Division of Medical Microbiology, University of British Columbia, Vancouver, Canada	242
Virus Laboratory - Laboratory Services Branch, Ontario Ministry of Health, Toronto, Canada	243

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

COMMENTS FROM THE EDITOR

The prompt arrival of your reports for this issue of the Arthropod-borne Virus Information Exchange was most gratifying. Also, a number of you informed me of changes in your address. For this, many thanks.

You will notice that the majority of articles in the present issue are reproductions of reports as they were submitted. This reflects the care with which the reports were prepared, saving our secretary considerable time. Keep up the good work! However, a few guidelines may help even further:

1. Watch the width of the typed page. If it is too wide to allow adequate margins in the bound copy, it must be either retyped here or reduced in the printshop. Length of a page is not so important, as the sheet can be cut and added to the following page.
2. Please head your report as has become a convention in the Info-Exchange: REPORT FROM THE
3. Sizes and shapes of tables often are dictated by the material presented. We can reduce size of tables here as necessary. However, if copies rather than originals are submitted, be certain that they are easily read, black-on-white. This also pertains to copies of text material. Thumbing through the issue you will see a number of reports that did not reproduce as clearly as could be wished. All reports are readable, which is the real purpose of the Info-Exchange, but if a little extra care can improve it, I'd appreciate your efforts.

As always, I stand ready to edit as may be necessary any reports from contributors who may have trouble with the English language. I am glad to help. However, no other language than English, please.

Thanks again for your fine cooperation. The deadline for reports for issue No. 33 is September 1, 1977. The address, as always:

Roy W. Chamberlain, Editor
Arthropod-borne Virus Information Exchange
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MONOGRAPH, "TICK-BORNE ENCEPHALITIS IN
CROATIA (YUGOSLAVIA)"

A 184-page monograph (in English, with summaries in Yugoslavian), "Tick-borne Encephalitis in Croatia (Yugoslavia)" came off the press in late 1976. It is by Professor Jelka Vesenjsek-Hirjan and coworkers of the University of Zagreb and associated organizations. Twenty authoritative articles on epidemiologic, ecologic and laboratory aspects of TBE are presented; see the table of contents reproduced on the next two pages.

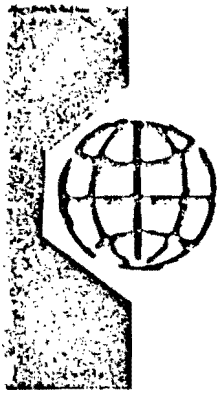
Undoubtedly this excellent publication will be of value to many of you. It can be obtained free of charge by merely requesting a copy from:

Charles H. Calisher
Vector-Borne Diseases Division
Center for Disease Control
Fort Collins, Colorado 80522, U.S.A.

SADRŽAJ — CONTENTS

Vesenjak-Hirjan, J.:		
Tick-borne Encephalitis in Croatia		
Krpeljni encefalitis u Hrvatskoj		1
Vesenjak-Hirjan, J., †Egri-Hećimović, E., Brudnjak, Z., Vince, V. Sooš, E.:		
Infections with Tick-borne Encephalitis Virus in the Pannonian		
Focus Stara Ves 1. Serological studies 1961—1964.		
Prokuženost na virus krpeljnog encefalitisa u panonskom žarištu		
Stara Ves 1. Serološka ispitivanja 1961—1964.		11
Vesenjak-Hirjan, J., Galinović-Weisglass, M., Brudnjak, Z.:		
Infections with Tick-borne Encephalitis Virus in Pannonian Focus		
Stara Ves 2. Serological studies in 1972.		
Prokuženost na virus krpeljnog encefalitisa u panonskom žarištu		
Stara Ves 2. Serološka ispitivanja 1972.		21
Vesenjak-Hirjan, J., Galinović-Weisglass, M., Brudnjak, Z.:		
Infections with Tick-borne Encephalitis Virus in Pannonian Focus		
Stara Ves 3. Persistence of HI, CF and N Antibodies to Tick-		
borne Encephalitis Virus		
Prokuženost na virus krpeljnog encefalitisa u panonskom žarištu		
Stara Ves 3. Perzistencija HI, KF i N antitijela za virus krpeljnog		
encefalitisa		29
Vesenjak-Hirjan, J., †Egri-Hećimović, E., Sooš, E.:		
Infections with Tick-borne Encephalitis Virus in Mediterranean		
Focus Nadsela (Island of Brač)		
Prokuženost na virus krpeljnog encefalitisa u mediteranskom ža-		
rištu Nadsela (otok Brač)		37
Brudnjak, Z., Vesenjak-Hirjan, J., Sooš, E.:		
Isolation of Tick-Borne Encephalitis Virus from Ticks and Pa-		
tients		
Izolacija virusa krpeljnog encefalitisa iz krpelja i bolesnika . . .		41
Brudnjak, Z.		
Some Physico-Chemical and Biological Properties of Tick-borne		
Encephalitis Virus Strains Isolated in Croatia		
Neka fizikalno-kemijska i biološka svojstva sojeva krpeljnog en-		
cefalitisa izoliranih u Hrvatskoj		49
Rulnjević, J., Zorinc, S., Košić, Z., Majerus, Lj., Fališevac, J.		
Clinical Characteristics of Tick-borne Encephalitis in Croatia		
Kliničke karakteristike krpeljnog encefalitisa u Hrvatskoj . . .		55
Galinić-Weisglass, M. and Vesenjak-Hirjan, J.		
Serological Study of Clinical Cases of Tick-borne encephalitis in		
Croatia		
Serološka studija kliničkih slučajeva krpeljnog encefalitisa u		
Hrvatskoj		67

<i>Bertović, S.</i>	Ecological-Vegetational Characteristics of TBE Foci in Croatia Ekološko-vegetacijske karakteristike prirodnih žarišta KE u Hrvatskoj	79
<i>Tovornik, D.:</i>	Seasonal and Diurnal Periodicity of the Tick <i>Ixodes ricinus L.</i> in the Pannonian Tick-borne Encephalitis Focus (Stara Ves) Sezonska i dnevna periodičnost krpelja <i>Ixodes ricinus L.</i> u panon- skom žarištu krpeljnog encefalitisa (Stara Ves)	99
<i>Tovornik, D.:</i>	Ecological Notes on Ticks (<i>Ixodidae</i>) on the Island of Brač Ekološki podaci o krpeljima (<i>Ixodidae</i>) na otoku Braču	105
<i>Tovornik, D. and Šooš, E.:</i>	On the Colour of the Unfed Female of <i>Ixodes Gibbosus Nuttall</i> , 1916 found in the Tick-borne Encephalitis Focus on the Island of Brač O obojenosti nenasisane ženke <i>Ixodes Gibbosus Nuttall</i> 1916 na- dene u žarištu krpeljnog encefalitisa na otoku Braču	115
<i>Džulić, B.:</i>	Ecologic Characteristics of Mammals on the Island of Brač and in the Area of Stara Ves Ekološke značajke sisavaca otoka Brača i okolice Stare Vesi	117
<i>Rucner, D.:</i>	Avifauna in Pannonian and Mediterranean Tick-borne Encephalitis Foci Značaj avifaune u nekim centrima zaraze arbovirusima u Hrvatskoj	121
<i>Grčević, N. and Vince, V.:</i>	Tick-borne Encephalitis induced in White Mice by some Yugoslav Virus Strains Krpeljni encefalitis izazvan na bijelom mišu nekim od jugosla- venskih sojeva virusa	127
<i>Vince, V. and Brudnjak, Z.:</i>	Serological Study of Mice Experimentally Infected with Tick- borne Encephalitis Virus Seroološka istraživanja kod miševa eksperimentalno inficiranih vi- rusom krpeljnog encefalitisa	151
<i>Vesenjak-Hirjan, J., Rulnjević, J., Šooš, E., Galinović-Weisglass, M., Beus, I.:</i>	Appearance of Tick-borne Encephalitis in the Mountain Range Medvednica Pojava krpeljnog encefalitisa na Medvednici	161
<i>Vesenjak-Hirjan, J. and Šooš, E.:</i>	The Role of Migrating Flocks of Sheep in the Spreading of Tick- borne Encephalitis Virus Uloga migrirajućih ovaca u širenju krpeljnog encefalitisa	167
<i>Šooš, E. and Brudnjak, Z.:</i>	Prophylaxis of Tick-borne Encephalitis Profilaksa krpeljnog encefalitisa	175



The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1976 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 1976, 157 mailings of Catalogue material were being made. During the year, 2 participants were dropped and 5 new participants were added to the mailing list. At the end of the year, 160 mailings of Catalogue material were being made, including 61 within the U.S.A. and 99 to foreign addresses. Distribution by continent was: Africa 15, Asia 17, Australasia 6, Europe 33, North America 72, and South America 17.

Abstracts and Current Information: A total of 628 abstracts or references were coded by subject matter and distributed to participants during 1976. Of this total, 540 were obtained from Biological Abstracts, 83 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 5 from current journals, personal communications, or other sources. A total of 10,222 references or units of information have been issued since the start of the program.

Registration of New Viruses: Twelve viruses were accepted for registration during 1976. Of this number, 7 viruses were submitted in the latter months of 1975 and either were under review or in various stages of processing at the conclusion of 1975. As of December 1975, there were 369 registered viruses in the Catalogue. With the 12 registrations during 1976, the total number of registered viruses stood at 381 as of December 1976. The viruses registered during 1976 are listed below.

<u>Virus Name</u>	<u>Recommended Abbreviation</u>	<u>Country</u>	<u>Source</u>	<u>Antigenic Group</u>
Aride	ARI	Seychelles, Indian Ocean	Ixodid ticks	
Bovine ephem- eral fever	BEF	Australia	Calf	
Ibaraki	IBA	Japan	Cattle	
Isfahan	ISF	Iran	Phlebotomines	VSV
Khasan . .	KHA	USSR	Ixodid ticks	
Klamath	KLA	USA	Rodent	
Olifantsvlei	OLI	South Africa	Mosquitoes	OLI
Paramushir	PMR	USSR	Ixodid ticks	
Razdan	RAZ	USSR	Ixodid ticks	
Sunday Canyon	SCA	USA	Argasid ticks	
Tamdy	TDY	USSR	Ixodid ticks	
Vesicular Stoma- titis Alagoas	VSA	Brazil	Mule	VSV

The above viruses all were isolated between 1959 and 1976. One was isolated in 1959 (IBA), one in 1962 (KLA), one in 1963 (OLI), one in 1964 (VSA), one in 1968 (BEF), three in 1971 (KHA, SCA, TDY), one in 1972 (PMR), two in 1973 (ARI, RAZ), and one in 1976 (ISF).

All 12 viruses were evaluated as possible arboviruses by the SEAS Subcommittee.

Antigenic Grouping: The Olifantsvlei serogroup has been constituted with the registration of Olifantsvlei virus. The Olifantsvlei antigenic group consists of Olifantsvlei and Bobia (DakAr 1569), which is presently unregistered. This serogroup has been classified within the Bunyamwera Supergroup (see Olifantsvlei virus registration card). Efforts to solicit the registration of Bobia virus have been unsuccessful.

Computerization of Information on Registered Viruses: Efforts were initiated in June 1975 to modify, update, expand, and reactivate a presently dormant computer program for storage and retrieval of information on registered viruses. At that time, information for 309 registered viruses was contained in the computer program. Every piece of information for each of the 309 viruses in the program was updated and verified for current validity. Upon completion of that aspect in early fall of 1976, the same kinds of information for 72 additional viruses was prepared for programming. Decisions are now being made about what additional kinds of information should be included in the program. Inclusion of this additional information will allow the program to be consistent with the kinds of information being solicited by the new registration forms and will enable the program to be current with respect to prevailing trends in knowledge and research endeavor.

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 381 viruses registered in the Catalogue as of December 1976. 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 49 distinct 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 Bunya virus 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 pre-

sented 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. Two isolates identified as Semliki Forest virus, one each from Aedes vexans and Culex pipiens, have been reported from the far east USSR. The distribution of Semliki Forest, which previously was detected only in Africa, is now extended to include Asia.

Tables 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 RSSE and other members of the same complex.

Within group B viruses with no proven arthropod vector (Table 8), Koutango virus recently has been reported to cause disease in man. The clinical manifestations are a fever with rash.

Israel turkey meningoencephalitis virus has been upgraded in arbovirus status from possible to probable arbovirus on the basis of re-evaluation by SEAS.

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 Anhembi, 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. Additional serologic data are still needed which substantiate the relationship of Bocas virus to members of the California group. Prior to the registration of Bocas, 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.

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.

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.

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.

Recently, five isolates of Wad Medani of the Kemerovo group were obtained from Hyalomma ticks in the eastern USSR. These mark the first isolations of Wad Medani in the USSR.

Table 18. Tick-borne groups other than group B viruses. Members of these five minor antigenic groups have not been classified taxonomically. Abu Hammad of the DGK serogroup has been isolated from Argas ticks collected in Iran. That extends the distribution of this virus to Asia as well as Africa.

Soldado virus of the Hughes group has now been isolated in Ethiopia, Seychelles Islands, and northern Wales. All isolations were from Ornithodoros ticks. Previously, Soldado virus had been isolated only in South America.

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.

The membership of the VSV group has been increased by two with the registration of VS-Alagoas and Isfahan. The Alagoas serotype was isolated from a mule in Brazil and appears to be serologically close to Cocal and VSI viruses. Isfahan virus was isolated from Phlebotomine flies collected in Iran. By neutralization, Isfahan is quite distinct from other members of the VSV group. Neutralizing antibody to Isfahan virus has been detected in human beings residing in four different provinces in Iran.

Table 22. Minor antigenic groups of viruses. These antigenic groups consist of members which are taxonomically unclassified.

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.

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 these rhabdoviruses have been implicated in disease of man or animals. While Flanders and Hart Park are listed as serologically ungrouped, they are in fact closely related to each other. It is still unresolved whether they form an antigenic group or complex or whether they are variants or subtypes of a single virus.

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. There have been a number of additions to this list in the past year. Sunday Canyon, which was isolated from Argas ticks in Texas, appears to be bunyavirus-like. Bovine ephemeral fever, taxonomically classified as a rhabdovirus, causes extensive outbreaks of disease in cattle. Though the disease was initially recognized and described in South Africa and though the virus was extensively studied there and in Japan, this registration was prepared by a laboratory in Australia. Likewise, considerable study of this virus and its disease in cattle have been conducted in Australia. Recently the virus also was isolated from cattle in Iran. Aride, from Amblyomma ticks in the Seychelles; Khasan, from Haemaphysalis ticks; Paramushir, from Ixodes; Razdan, from Dermacenter; and Tamdy, from Hyalomma all are taxonomically unclassified and have been registered in the past year. The latter four viruses were isolated in the eastern USSR.

Table 27. Ungrouped viruses, no arthropod vector known. Two recently registered viruses have been added to the list in Table 27. Klamath, a rhabdovirus, has been isolated from rodents in Oregon and Alaska. Ibaraki, an orbivirus, causes a Bluetongue-like disease in cattle and has been isolated in Japan, Indonesia, and Taiwan. There is no serological evidence of a relationship between Ibaraki and Bluetongue.

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 85% 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. Approximately 50% 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 25% 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 204 (54%) 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.

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TABLE 1

ALPHABETICAL LISTING OF 381 VIRUSES REGISTERED AS OF 31 DEC. 1976
WITH RECOMMENDED ABBREVIATIONS AND ANTIGENIC GROUPING

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
ABSETTAROV	ABS	B	AVALON	AVA	SAK
ABU HAMDAD	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	CAL	CHANDIPURA	CHP	VSV
BORACEA	BOR	ANB	CHANGUINOLA	CGL	CGL
BOTAMBI	BOT	SBU	CHARLEVILLE	CHV	
BOTEKE	BTK	BTK	CHENUDA	CNU	KEM
BOVINE EPHEMERAL FEVER	BEF		CHIKUNGUNYA	CHIK	A
BOUBOUI	BOU	B	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	
JAREY ISLAND	CI	B	DUGBE	DUG	NSD
CATU	CATU	GMA	EAST. EQUINE ENC.	EEE	A
CHACO	CHO	TIM	EDGE HILL	EH	B
CHAGRES	CHG	PHL	ENTEBBE BAT	ENT	B

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
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		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		JUGRA	JUG	B
HAZARA	HAZ	CON	JUNIN	JUN	TCR
HUACHO	HUA	KEM	JURONA	JUR	SBU
HUGHES	HUG	HUG	JUTIAPA	JUT	B

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

*Rabies related

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
MAPRIK	MPK	MAP	NAIROBI SHEEP DIS.	NSD	NSD
MARBURG	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
MUCAMBO	MUC	A	ORUNGO	ORU	
MURRAY VALLEY ENC.	MVE	B	OSSA	OSSA	C
MURUTUCU	MUR	C	OUANGO	OJA	

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

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
SILVERWATER	SIL	KSO	TETNANG	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		USUTU	USU	B
TANJONG RABOK	TR		UUKUNIEMI	UUK	UUK
TATAGUINE	TAT		VELLORE	VEL	PAL
TEMBE	TME		VEN. EQUINE ENC.	VEE	A
TEMBUSU	TMU	B	VENKATAPURAM	VKT	
TENSAW	TEN	BUN	VS-ALAGOAS	VSA	VSV
TETE	TETE	TETE	VS-INDIANA	VSI	VSV

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
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				
WYEOMYIA	WYO	BUN			
YAQUINA HEAD	YH	KEM			
YATA	YATA				
YELLOW FEVER	YF	B			
YOGJE	YOG				
ZALIV TERPENIYA	ZT	UUK			
ZEGLA	ZEG	PAT			
ZIKA	ZIKA	B			
ZINGA	ZGA				
ZINGILAMO	ZGO	BTK			
ZIRQA	ZIR	HUG			

HEW, PHS, CDC

Table 2. Antigenic Groups of 381 Viruses Registered in Catalogue

<u>Antigenic Group</u>	<u>Abbreviation</u>	<u>No. Registered Viruses in Group</u>	<u>%</u>
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	58	15.2
Bakau	BAK	2	0.5
Bluetongue	BLU	1	0.3
Boteke	BTK	2	0.5
Bunyamwera Supergroup		88	23.1
Bunyamwera	BUN	18	
Bwamba	BWA	2	
C	C	11	
California	CAL	12	
Capim	CAP	6	
Guama	GMA	6	
Koongol	KOO	2	
Olifantsvlei	OLI	1	
Patois	PAT	4	
Simbu	SIM	15	
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
Hughes	HUG	4	1.0
Kaisodi	KSO	3	0.8
Kemerovo	KEM	16	4.2
Kwatta	KWA	1	0.3
Malakal	MAL	2	0.5
Mapputta	MAP	3	0.8
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	20	5.2
Qalyub	QYB	2	0.5
Ouaranfil	QRF	2	0.5
Sakhalin	SAK	4	1.0
Tacaribe	TCR	9	2.4
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		89	23.4
	Total	381	

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

<u>Decade</u>	<u>Continent</u>	<u>Country</u>	<u>Virus</u>
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	CNU, QRF, QYB, SIN, WM
		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	Australia
	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.
		Colombia	GRO, NAV
		Trinidad	ARU, BIM, BSB, IERI, KRI, LUK, MAN, MAY, MEL, NEP, ORO, TCR, TNT.

* Isolated in U.S.A. Laboratory

Table 3. (Continued)

Decade	Continent	Country	Virus	
1960-69	Africa	Cameroon	NKO,OKO	
		Cent.Afr.Rep.	BAG,BGN,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	
	West Germany		MBG	
	N. America	Canada	SIL	
		Guatemala	JUT*	
		Mexico	MNT,TLA*	
		Panama	AGU,CHG,CHI,CGL,FRI,GAM,JD,LAT,MAD, MAT,OSSA,PAR,PAT,PT*,ZEG	
	S. America	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	
		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,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***
	Asia	India	CG
		Iran	ISF*
		Malaysia	BC,CI
	Australasia	U.S.S.R.	BKN,IK,KHA,KSI,PMR,RAZ,SOK,TDY
		Australia	NGA,NUG,TAG,WAL,WGR
	Europe	Germany	EYA,TET
		Scotland	CM,CW
		U.S.S.R.	BAKU
	N. America	Canada	AVA,BAU*,GI*
		Panama	CAC,CAI,NIQ
		U.S.A.	NOR,SCA,YH

* Isolated in U.S.A. laboratory

** Isolated in Panama laboratory

*** Isolated in Egypt laboratory

Table 4. Initial Isolation of 381 Registered Viruses
by Continent, Country, and Chronological Period

Continent	Country or Area	Before 1930	1930 -39	1940 -49	1950 -59	1960 -69	1970 -76	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
	Totals	4	4	5	28	42	22	105
ASIA	Cambodia					1		1
	India				12	9	1	22
	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	12	70
AUSTRAL- ASIA and PACIFIC ISLANDS	Australia				1	25	5	31
	Hawaii			1				1
	Johnston Island					1		1
	New Guinea			1				1
	New Zealand					1		1
	Philippines				2			2
	Totals	0	0	2	3	27	5	37
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	3	46
	Totals	1	3	3	14	45	9	75
SOUTH AMERICA	Argentina				1			1
	Bolivia					1		1
	Brazil			1	18	23		42
	Colombia			3	2	1		6
	Peru					2		2
	Surinam					1		1
	Trinidad				13	5		18
	Venezuela		1					1
	Totals	0	1	4	34	33	0	72
	Grand Totals	6	10	19	109	184	53	381

TABLE 5. GROUP A ARBOVIRUSES

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. Cullicine	Anopheiline	Ticks Ixodid	Argasid	Phlebotomine	Other Culicoides	Other	Man	Other Primates	Rodents										
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					SEAS RATING *	TAXONOMIC STATUS		
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America			South America	HUMAN DISEASE
	Mosq. Culexine	Ancpeline	Ticks Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds						Bats			Marsupials
Alfuy	+									+								20	Flavivirus	
Bagaza	+																	22		
Banzi	+						+								+			20		
Boubou1	+	+						+										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					SEAS RATING *	TAXONOMIC STATUS				
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America			South America	HUMAN DISEASE		
	Mosq. Culicine	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats									Marsupials	Other	Sentinel's
Absettarov			+			+												+	+	20	Flavivirus	
Hanzalova			+			+												+	+	20		•
Hypr			+			+												+	+	20		•
Kadam			+			+												+	+	20		•
Karshi				+																21		•
Kumlinge			+																	22		•
Kyasanur Forest disease			+		+															20		•
Langat			+			+														20		•
Louping 111			+																	20		•
Omsk hem. fev.			+																	20		•
Powassan			+																	20		•
Royal Farm				+																20		•
RSSE			+																	22		•
Tyulenty			+																	20	•	
			+																	21	•	

* See footnote Table 5

TABLE 8. GROUP B VIRUSES, NO ARTHROPOD VECTOR DEMONSTRATED

VIRUS	ISOLATED FROM										ISOLATED IN						SEAS RATING *	TAXONOMIC STATUS		
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America			HUMAN DISEASE	
	Mosq. Culicine	Ticks Ixodid Anophelinae	Phlebotomine Argasid	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats									Marsupials	Other
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	"	
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					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection			Lab Infection
	Mosq. Culicine	Anopheleine Ixodid	Argasid	Phlebotomine	Culicoides Other	Man	Other Primates	Rodents	Birds	Bats										
Anhembí	+																	22	Bunyavirus *	
Bataí	+	+																22		
Birao	+	+																22		
Bunyamwera	+					+							+					20		
Cache Valley	+	+													+			20		
**Calovo	+	+																22		
Germiston	+					+	+					+					+	20		
Guaroa	+	+				+	+								+			20		
Ilesha	+	+				+	+								+			20		
Kairi	+						+						+					20		
Lokern	+							+							+			20		
Maguari	+	+											+					20		
Main Drain	+														+			20		
Northway	+														+			21		
Sororoça	+																	22		
Tensaw	+	+																20		
Tlacotalpan	+	+																22		
Wyeomyia	+	+				+												21		

* See footnote Table 5
 ** May be strain of Bataí

TABLE 10. BUNYAVIRA 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
	Mosq. Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds										
<u>BWAMBA GROUP</u>																					
Bwamba		+						+													+
Pongola	+	+																			
<u>GROUP C</u>																					
Apeu	+							+							+						+
Caraparu	+							+		+					+						+
Gumbo Limbo	+							+		+					+						+
Itaquí	+							+		+					+						+
Madrid	+							+		+					+						+
Marituba	+							+		+					+						+
Murutucu	+							+		+				+	+						+
Nepuyo	+							+		+		+			+						+
Oriboca	+							+		+		+			+						+
Ossa	+							+		+					+						+
Restan	+							+		+					+						+

* See footnote Table 5

TABLE 11. BUNYAMERA SUPERGROUP: CALIFORNIA AND CAPIM 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										
Culicine	Anopheline	Ixodid	Argasid																		
<u>CALIFORNIA GROUP</u>																					
Bocas	+									+						+				22	Bunyavirus
California Enc.	+															+				20	•
Inkoo	+															+				22	•
Jamestown Canyon	+					+							+			+				20	•
Jerry Slough	+															+				20	•
Keystone	+	+											+			+				20	•
La Crosse	+															+				20	•
Melao	+							+								+				21	•
San Angelo	+															+				22	•
Snowshoe Hare	+												+			+				20	•
Tahyna	+	+														+				20	•
Trivittatus	+															+				21	•
<u>CAPIM GROUP</u>																					
Acara	+																			21	Bunyavirus
Bushbush	+																			20	•
Capim	+																			20	•
Guajara	+																			20	•
Juan Diaz	+																			22	•
Moriche	+																			22	•

* See footnote Table 5

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

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

* See footnote Table 5

TABLE 13. BUNYAMWERA SUPERGROUP: SIMBU 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. Cullicine	Anopheleine	Ticks Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds										
Aino	+					+														22	Bunyavirus
Akabane	+					+														21	"
Buttonwillow						+														20	"
Ingwavuma	+					+				+										20	"
Manzanilla								+												22	"
Mermet										+										22	"
Nola	+																			20	"
Oropouche	+							+												21	"
Sabo						+														22	"
Sango	+					+														22	"
Sathuperi	+					+														22	"
Shamonda						+														22	"
Shuni	+					+														22	"
Simbu	+																			21	"
Thimiri										+										22	"

* See footnote Table 5

TABLE 14. BUNYAVIRERA SUPERGROUP: TETE GROUP AND UNASSIGNED VIRUSES

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SVAS 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										
Culicine	Anopheline	Ixodid	Argasid																	
<u>TETE GROUP</u>																				
Bahig																			22	Bunyavirus
Matruh			++																22	"
Tete																			22	"
Tsuruse									+++										22	"
<u>UNASSIGNED - "SBU"</u>																				
Botambi	+																		22	Bunyavirus
Gamboia	+																		22	"
Guaratuba	+																		21	"
Jurona	+								+										22	"
Kaeng Khoi										+									22	"
Minatitlan																			22	"
Mirim	+																		20	"

* See footnote Table 5

TABLE 15. PHLEBOTOMUS FEYER 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										
Cuticine	Anophelinae	Ixodid	Argasid																	
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		
Salehabad				+														22		
SF-Maples				+														20		
SF-Sicilian				+														20		

* 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	
	Mosp.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds											Bats
	Culicine	Anopheline	Ixodid	Argasid																	
<u>CHF-CONGO GROUP</u> Congo Hazara			+			+						+			+			+	+	20 22	Bunyavirus-like "
<u>KAISODI GROUP</u> Kaisodi Lanjan 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. Culicine	Ticks Ixodid Anopheline	Argasid	Phlebotomine	Culicoides Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials Other										
<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 18. TICK-BORNE GROUPS OTHER THAN GROUP B VIRUSES

40

	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. Culicine	Ticks Ixodid Anophelline	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats											Marsupials	Other
<u>DERA GHAZI KHAN GROUP</u> Abu Hammad Dera Ghazi Khan Kao Shuan Pathum Thanf Pretoria			+	+																	22 22 22 22 22	Unclassified " " " "	
<u>HUGHES GROUP</u> Hughes Punta Salinas Soldado Zirqa			+	+					+													21 22 22 22	Unclassified " " "
<u>QALYUB GROUP</u> Bandia Qalyub			+	+				+														22 22	Unclassified "
<u>QUARANFIL GROUP</u> Johnston Atoll Quaranfil			+	+			+	+										+				20 20	Unclassified "
<u>SAKHALIN GROUP</u> Avalon Clo More Sakhalin Taggert			+	+					+													21 22 22 22	Unclassified " " "

* See footnote Table 5

TABLE 20. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					SEAS RATING *	TAXONOMIC STATUS			
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America			South America	HUMAN DISEASE	
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Nan	Other Primates	Rodents	Birds	Gats									Marsupials	Other
Culicine	Anopheline	Ixodid	Argasid																		
<u>AFRICAN HORSE SICKNESS</u> African horsesickness					+							+		+					20	Orbivirus	
<u>BLUETONGUE GROUP</u> Bluetongue					+							+		+					20	Orbivirus	
<u>CHANGUINOLA GROUP</u> Changuinola							+											+	21	Orbivirus	
Irituia								+											22	"	
<u>CORRIPARTA GROUP</u> Acado	+																		22	Orbivirus	
Corriparta	+								+										22	"	
<u>EHD GROUP</u> Epizootic hem. dis.														+					21	Orbivirus	
<u>EUBENANGEE GROUP</u> Eubenangee	+	+																	22	Orbivirus	
Pata	+																		22	"	
<u>PALYAM GROUP</u> D'Aguiar					+														22	Orbivirus	
Kasba	+																		22	"	
Palyam	+																		22	"	
Vellore	+																		22	"	
<u>WARREGO GROUP</u> Mitchell River																			22	Orbivirus	
Warrego																			22	"	

* 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	Australasia	Europe	North America	South America	Natural Infection			Lab Infection
	Mosp. Culicine	Anopheline	Ticks Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds										
<u>KWATTA GROUP</u> Kwatta	+																			22	Rhabdovirus
<u>MOSSURIL GROUP</u> Kamese Mossuril	+									+										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	22 22	22 22	
	Mosq.	Ticks	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other											
<u>BOIJEKE GROUP</u> Botofe Zirgilano	+					+															Unclassified "
<u>MALAKAL GROUP</u> Matakal Puchong																					Unclassified "
<u>NATARIYA GROUP</u> Burg el Arab Garba Natariya							+++														Unclassified "
<u>NYANDO GROUP</u> Nyando																					Unclassified
<u>TIMBO GROUP</u> Chaco Timbo																					Unclassified "

* See footnote Table 5

TABLE 23. TACARIBE (LCM) GROUP VIRUSES

VIRUS	ISOLATED FROM		ISOLATED IN		HUMAN DISEASE		SCAS RATING *		TAXONOMIC STATUS		
	ARTHROPODS		VERTEBRATES								
Amapari Junin Lassa Latino Machupo Parana Pichinde Tacaribe Tamiari	Mosq.	Culicine							24	Arenavirus	
		Anopheline							24	"	
	Ticks	Ixodid								24	"
		Argasid								24	"
			Other	+	+					24	"
			Man	+	+	+				24	"
			Other Primates							24	"
			Rodents	+	+	+	+	+	+	24	"
			Birds							24	"
			Bats							24	"
			Marsupials							24	"
			Other							24	"
			Sentinels							24	"
			Other							24	"
		Africa						+	24	"	
		Asia							24	"	
		Australasia							24	"	
		Europe							24	"	
		North America							24	"	
		South America							24	"	
		Natural Infection							24	"	
		Lab Infection							24	"	

* See footnote Table 5

TABLE 24. UNGROUPED MOSQUITO-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			
	Mosg.	Culicine	Anopheline	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents									Birds		
Rift Valley fever	+						+	+										+	20	Bunyavirus-like	
Tataguine	+																		21	"	
Witwatersrand	+																		21	"	
Japanaut																			21	Orbivirus	
Lebombo																			21	"	
Orungo																			22	"	
Umatilla																			20	"	
Nodamura																			23	Picornavirus	
Cotia																			24	Poxvirus	
Flanders																			22	Rhabdovirus	
Hart Park																			21	"	
Joinjakaka																			22	"	

*See footnote Table 5

TABLE 25. UNGROUPED MOSQUITO-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE	SEAS RATING *	TAXONOMIC STATUS		
	ARTHYROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America				South America	Natural Infection
	Moso.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials						Other	Sentinelis			
Arkonam	+																			22	Unclassified " " " " " " " " " " " " " " " " " "
Aruac	+																			21	
Bangoran	+																			22	
Belmont	+																			22	
Gomoka	+																			22	
Ieri	+																			22	
Kowanyama	+																			22	
La Joya	+																			22	
Minnal	+																			22	
Nkolbisson	+																			22	
Okola	+																			22	
Oubangui	+																			22	
Pacora	+																			22	
Tanga		+																		22	
Tembe		+																		22	
Trinit	+																			21	
Venkatapuram	+																			22	
Wongorr	+																			22	
Yata	+																			22	
Zinga	+																			22	

47

*See footnote Table 5

TABLE 26. UNGROUPED TICK-, CULICOIDES-, OR PHEBOTOMUS-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. Culicine	Anophelinae	Ixodid	Argasid	Phebotomine	Culicoidea	Other	Man	Other Primates	Rodents	Birds											Bats
Bhanja			+				+		+						+	+				+	22	Bunyavirus-tik
Lone Star			+																		22	"
Sunday Canyon				+													+				22	"
African swine fever				+											+			+++			20	Iridovirus
Barur			+						+						+	+					22	Rhabdovirus
Bovine ephemeral fever						+									+	+					22	"
Aride			+												+						22	Unclassified
Batken	+		+												+						22	"
Charleville					+										+						22	"
Chobar Gorge				+											+						22	"
Dhori			+												+						22	"
Issyk-Kul			+	+											+	+					22	"
Keterah			+	+											+	+					21	"
Khasan			+	+											+	+					22	"
Matucare				+															+		22	"
Ngaingan						+															22	"
Nyamanini				+																	22	"
Paramushir			+	+						+					+						21	"
Razdan			+	+											+	+					22	"
Sawgrass			+	+											+						22	"
Tamdy			+	+											+						22	"
Tettnang			+	+													+				22	"
Upolu				+																	22	"
Wallal						+															22	"
Wanowrie	+		+												+	+					22	"

48

* See footnote Table 5
 **Cuba

TABLE 27. UNGROUPED VIRUSES: NO ARTHROPOD VECTOR KNOWN

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SCAS 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										
Nariya							+										+		23	Paramyxovirus	
Kern Canyon									+										23	Rhabdovirus	
Klamath							+										+		22	"	
Lagos Bat									+										24	"	
Mount Elgon Bat									+										23	"	
Navarro									+								+		22	"	
Ibaraki												+				+			22	Orbivirus	
Almpiwar												+				+			21	Unclassified	
Bangui																	+		22	"	
Bimbo																			22	"	
Bobaya									+										22	"	
Gossas									+										23	"	
Ippy									+										22	"	
Kammavanpettai									+								+		22	"	
Kannamangalm									+										22	"	
Keuraliba									+										22	"	
Kolongo									+										22	"	
Landjia									+										22	"	
Le Dantec									+										22	"	
Marburg									+								+		23	"	
Marco									+										22	"	
Quango									+								+		22	"	
Saint-Floris									+										22	"	
Salanga									+										22	"	
Sandjimba									+										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	58	18	24	12	7	10	6	44	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	12	1	0	0	2	9	2	10	2	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	15	9	5	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
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
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
NDO	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	20	4	4	0	2	8	6	18	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	89	38	23	11	4	12	10	80	2	3	0	0
Totals	381	115	92	43	33	87	87	322	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		Mites	Other	1	2	3
				Flies	Culicoides					
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	58	28	15	0	0	1	1	39	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	12	12	0	0	0	0	1	11	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	15	9	0	0	8	0	0	7	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
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
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	20	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
THO	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	89	34	21	2	3	0	1	51	4	0
Totals	381	192	87	20	21	9	6	274	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	58	26	3	17	14	14	1	5	6	27	6	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	12	2	0	3	0	1	0	0	1	5	1	0	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	15	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
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
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	20	5	0	5	2	0	1	0	2	9	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	89	9	1	13	14	8	0	6	3	42	3	0	0	0	0
Totals	381	86	9	81	59	30	17	32	30	154	41	11	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	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	58	27	23	30	52.0
Bakau	2	0	0	0	
Bluetongue	1	0	0	0	
Boteke	2	0	0	0	
Bunyamwera	18	4	2	5	27.8
Bwamba	2	1	0	1	50.0
C	11	9	2	9	81.8
California	12	4	0	4	33.0
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	15	2	1	2	13.3
Tete	4	0	0	0	
SBU	7	0	0	0	
Changuinola	2	1	0	1	50.0
Colorado tick fever	2	1	1	0	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	
Hughes	4	0	0	0	
Kaisodi	3	0	0	0	
Kemerovo	16	1	1	1	6.3
Kwatta	1	0	0	0	
Malakal	2	0	0	0	
Mapputta	3	0	0	0	
Matariya	3	0	0	0	
Mossuril	2	0	0	0	
Nairobi sheep dis.	3	3	2	2	100.0
Nyando	1	1	0	1	100.0
Palyam	4	0	0	0	
Phlebotomus fever	20	5	0	5	25.0
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	89	9	5	9	10.1
Totals	381	90	51	94	24.7

TABLE 32. EVALUATION OF ARTHROPOD-BORNE STATUS
OF 381 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	58	29	8	14	2	5	37	63.8	7	13.0
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	
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	12	7	2	3	0	0	9	75.0	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	15	3	3	9	0	0	6	40.0	0	
TETE	4	0	0	4	0	0	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	
HUG	4	0	1	3	0	0	1	25.0	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	
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	20	3	5	12	0	0	8	40.0	0	
QYB	2	0	0	2	0	0	0		0	
QRF	2	1	0	1	0	0	1	50.0	0	
SAK	4	0	0	4	0	0	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	2	1	4	0	0	3	42.9	0	
WAR	2	0	0	2	0	0	0		0	
Ungrouped	89	3	9	68	6	3	12	13.5	9	10.1
Totals	381	97	54	204	8	17	152	39.9	25	6.6

The isolation of Hughes group viruses in New Zealand

During the course of continuing investigations on tick-borne viruses in New Zealand six isolates have recently been identified by complement fixation, as members of the Hughes group. All six agents, which were isolated by intracerebral inoculation of newborn mice, came from Ornithodoros capensis ticks collected at seabird breeding colonies on the East Coast of New Zealand (Table 1).

Infected mouse brains were extracted with tween 80 and ether to prepare complement fixing antigens which were tested against NIH reference hyperimmune fluids and hyperimmune ascitic fluids prepared against isolates T469 and T497. The results, shown in Table 2, indicate that the isolates are closely related to, or identical with Hughes virus.

Although viruses of the Hughes group have been isolated from ticks collected in the North Pacific region along the West Coast of North America, this is the first record of isolations from the South-west Pacific area.

(F. J. Austin)

TABLE 1

Hughes group viruses isolated from *O. capensis* in New Zealand

Isolate	Date of tick collection	Locality	Composition of tick pool	Associated bird species
T 1	10 Sept. 1969	Kaikoura 42°25' S; 173°42' E	1 male 9 females	<i>Larus novaehollandiae scopulinus</i> (red billed gull) <i>Sterna striata</i> (white fronted tern)
T26	16 Mar. 1972	Karitane 45°39'S; 170°40'E.	10 females	<i>S. striata</i>
T414	18 Dec. 1972	Cape Kidnappers 39°38'S; 177° 5'E.	21 females	<i>Sula bassana serrator</i> (gannet)
T469	27 Oct. 1974	Sumner 43°33'S; 172°47'E.	24 nymphs	<i>L. n. scopulinus</i>
T497	19 Nov. 1974	Kaikoura	22 females	<i>S. striata</i>
T500	19 Nov. 1974	Kaikoura	21 nymphs	<i>S. striata</i>

TABLE 2

Relationship of isolates by complement fixation

Antigens	Hyperimmune ascitic fluids			
	Polyvalent 5 (Hughes, Soldado, Sawgrass, Matucare, Lone Star)	Hughes	T 469	T 497
Lone Star	64/≥64*	<8/<16	<8/<16	<8/<16
T 1	16/4	32/64	≥640/320	NT [#]
T 26	16/16	32/128	≥640/640	NT
T 414	16/8	32/64	≥640/160	NT
T 469	NT	32/128	≥640/320	≥640/320
T 497	16/8	64/64	≥640/320	≥640/160
T 500	16/4	32/32	320/80	≥640/80

* = reciprocal antibody titre/reciprocal antigen titre

NT = not tested.

Antigens T 1; T 26; T 414; T 497 and T 500 did not fix complement with NIH hyperimmune ascitic fluids:

Lone Star

Sawgrass

Polyvalent 3 (Koongol; Wongal; Bakau; Ketapong; Mapputta)

Polyvalent 4 (Nyamanini; Uukuniemi; Grand Arbaud; Thogoto)

Polyvalent 10 (Upolu; Dera Ghazi Khan; Wanowrie; Dhori)

Polyvalent Quarafil (Quarafil; Johnston Atoll; Qalyub; Bandia;
Kasodi; Lanjan; Silverwater)

Polyvalent Kemerovo (Kemerovo; Chenuda; Mono Lake; Wad Medani;
Tribec; Huacho)

Polyvalent Group B (34 viruses)

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

A research program on dengue and hemorrhagic fever was initiated in 1975 as dengue I virus was spreading over the islands of South Pacific. Studies were conducted in New Caledonia, Loyalty Islands, New Hebrides, Wallis, Futuna, and Salomon Islands, in connection with the Special Project on Dengue, promoted by the South Pacific Commission.

Virus isolations

1) From patients suffering dengue-like disease : between april 1975 and december 1976, 88 strains were obtained from blood samples taken in acute phase : 85 by intra-cerebral inoculation to suckling mice : 3 by intra-thoracic inoculation to Aedes aegypti only. 44 are identified as dengue 1 virus : the remainder is under examination.

2) From mosquitoes : 3 strains were isolated by intra-cerebral inoculation to baby mice of centrifuged ground mosquitoes suspension. Species found naturally infected were :

Aedes aegypti : 15 ♀ ♀ from Lolowai, Aoba, New Hebrides, July 1975
Dengue 1.

2 ♀ ♀ from Nouméa, New Caledonia. April 1976.
Identification pending.

Aedes polynesiensis = 2 ♀ ♀ from Ono, Futuna, Horne Islands. November 1976. Identification pending.

Serology and epidemiology

New Caledonia

At the beginning of the studies there was no evidence of dengue transmission since the outbreak of dengue 2 of 1971-1972.

In march 1975, a woman and her two daughters, recently arrived from France, were struck by a dengue-like disease : IH tests, 11 days after the onset, showed significant titers of anti-dengue seric antibodies (1/1280-1/1280-1/20, respectively). Soon after, 3 cases were found among people already hit by dengue 2 virus in 1972. It was then obvious that another serotype was involved as proven by subsequent isolations of dengue 1 strains.

In Noumea, the main town, virus transmission was markedly affected by Aedes aegypti control measures : two aerial ultra-low volume sprayings of malathion (225 ml/ha - 3 fl. oz/acre) in april-may 1975 - cold fogging of ULV malathion (600 ml/ha) from ground, in april-may 1976.

Monthly records of serologic diagnosis (positive/total), performed for dengue suspected cases, clearly demonstrate the efficiency of aerial treatments :

	<u>JAN</u>	<u>FEB</u>	<u>MAR</u>	<u>APR</u>	<u>MAY</u>	<u>JUNE</u>	<u>JULY</u>	<u>AUG</u>	<u>SEPT</u>	<u>OCT</u>	<u>NOV</u>	<u>DEC</u>
<u>1975</u> :	0/0	0/1	4/6	3/47	3/42	1/18	0/22	0/16	0/11	1/21	0/31	1/23
<u>1976</u> :	2/4	2/14	16/74	122/519	65/307	29/124	7/33	4/22	3/17	0/16	0/5	3/54

TOTAL 1975 : 12/207
 1976 : 253/1189

Sentinel survey by periodic serum examination of young soldiers in Nandaf and Noumea was negative.

Blood samples collected by the blood bank and by Institut Pasteur for laboratory tests were systematically examined for dengue antibodies :

in 1975, on a total of 2194, 756 (34 %) were positive (IH test $\geq 1/10$) in 2 sera, titer was over 1/1280 and may be interpreted as a response to an asymptomatic recent infection.

in 1976, 1411 sera were tested :

A fatal case occurred in Noumea, in april 1976 ; a dengue 1 strain was isolated from the blood of a man of 24, from Wallis Island, deceased after he displayed signs of encephalitis and a 3 days coma. The virus was re isolated and identified as dengue 1 by Dr. Leon Rosen, Pacific Research Section, NIAID, Honolulu.

Virus isolation was successful in 46 other patients who contracted their infection in the following localities : Bourail (1), Kone (5), La Ton-touta (1), Nepoui (1), Noumea (37), Poya (1) ; 2 strains were also recovered from people infected in Papeete, Society Islands.

Aedes aegypti, was found naturally infected in Noumea ; it was apparently responsible of the virus transmission.

Loyalty Islands - Ouvea

From June to September 1975, dengue was suspected in 17 patients. Examination of 16 paired sera showed 2 primary and 1 secondary responses ; 2 dengue 1 strains were isolated from blood taken in acute phase.

A survey on 220 samples collected on filter paper discs disclosed a recent infection in a girl of 11 ; 9 % sera had antibodies that may be considered as traces of 1972 dengue 2 outbreak.

Aedes aegypti was the lone mosquito species found around the homes of sick people.

New Hebrides

First cases were seen in January 1975, among the population of Vila, Vate Island. From March to July 1975, 216 serologic diagnosis were performed on 43 paired sera and 173 unique blood samples and interpreted as follows :

25 primary responses
22 secondary responses
28 presumptive recent infections.

Virus was isolated from the blood of 27 patients and from Aedes aegypti collected in Aoba.

Localities affected : Vila and Malapoa (Vate) - Catholic mission in Wala Rano (Mallicolo) - Lolowaf (Aoba).

Wallis Islands

Apparently, virus was imported from Noumea at the fall of June 1976, examination of 10 paired sera from suspected cases indicated 4 primary and 4 secondary responses to a recent infection.

No viral strain was obtained from human cases neither from 98 Aedes polynesiensis collected during an entomological survey, in November 1976. At the same time, only 1 Aedes aegypti ♂ was caught.

Horne Islands - Futuna

About 30 suspected cases were noticed in October 1976. From 8th to 10th November, 24 blood samples and 55 female mosquitoes were collected by a team of the Institut Pasteur and shipped to Noumea in liquid nitrogen; 11 virus isolations were obtained from human blood and 1 from Aedes polynesiensis (2 ♀ ♀). Aedes aegypti was not seen during the survey and never recorded before from Futuna or Alofi. IH tests on 12 paired sera were positive.

Solomon Islands

In March 1975, several localities were visited for an entomological and serological survey including: Honiara (Guadalcanal) Tulagi (Florida), Auki (Malaita), Munda (New Georgia), Yandina (Russell), Gizo (Western District). On 63 sera, 3 had antibodies (1 from Malaita, 2 from Western District). Numerous larvae of Aedes quasiscutellaris, albolineatus and albilabris were collected but none of Aedes aegypti.

(P. FAURAN, G. LE GONIDEC)

SUMMARY OF RESEARCH

Most of the Institute's research programme in 1975-1976 continued to be in interlocking areas of microbiology-epidemiology-entomology-biochemistry-oncology, with concentration of interests on the viruses transmitted by insects or ticks, cancer and health problems of Aborigines. The following section provides summaries introductory to the more detailed accounts later in the report.

INFECTIOUS DISEASE IN AUSTRALIA

The arthropod-borne viruses of Australia, growing in number each year with isolations by this Institute and other research groups, provide the subject matter for research by several groups, concerned with various aspects of the viruses, the diseases they produce, the insects or ticks that transmit them and the animals that act as hosts. Associated work continues on two rickettsial diseases.

Epidemiological studies of arboviruses

The year saw completion of studies on the large and important outbreak of Murray Valley encephalitis (MVE) in Australia in 1974, with two intriguing observations for future study. Two patients showed rises in antibody titre

suggestive of recrudescent infection more than 150 days after onset of disease, in one case at least in circumstances which made a second infection unlikely. Several other patients, clinically acceptable as cases of Murray Valley encephalitis, had antibody responses more suggestive of infection with Kunjin virus. Several explanations for this finding are possible, but clearly Kunjin virus must be kept in mind as a possible cause of serious disease.

The 1974 epidemic was the first to cause recognized clinical disease in central Australia. We have previously reported antibody surveys which showed widespread subclinical infection with MVE and other viruses in that year.

Subsequent work, much assisted by several colleagues who provided serum samples from their files, showed that this was no "virgin soil" epidemic. Antibody to MVE virus was found in a proportion of Aborigines at most centres tested, and the age of some reactors indicated infection between known disseminations in 1951 and 1974. The results clearly define an area for future study.

Several long-term projects continued to give results of interest. The Institute has maintained surveillance of arbovirus infection at Charleville, in south-west Queensland, since 1964. This year sentinel chickens developed antibody

to Sindbis in January-March, and mosquitoes collected in February yielded many virus strains. No serological evidence was found of MVE virus infection at Charleville this year, but most of the viruses isolated are not yet identified. The Institute, CSIRO Division of Animal Health and the Queensland Department of Primary Industries also collaborated to monitor arbovirus infection in the Flinders River basin in north Queensland. This programme, in its second year, again showed evidence of infection by several togaviruses, including MVE virus, in late summer.

The Institute has for some years been designated as a World Health Organization Centre for Arbovirus Reference and Research. In this capacity it continued to receive virus strains for study from other Australian research units. Several were of particular interest, including a strain of Upolu virus from a bird (submitted by the John Curtin School of Medical Research, Canberra), a "new" flavivirus related to Tyuleniy virus (submitted by CSIRO Division of Animal Health, with final identification by Dr J. Casals, Yale Arbovirus Research Unit, New Haven, U.S.A.), and over 40 isolates from the Northern Territory (submitted by CSIRO Division of Animal Health) including several "new" viruses and many new host and geographical records.

Studies on the role of native animals, particularly water birds, in the epidemiology of MVE and other arboviruses were initiated. Population studies of water birds commenced at Lake Bullawarra near Thargomindah in western Queensland. Information is being sought on the breeding, dispersal and migration of birds and the occurrence and timing of infection among them. It is hoped to expand these studies into more detailed investigations of the nature of arbovirus infection in wild animals and into ecological considerations of the interactions between the vertebrate hosts and the arthropod vectors in the field.

Entomology

Mosquito biology and systematics.- Taxonomic studies were concerned principally with Culex, Aedes and Bironella. Four new undescribed species were recognised, two Aedes from north-west Australia and a Culex and a Bironella from north Queensland. In preliminary studies of the Culex annulirostris complex, distinctive forms were recognised among larvae and adults from northern Australia, but good series of reared specimens will be needed to resolve whether several species are currently identified as C. annulirostris.

Investigation of a mosquito problem at Gladstone

showed that Culex sitiens was breeding in plague proportions in a recently impounded tidal area. At Hinchinbrook Island the saltmarsh mosquito, Aedes vigilax, was breeding extensively in rock pools on the headlands.

Over 30 collections received for identification included the first Queensland record of Aedeomyia venustipes. Enquiry showed 23 individuals or institutions to be working on mosquitoes in Australia.

Mosquito ecology and mosquito-virus relationships.-

A programme of field work on factors that contribute to the importance of the mosquito, Culex annulirostris, as a vector of arboviruses, especially MVE and Ross River viruses, was completed and data are being analysed for publication. The study demonstrated that C. annulirostris prefers to feed on calf, dog, feral pig, grey kangaroo, man and domestic fowls in that order when tested in an experimental situation, but identification of the source of host blood of naturally-fed mosquitoes at Kowanyama indicated that dogs are severely attacked compared to the human population in the village. The mosquito was collected throughout all seasons at both Kowanyama and Charleville and large numbers of physiologically old mosquitoes, possibly capable of transmitting virus, were taken in both summer and winter. Some degree of success was achieved in assessing the age of C. annulirostris by

using a quick technique applicable to biting midges. Study of a population of C. annulirostris from Long Pocket demonstrated that it was an excellent vector of Murray Valley encephalitis virus but probably only moderately efficient with Ross River virus. Other mosquito-virus studies are outlined.

In total, 62,724 female mosquitoes were collected for virus isolation and 8,051 blood-engorged females collected resting during the day on gauze mosquito screening and under eaves of houses, in cardboard boxes in grass and leaf litter, in trees and flying during evenings and early mornings. These studies have allowed us to take a broad overview of C. annulirostris but there are still other aspects that are worthy of investigation.

Acarology

Even at the level of mechanical *description*, much useful and relevant work clearly remains to be done on the classification of mite parasites of Australian vertebrates. Study of material involving three aspects of the family Dermanyssidae is under way.

Dermanyssid classification has traditionally been based on females, but a range of previously unknown males and immatures was illustrated and described. In this difficult group, the somewhat unidirectional adaptational "pressure"

applied by parasitism has compounded the structural conservatism seen in free-living members of the family, and any avenue likely to afford useful classificatory data should be explored. Several collections received from collaborating scientists were incorporated into the body of data prepared for publication. Among this material, some mites from mobile hosts, e.g. birds and bats, were found to have much wider geographic distributions than previously recognized.

Initial scanning of an important circum-Australian collection from rodents, including several seldom-seen species, indicated many novelties. A good start was made to illustrating this material, which will be worked up as a single paper.

Two supporting papers on Oriental members of dermanyssid groups also present in Australia were written.

An initial collection of mites from small mammals from rain forest at Bamaga, near the tip of Cape York Peninsula, continued to yield taxa otherwise unknown in Australia, and with clear Papuan/Oriental connexions.

General collecting was undertaken for a month at Kowanyama, near the east coast of the Gulf of Carpentaria, in an area of mixed tropical woodland and tussock grassland.

Among several lots of mites submitted as of possible

economic importance were the vectors of scrub typhus in the Solomons and the New Hebrides.

A major effort this year was devoted to clearing the back-log of curatorial tasks left incomplete over a long understaffed period.

Laboratory studies of arboviruses

Biochemistry of orbiviruses.- Orbiviruses include pathogenic agents of man (Colorado tick fever), domestic animals (bluetongue of sheep and African horse-sickness) and native animals (epizootic haemorrhagic disease of deer). None of these diseases are known in Australia, but a number of orbiviruses have been isolated from mosquitoes and biting midges in the arbovirus isolation programme conducted by the Institute over the last seventeen years.

The orbiviruses are divided into groups depending on their serological reactions; the Australian viruses are classified into five groups. Of these, Eubenangee and Corriparta group viruses have also been found in Africa, and D'Aguilar virus is a member of the Palyam group found also in India and Africa; the Wallal and Warrego group viruses have so far been found only in Australia. The serological groups are composed of large numbers of viruses or virus types, and the antigenic diversity within these groups forms the basis of our studies of orbiviruses.

We are able to analyse the ribonucleic acid from orbiviruses by gel electrophoresis. The patterns of separation depend on the sizes of the RNA fragments and the orbiviruses show a characteristic pattern of ten pieces of RNA. Each of these RNA pieces is the genetic code for virus proteins synthesized in infected cells. The pattern of RNA segments of viruses within serological groups can be correlated with the known antigenic properties of the proteins; for example the RNA segments coding for proteins on the surface of the viruses are recognisably distinct in the gel electrophoresis patterns. The proteins on the surface would be involved in the neutralization of virus by antibody produced to them in infected animals.

We are testing the hypothesis that new types of viruses arise by exchange of certain RNA segments in cells infected simultaneously with two viruses. Our work with temperature-sensitive mutants of two viruses shows that they recombine with high frequency. A random mix of RNA segments in cells infected with two viruses would produce recombination frequencies of 25% and we interpret our results as suggesting interchange of segments.

In order to recognize equivalent RNA segments in different viruses we are developing techniques to "finger-print"

each segment. The use of such techniques in conjunction with the more usual cross-hybridization of RNA molecules will allow us to determine the relationship between viruses at a molecular level.

Biochemistry of Ross River virus.- Work has been aimed at developing a process to produce quantities of the structural proteins of Ross River virus for use in a study of their immunogenicity and to characterize inter-strain relationships of the structural components. A procedure was developed to purify the virus from culture fluid avoiding all processes which may have been physically or chemically damaging to the particle. It involved concentration of virus suspension by filtration and sedimentation of the concentrate through a sucrose solution on to a sucrose cushion. Initially, this material was then subjected to electrofocussing but virus was concentrated at pH 4.1. It was felt that exposure of virus for long periods to this pH may be detrimental. Accordingly the focussing was abandoned and material from the sucrose sedimentation was centrifuged in a sodium tartrate equilibrium gradient until it reached its equilibrium position. Analysis of the opalescent band at a density of 1.18 gm/ml showed the three structural polypeptides characteristic of Ross River virus while

electron microscopic examination of the same band showed fields of tightly packed particles 60-70 nm in diameter.

Experiments to find a satisfactory technique to separate the structural components of the virion suggested that the non-ionic detergent NP40 was most effective. Purified virus treated with NP40 was found to yield very satisfactory separation of envelope and nucleocapsid proteins. It is felt that this technique will allow production of any quantities needed.

(R. L. Doherty)

(Received November 12, 1976 - Editor)

REPORT FROM CSIRO DIVISION OF ANIMAL HEALTH LABORATORIES AT :

PRIVATE BAG NO. 1, PARKVILLE, VICTORIA, 3052, AUSTRALIA &

PRIVATE BAG NO. 1, GLEBE, NEW SOUTH WALES, 2037, AUSTRALIA

Akabane Disease : Field Isolation of the Virus from Ovine
Foetuses

Since the major outbreak of epizootic congenital bovine arthrogryposis and hydranencephaly, caused by Akabane virus (Simbu serogroup, Bunyaviridae), in south-eastern New South Wales in 1974, sentinel flocks of sheep have been maintained in this area. There were 2 sentinel flocks near Sydney, at Bringelly (in co-operation with W.J. Hartley, University of Sydney) and at Badgery's Creek. There were approximately 50 ewes in each group and their sera was tested at monthly intervals for the presence of neutralizing antibodies against Akabane virus strain B8935.

None of the ewes sero-converted during 1975, indicating that Akabane virus had not infected sheep in the area during the year. Between February and April, 1976, a high proportion of the ewes in each flock became infected with Akabane virus, as indicated by the presence of serum neutralizing antibodies. 43% sero-converted at Badgery's Creek and 73% at Bringelly. At Badgery's Creek 31 of the 49 ewes in the sentinel flock were mated between January 28 and February 26, 1976. Of the mated group, 11 had sero-converted at the bleed on March 18. These ewes were killed and autopsies performed in an attempt to isolate Akabane virus.

Of the 11 ewes sacrificed, only 8 were pregnant. The gross examination of the foetuses (at 72 to 89 days gestation) showed 1 foetus was mummified, 1 foetus had arthrogryposis and hydranencephaly, and the remaining 6 foetuses appeared normal. None of the foetuses possessed serum neutralizing antibodies against Akabane virus.

Virus was isolated from the deformed foetus (D385) at 89 days gestation and from a grossly normal foetus (D410) which had microscopic lesions of porencephaly, at 74 days gestation. In D385, virus was isolated from the placentomes ($10^{3.7}$ to $10^{5.0}$ TCID₅₀/g), foetal fluids ($10^{2.7}$ TCID₅₀/ml), and foetal membranes ($10^{4.2}$ TCID₅₀/g) but not from the lung, muscle, thymus, heart, kidney, spleen, liver, stomach contents and cerebro-spinal fluid. In D410, virus was isolated from the placentomes ($10^{4.0}$ TCID₅₀/g), foetal fluids ($10^{5.3}$ TCID₅₀/ml), foetal membranes ($10^{5.5}$ TCID₅₀/g), lung ($10^{4.0}$ TCID₅₀/g), muscle ($10^{2.0}$ TCID₅₀/g), cerebellum ($10^{2.0}$ TCID₅₀/g), cerebrum ($10^{4.0}$ TCID₅₀/g) but not from thymus, heart, kidney, spleen, and liver.

Cross-neutralization tests were performed to compare the 2 isolates (Akabane/D385 and Akabane/D410) with our reference strain B8935 of Akabane virus. No antigenic difference could be demonstrated among the 3 isolates. It was therefore concluded that Akabane virus (strain B8935) isolated from the biting midge Culicoides brevitarsis and which has been demonstrated by us to cause congenital deformities in lambs, when ewes were experimentally infected at 30 to 36 days gestation, is antigenically identical, by the cross-neutralization test, with the 2 isolates made in the field from the ovine foetuses.

In the period June to September, 1977, there was an epizootic of congenital deformities in lambs, associated with Akabane virus, in the area west and south of Sydney, New South Wales. Micrencephaly has been the main deformity seen in these lambs, although some have shown arthrogryposis and hydranencephaly. There has only been a small number of cases of bovine arthrogryposis and hydranencephaly, possibly because there was a smaller number of susceptible pregnant cows after the major epizootic of Akabane disease in 1974.

(A.J. Della-Porta, M.L. O'Halloran, I.M. Parsonson, W.A. Snowdon and M.D. Murray).

PRELIMINARY STUDIES ON A POSSIBLE NEW RHABDOVIRUS (KUNUNURRA VIRUS)
ISOLATED FROM MOSQUITOES

A possible new rhabdovirus (strain OR194 - Kununurra virus) had been isolated from a pool of 39 mosquitoes (*Aedeomyia catanticta*) by i.c. inoculation in suckling mice. The exact location of mosquito sample collection was reported in *Arthropod-Borne Virus Information Exchange*, No. 31, 93-98, 1976. The method of mosquito collection and sampling was described elsewhere (Liehne *et al.*, *Aust. J. exp. Biol. med. Sci.*, 54(5), 487-497, 1976).

Morphology

The virus particles are bullet-shaped; typical of members of rhabdovirus group. The average dimensions are 70 nm x 130 nm, as measured by electron microscopy. The virus particles are observed to accumulate in the cytoplasm in lattice formation.

Chemical sensitivity

	Titre before treatment	Titre after treatment
Ether (1:1)	4.7*	<1
Sod. deoxycholate (1:1000)	4.5	<1

*Log₁₀ TCID₅₀/0.025 ml.

Cell culture systems

VERO cells	(i) CPE observed on the 3rd day.
	(ii) Irregular-shaped plaques (0.5 mm-1 mm) formed under agar medium after 4 days incubation.
BHK-21 cells	(i) CPE observed after 2-3 days. (ii) No plaque formation.
PS cells	No CPE observed.

Other properties

From acridine orange staining study, OR 194 is probably a single-stranded RNA virus.

Antigen of OR 194 was prepared from infected suckling mouse brains by the sucrose-acetone extraction method. This antigen showed no haemagglutinating ability with gander erythrocytes over a pH range of 5.9-7.4. However, it is a potent antigen in complement-fixation tests.

Antigenic relationship

Antigen of isolate OR 194 failed to react in CF tests with immune sera to the following viruses:

Aino	Johnston Atoll	Sindbis
Almpiwar	Joinjakaka	Taggert
Belmount	Kowanyama	Trubanaman
Bovine Ephemeral Fever	Kunjin	Upolu
Charleville	Mapputta	Wallal
Corriparta	Mitchell River	Wongal
D'Aquilar	Ngaingan	Wongorr
Eubenangee	Nugget	

Antigen OR 194 also failed to react in CF tests with immune ascitic grouping fluids obtained from the National Institutes of Health, Bethesda, Maryland, U.S.A.:

Group C	Polyvalent Patois	Polyvalent 6
Group Guama	Group Capim	Polyvalent 7
Group Simbu	Polyvalent Palyam	Polyvalent 8
Group VSV	Group Kemerovo	Polyvalent 9
Group Bunyamwera	Polyvalent Congo	Polyvalent 10
Group California	Polyvalent 1	Polyvalent 12
Group Tacaribe	Polyvalent 2	Polyvalent Rabies
Group Phlebotomus	Polyvalent 3	
Poly. Anopheles A	Polyvalent 4	
Polyvalent Bwamba	Polyvalent 5	

(N.F. Stanley, M.P. Alpers, K.H. Chan, S. Paul, A. Wright, D. Britten).

JAKARTA, INDONESIA

Epidemic DHF in Central Java

During October, November and December, 1976 an outbreak of dengue occurred in Bantul, a rural area approximately 25 kilometers south of Yogyakarta, Central Java. Bantul is a very densely populated agricultural area with rice and sugar cane as the most common crops. Kampongs (small villages) where the majority of people live, occur as islands of vegetation and houses surrounded by rice paddy and sugar cane. Sporadic cases of DHF have been reported in the area since 1973 when reporting was first started, but epidemic dengue has never been a problem.

In late October, 1976 and coinciding almost exactly with the onset of the rainy season, the number of cases of DHF began to increase (Table 1) and peaked in December. The illness affected primarily children. In serologically confirmed or presumptive cases, the modal age was 7 years, similar to that observed in Jakarta. The male to female ratio was 1:1.5.

Most patients had a rather severe febrile illness characterized by acute onset, fever, nausea, vomiting and headache (Table 2). A positive tourniquet test was the most common hemorrhagic manifestation observed although epistaxis and hematemesis were not uncommon (Table 3).

Four fatal cases of DHF/DSS were studied. Three of these were confirmed virologically by isolation of virus from the acute serum specimen. Of these 3, one had no detectable dengue HI antibody in a serum taken on the fourth day of illness and another had a titer of 10 in a serum taken on day 3 of illness. The other child had a dengue HI antibody titer of 80 in a serum also taken on day 3 of illness. Thus 1 and possibly 2 of the fatal cases were probably primary infections.

Of 58 patients who had a 4-fold or greater rise in dengue HI antibody between the acute and convalescent sera, virus has been isolated from 41 or 71%. The high virus isolation rate alone suggested high circulating virus titers in the blood and this was confirmed by titration of the acute sera. These titrations have now been completed on a majority of the positive patients and many had very high viremias of over 10^8 MID₅₀ per ml. Serial blood samples could not be taken and it was therefore not possible to determine how long the viremia lasted in these patients. However, most of the virologically positive sera were taken on days 2, 3 and 4 of illness whereas the majority of virologically negative sera were taken on days 4, 5 and 6. The viruses have not yet been typed.

Previous studies on mosquito susceptibility have shown that with the

Hawaiian strain of A. albopictus, 100% infection rates were obtained when the mosquitoes were fed on a patient circulating $10^{8.50}$ MID₅₀ per ml of dengue type 2 virus (D.J. Gubler and L. Rosen, unpublished data). A similar occurrence in Bantul could explain the explosive nature of that epidemic. Only 40 adult A. aegypti females were collected from Bantul for virus isolation. These were divided into 4 pools of 10 each and inoculated into mosquitoes. Virus was isolated from 2 of the 4 pools suggesting a relatively high infection rate in the wild A. aegypti population. Unfortunately, no A. albopictus were collected for virus isolation. Susceptibility tests with the A. aegypti and A. albopictus from Bantul are in progress, but are not yet complete. However, preliminary results (Table 4) indicate that the Bantul A. albopictus may be more susceptible than A. aegypti to oral infection with all 4 dengue serotypes.

Larval mosquito surveys were carried out in 3 representative Kampongs in the Bantul area. Both A. aegypti and A. albopictus were abundant in all 3 villages. A. aegypti was found breeding primarily in cement water holding tanks both indoors and outdoors, whereas the A. albopictus were breeding mostly in cut bamboo stumps outdoors. The A. aegypti premise index (percentage of households with A. aegypti) was 49.5% whereas nearly every house had several clumps of bamboo and therefore A. albopictus. The Breteau index for A. aegypti was 32.3. The data suggest that A. aegypti was widespread and could have been responsible for transmission of the epidemic. The abundance of A. albopictus in the area, the apparent higher susceptibility of this species and the correlation between the start of the epidemic and the beginning of the rainy season, however, suggest that A. albopictus was also involved.

(D.J. Gubler, Suharyono, S. Nalim and I. Lubis).

Table 1

Numbers of reported dengue hemorrhagic fever patients and rainfall
in Bantul, Central Java, by month

Month	Rainfall (mm)	No. DHF Cases
January 1976	286	4
February	148	-
March	377	2
April	74	-
May	22	1
June	-	-
July	-	-
August	8	-
September	-	2
October	143	23
November	245	125
December	98	217

Table 2

Clinical manifestations observed in 82 confirmed dengue patients ¹ from Bantul, Central Java, 1976

Sign or Symptom	Number	%
Fever	82/82	100.0
Nausea/vomiting	51/82	62.2
Headache	48/82	58.5
Abdominal pain	32/82	39.0
Cough	19/82	23.2
Rhinitis	10/82	12.2
Sore throat	8/82	9.8
Diarrhea	6/82	7.3
Constipation	4/82	4.9
Backache	2/82	2.4
Pruritis	1/82	1.2
Myalgias	0/82	0
Joint pains	0/82	0

¹. Includes patients with 4-fold or greater rise in HI antibody between acute and convalescent sera plus patients with a titer of 1280 or greater in the acute specimen.

Table 3

Hemorrhagic manifestations in patients ¹ with serologically confirmed dengue fever, Bantul, Central Java, 1976

Hemorrhagic Manifestation	Number	%
Positive tourniquet test	70/82	85.4
Petechial rash	2/82	2.4
Epistaxis	16/82	19.5
Hematemesis and/or Melena	6/82	7.3
Gum Bleeding	2/82	2.4
Ecchymoses	3/82	3.7
Hematoma	1/82	1.2

¹. Includes patients with 4-fold or greater rise in HI antibody between acute and convalescent sera plus patients with a titer of 1280 or greater in the acute specimen.

Table 4

Comparative susceptibility of Aedes aegypti and A. albopictus from Bantul to oral infection with dengue types 1, 2, 3 and 4

Titer* Mosquito	D1	D2	D3	D4
	10^8	10^8	4×10^8	2×10^7
<u>A. aegypti</u> Jakarta	13/20** (65.0)	6/20 (30.0)	8/20 (40.0)	5/17 (29.4)
<u>A. aegypti</u> Bantul	4/16 (25.0)	2/6 (33.3)	2/5 (40.0)	1/12 (8.3)
<u>A. albopictus</u> Bantul	10/20 (50.0)	13/20 (65.0)	10/20 (50.0)	12/20 (60.0)

* MID_{50}/ml

** No. infected/No. tested (Percent infected)

Experimental viraemia and transmission of JE virus by mosquitoes in
Ardeid birds

Viraemia studies were carried out with three species of birds belonging to the family Ardeidae viz. Ardeola grayii (Pond heron), Bubulcus ibis (Cattle egret), Egretta garzetta (Little egret) and one species from the family Phalacrocoracidae, Phalacrocorax niger (Little cormorant). The birds were trapped in the rice growing districts of Krishna and West Godavari of Andhra Pradesh, in South India. The age of the birds varied from 1/2 to 5 months approximately. Thirteen pond herons, 8 cattle egrets, 9 Little egrets and 2 Little cormorants were inoculated intramuscularly with 2.7 to 3.5 dex LD₅₀ of JE virus of the 3rd mouse passage level which was originally isolated from a pool of Culex whitmorei mosquitoes collected from the Krishna district of Andhra Pradesh. The birds were bled daily from day 2 PI to day 8 PI. Heparinized blood was inoculated (0.02 ml.) intracerebrally (i.c.) in 2-4 days old baby mice and the mice were observed for sickness for 21 days. The brains from sick mice were tested for the presence of JE virus by the CF test using aqueous saline extract of the mouse brain.

As detailed below all the 13 pond herons tested and all the 8 cattle egrets tested circulated the virus for a period of at least 2-3 days, usually starting from day 2 PI.

Bird species	Proportion of bird circulating virus	Onset of viraemia day	Duration of viraemia	Virus titre
<u>Ardeola grayii</u>	13/13	2	2-3 days	Virus traces to 2.5 dex LD ₅₀
<u>Bubulcus ibis</u>	8/8	2-3*	2-3 days	Virus traces to 1.9 dex LD ₅₀
<u>Egretta garzetta</u>	0/9	No virus detected		
<u>Phalacrocorax Niger</u>	0/2	No virus detected		

*One bird circulated traces of virus on day 7 and 8 PI only.

The titres of virus varied from traces of virus to 2.5 dex LD₅₀ in Pond herons and from traces of virus to 1.9 dex LD₅₀ in Cattle egrets. However, Little egrets and Little cormorants failed to circulate the virus. Attempts were also made to infect Culex tritaeniorhynchus mosquitoes from the laboratory colony by feeding them on these infected birds.

Bird-mosquito-bird transmission of virus was achieved between Ardeola grayii-C. tritaeniorhynchus-Ardeola grayii; Ardeola grayii/Bubulcus ibis-C. tritaeniorhynchus-white leghorn chick and white leghorn chick-C. tritaeniorhynchus-Ardeola grayii.

(R. S. Soman, F. M. Rodrigues, S. N. Guttikar and P. Y. Guru)

Reference:

R.S. Soman, F.M. Rodrigues, S.N. Guttikar and P.Y. Guru. (1977). A study of the role of wild birds in the epidemiology of Japanese encephalitis virus in India.

I. Experimental viraemia and transmission of virus by mosquitoes in Ardeid birds.

Paper submitted for publication in the Indian Journal of Medical Research.

Establishment of cell lines from ticks

Continuous cell lines from three species of ticks, viz., Haemaphysalis spinigera, Haemaphysalis obesa and Rhipicephalus sanguineus have been established at this laboratory. These cell lines were derived from the imaginal tissues developing inside the nymphal exuvium. The tissue was obtained by dissecting the surface sterilized nymphs during moulting phase, held at 30°C, five to eight days after engorgement. All the developing tissue excepting the alimentary canal, the rectal sac and the malpighian tubules were utilized for preparing the cultures. The growth medium consisted of L-15, supplemented with 10 percent tryptose phosphate broth and 10 percent foetal calf serum. The current passage level of these cell lines are as follows:

Tick species	Designation of cell line	Passage level
<u>Haemaphysalis spinigera</u>	ATC-281/282	23
" "	ATC-309	10
<u>Haemaphysalis obesa</u>	ATC-304	14
" "	ATC-311	7
<u>Rhipicephalus sanguineus</u>	ATC-307	8
" "	ATC-308	8

(P.Y. Guru, Vijai Dhandu and N.P. Gupta)

Susceptibility of tick cell lines to arboviruses

Haemaphysalis spinigera cell line (ATC-309) has been tested for its susceptibility to certain arboviruses. Kyasanur Forest disease, Ganjam, Phanja, Kaisodi and Wad Medani, all of which are tick borne viruses multiplied in this cell line. However, no cytopathic effect was apparent. Japanese encephalitis, West Nile and Chikungunya, all of which are mosquito borne viruses did not show multiplication. Sindbis virus, which has been isolated from mosquitoes as well as mites showed multiplication, albeit at low titres. Chandipura, a rhabdovirus, which has been isolated from sandflies, and could be transmitted through the bite of mosquitoes did not show multiplication.

Studies on the susceptibility of other tick cell lines are in progress.

(P.Y. Guru, Kalyan Banerjee and Vijai Dhanda)

Chalcids (Hymenoptera : Encyrtidae) parasitizing ticks in India

So far two species of chalcids parasitizing ticks have been reported in India. These are Ixodiphagous mysorensis Mani, parasitizing soft ticks of the genus Ornithodoros and Hunterellus hookeri Howard, parasitizing the Ixodid ticks. The validity of the former has been the subject of doubt, as it has been considered a synonym of H. hookeri.

During our studies, we came across two species, both parasitizing Ixodid ticks. H. hookeri was found to parasitize Hyalomma anatolicum, and a new species, close to H. hookeri was found to parasitize Haemaphysalis bispinosa. Both the species were collected from the moulting nymphs inside the cattle sheds in several localities in Maharashtra and Karnataka states. Colonies of both are being maintained in the laboratory. In addition to certain morphological differences, the two species differ in host predilection. While H. hookeri parasitizes mainly the nymphs of Hyalomma, the new species shows specificity to members of the genus Haemaphysalis.

The potential of these two species for the biological control of ticks, particularly inside the cattle sheds is being explored.

(G. Geevarghese and Vijai Dhanda)

Susceptibility of *Phlebotomus papatasi* to Chandipura (CHP) virus

Chandipura, a Rhabdovirus belonging to VSV group, was isolated in India from two febrile human cases and a pool of wild-caught sandflies (*Phlebotomus* sp.). Experiments were therefore conducted to evaluate the susceptibility and vectorial potential of *P. papatasi* to CHP virus.

Females of *P. papatasi* were infected by feeding on suckling swiss albino mice circulating 6-8 dex of virus. Transmission of the virus to suckling mice by bite of the infected females was successfully achieved on 4th and 7th days after the infective blood meal. The virus persisted in the infected *P. papatasi* up to 14 days. The eggs laid by infected females were tested and the virus was recovered from two batches of eggs, suggesting the possibility of transovarial transmission.

(Govind B. Modi and M.K. Goverdhan)

REPORT FROM VIROLOGY SECTION NATIONAL HEALTH LABORATORY
RANGOON, BURMA

Serological evidences of West Nile infection in Burma

During 1976, blood samples were taken from apparently healthy people and domestic animals from various parts of Burma to determine Japanese B Encephalitis prevalence.

While undertaking haemagglutinating inhibition (HI) tests on these sera, all four Dengue serotypes and West Nile haemagglutinating antigens were also included to confirm the specificity of the detected JBE antibody.

Unexpectedly, monotypic HI antibody responses to West Nile virus was detected in some sera of Rangoon, Mandalay, Myitkyina and Moulmein areas. So far there has been no reports of either clinically or by laboratory investigation on the presence of West Nile virus in Burma.

Examples of monotypic HI antibody responses to West Nile virus is shown in the table attached.

(Drs. Soe Thein & Than Swe)

Table showing examples of monotypic HI antibody responses:

CODE NO.	DEN 1	DEN 2	DEN 3	DEN 4	JEE	WN	AREA
H779	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	40	RANGOON
H950	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	40	
H954	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	40	
H958	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	80	
H959	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	80	
H576	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	40	MANDALAY
H588	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	10	
Hr1	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	80	
P1	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	20	
P3	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	20	
H213	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	40	MYITKYINA
H214	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	80	
H242	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	80	
H216	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	10	
H254	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	10	
H1253	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	40	MOUNGHEIN
H1412	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	40	
H1415	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	40	
H1137	∠ 10	∠ 10	∠ 10	∠ 10	∠ 10	40	
H1297	∠ 20	∠ 20	∠ 20	∠ 20	∠ 20	80	

ABBREVIATIONS: H=Human, P=Pig, Hr=Horse

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF HONG KONG,
PATHOLOGY BUILDING, QUEEN MARY HOSPITAL COMPOUND,
HONG KONG

An affinity chromatography procedure employing dodecylamine attached to sepharose beads to remove serum lipoprotein inhibitors of HA is under investigation for its suitability for detecting presumptive JE virus antibody in HAI titrations. Comparison of HAI antibody titres obtained by this and the conventional kaolin procedure with those obtained by VN for JE virus show a higher correlation coefficient for the affinity chromatography procedure. This is more apparent with reptile than human sera. Results obtained to date also suggest that it is essential to use fresh sera for effective lipoprotein removal. Freeze-thawing and ageing of sera cause structural alterations to lipoproteins rendering them less susceptible to removal by affinity chromatography and other procedures.

JE virus is the only arbovirus recorded to date in Hong Kong. This situation is being investigated further in conjunction with the London School of Hygiene and Tropical Medicine in a serological survey for arbovirus antibodies in animals and dwellers in the rural/urban areas of the New Territories, Hong Kong.

(K. F. Shortridge)

REPORT FROM THE BUREAU OF RESEARCH LABORATORIES
PHILIPPINE DEPARTMENT OF HEALTH, MANILA AND THE
U.S. NAVAL MEDICAL RESEARCH UNIT NO.2, TAIPEI, TAIWAN

An HI test survey for antibodies to Dengue virus antigen among the residents of different parts of the Philippines from 1972 to 1975

Cases and deaths due to hemorrhagic dengue fever in the Philippines were first noted in 1954, when it was first described as a clinical entity in the Philippines by Quintos and Lim. Since then it has been reported every year. It may be noted from Table I that the morbidity rates for the whole country was high in 1966. The rates after 1966 ranged from 1.2 to 4.0 per 100,000. The highest mortality rate was also in 1966.

Results of the HI studies from 1963 to 1964, using Dengue 1 as a representative Group B antigen, performed on sera obtained from residents of different parts of the Philippines, are summarized in Table II. It is noted that 77% and 85% of samples from residents of Manila and its suburbs, respectively, had HI antibodies to dengue, and 48.3% to 56% of samples from other parts of the country except those from the Mountain Province.

Recently in 1972 to 1975, surveys for Dengue HI antibody were done in connection with an on-going infectious disease survey, a joint project of the Philippine Department of Health and the U. S. Naval Medical Research Unit No. 2 at Taipei. The tests were performed at the NAMRU-2 laboratory employing standard methods.

The results are shown in Table III. While the number of samples from the Mountain Province in 1964 and 1974 are not comparable, it is noted that in 1974, 38.6% of the 1609 examined had Dengue HI antibodies, only 7 with a titer of 1:80 and all the rest a titer of 1:10 to 1:40. Only 54.8% of the 1212 examined from Sta. Cruz, Marinduque had similar antibodies; 16 had a 1:80 titer, one a 1:160 titer and all the rest, 1:10 to 1:40. Seventy-four per cent to 93.7% of the residents of the other parts of the country had dengue antibodies. Manila was not included in the survey but an examination of the sera from patients confined for other diseases showed 63.3% with dengue antibodies. Of the 9061 persons of all ages in various parts of the country tested from 1972 to 1975, 72.5% have antibodies to dengue antigen.

Table IV summarizes the results of the HI test of sera of children below 10 years of age from different parts of the country. Children from Benguet, Mountain Province and Sta. Cruz, Marinduque had a lower exposure rate of 8.1% and 17.4%, respectively. The highest rates were noted among children from Candelaria, Quezon and Northern Samar.

Table V shows the results of those examined in the age group above 10 years but below 20 years. Again it is noted that a low rate of 11.8% was found from those coming from Benguet, followed by 30.8% from Sta. Cruz, Marinduque. Sixty-one per cent to 88% of those from other parts of the country had antibodies in this age group.

Unlike other places, people examined from Candelaria registered 49.5% reactivity to Chikungunya virus; the highest titers observed were also 1:320 and 1:640 as with dengue antigen. Forty-two point eighty five per cent of those above 10 years but below 20 years and 60% of children below 10 years had antibodies to Chikungunya antigen. Forty-three point four per cent of both groups had antibodies to dengue.

While a high percentage of the population has antibodies to dengue virus in a wide geographical area, there has been no unusual increase in the morbidity rate from dengue hemorrhagic fever except in 1974 when there was an increase in the City of Manila. It is felt that most of the dengue infections in the country have been mild and often seen as a common cold "influenza" or a seasonal fever, especially since a diagnostic laboratory work-up is not a routine procedure. Likewise there has been much improvement in the environmental sanitation of the country.

(V. Basaca-Sevilla, J. H. Cross, T. Banzon and J. S. Sevilla)

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TABLE I. PHILIPPINE H-FEVER MORBIDITY AND MORTALITY*
1958 - 1974

Year	Morbidity		Mortality	
	No.	Rate**	No.	Rate**
1958	94	0.4	34	0.1
1959	40	0.1	11	0.04
1960	551	2.0	40	0.1
1961	1459	5.1	33	0.1
1962	134	0.5	62	0.2
1963	189	0.6	74	0.2
1964	759	2.4	169	0.5
1965	652	2.0	109	0.3
1966	9384	28.0	250	0.7
1967	1371	4.0	105	0.3
1968	1116	3.1	115	0.3
1969	1336	3.6	103	0.3
1970	922	2.5	83	0.2
1971	438	1.2	34	0.09
1972	1570	4.0	83	0.2
1973	710	1.8	214	0.5
1974	665	1.6	153	0.4

* Taken from the data of the DIC

** Rate per 100,000

Table II. Results of HI test of 328 Serum Samples
 Collected from adult residents from
 Different Islands of the Philippines
 from November, 1963 to August, 1964*

Island	Place	Total No. of samples examined	HI reaction with dengue		HI reaction with chi- kungunya	
			No.	%	No.	%
Luzon	1. Manila & Suburbs	153	41	77	11	20.8
			130	85	30	20.9
	2. Panga- sinan	18	10	53	1	5.5
	3. Sorsogon	25	14	56	1	4.0
	4. Albay	23	12	52	5	21.7
	5. Mountain Province	7	0	0	0	0
Visayas	1. Negros Occiden- tal	21 ** (children) 29	1	4.8	0	0
			14	48.3	2	6.8
	2. Palawan	22	11	50	4	18.1
Mindanao	1. Davao	30	15	50	6	20.0

* Data from V. Basaca-Sevilla & S. B. Halstead

** Include children from 7 - 16 years old

Table III. Results of the HI Tests with Dengue Virus Antigen on 9061 Serum Samples Collected from Residents of Different Islands of the Philippines (1972 - 1975)

Island	Province	Total no. examined	Sera reacting with Dengue Virus Antigen		Year of
			no.	%	
	Ilocos	842	789	93.7	1972
Luzon	Mountain Province (Benguet)	1609	622	38.6	1974
	Cagayan (Buguey)	614	494	80.4	1975
	Quezon (Candelaria)	1122	1031	91.9	1975
Marinduque	Marinduque (Sta. Cruz)	1212	664	54.8	1973
Samar	Northern Samar	1201	1008	83.9	1973
Cebu	Toledo	958	836	87.2	1973
Mindanao	Davao	1503	1122	74.7	1974
	TOTAL	9061	6566	72.5	
	Manila *	90	57	63.3	1974

* Sera from patients confined for other diseases

Table IV. Results of the HI Test with Dengue Virus antigen on 1047 Serum Samples Collected from Children Below 10 years of Age from Different Parts of the Philippines (1972 - 1975)

Place	: Number of: No. of sera with titer at serum : Total :								: Reactive: % Reactive	
	: sera	: dilution at:								: Sera
: examined	: 1	: 1	: 1	: 1	: 1	: 1	: 1	: 1	: 1	
:	: 10	: 20	: 40	: 80	: 160	: 320	: 640	:	:	
Mountain Province (Benguet)	197	14	2						16	8.1
Cagayan (Buguey)	43	9	7						16	37.2
Quezon (Candelaria)	25	10	9	3					22	88.0
Marinduque (Sta. Cruz)	219	22	9	6		1			38	17.4
Northern Samar	236	57	74	46	12	1	1		191	80.9
Toledo (Cebu)	248	38	51	49	15	6		1	160	64.5
Davao (Mindanao)	79	12	10	6	6	1			35	44.3
TOTAL	1047	162	162	110	33	9	1	1	478	45.6
Manila *	47	10	4	1		1			16	34.0

* Sera from patients confined for other diseases

Table V. Results of the HI Tests with Dengue Virus Antigen on 2345 Serum Samples Collected from Persons 10 - 19 Years Old from Different Parts of the Philippines (1972 - 1975)

Place	: Number of sera examined	: No. of sera with titer at dilution at:	: serum	: Total Reactive Sera	: % Reactive sera				
		: <u>1</u> : <u>1</u> : <u>1</u> : <u>1</u> : <u>1</u> : <u>1</u> : <u>1</u> :							
		: 10 : 20 : 40 : 80 : 160 : 320:640 :							
Benguet	: 365	: 30	: 9	: 3	: 1	: :	: :	: 43	: 11.8
Buguey	: 197	: 53	: 38	: 36	: 4	: 1	: :	: 132	: 67.0
Candelaria	: 539	:135	: 193	: 102	: 28	: 11	: 6	: 475	: 88.1
Sta. Cruz	: 377	: 57	: 30	: 24	: 5	: :	: :	: 116	: 30.8
Northern Samar	: 262	: 68	: 92	: 42	: 13	: 3	: :	: 218	: 83.2
Toledo	: 138	: 26	: 38	: 32	: 20	: 3	: :	: 119	: 86.2
Davao	: 467	: 78	: 99	: 73	: 31	: 3	: 1	: 285	: 61.0
TOTAL	: 2345	:447	:499	:312	:102	: 21	: 7	: 1388	: 59.1
Manila *	: 27	: 9	: 11	: 1	: 3	: :	: 1 : 1	: 26	: 96.2

* Sera from patients confined for other diseases

REPORT FROM DEPARTMENT OF MICROBIOLOGY

KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

We previously reported that cultures of a human leukemic leucocyte line(J-111) supported growth of DEN-1 virus (Information Exchange, No. 30, p. 91, March 1976). Subsequently we examined the infected J-111 cells by electron microscopy.

The J-111 cells (provided by the Flow Laboratories, U. S. A. and the Dainippon Pharmaceutical Co., Ltd., Japan) were cultivated in bottles and infected with DEN-1 virus Mochizuki strain in the form of infected mouse brain homogenate. At intervals after infection the culture fluids were taken to determine their infective titers by plaque assays on BHK-21 cell monolayers. The maximum titers, ca. 10^6 PFU/ml, were usually obtained 7 to 10 days after the beginning of incubation at 37C, which were maintained for further few weeks, forming a plateau. The cells harvested during the logarithmic or stationary stage of virus infection were washed with phosphate buffer saline, double-fixed with glutaraldehyde and osmium tetroxide, embedded in epon, thin-sectioned and examined with an electron microscope. The BHK-21 cells were similarly examined for comparison.

Significant pictures revealed in the infected J-111 cells were: (i) Emergence of mature virions in cisternae of the endoplasmic reticulum and in cytoplasmic vesicles; (ii) formation of lamellar and crystalloid structures; (iii) appearance of "nucleoid" particles in the cytoplasm; and (iv) "budding" of viral particles from the surface and/or internal membranes.

Some of these images were essentially the same as those reported by previous investigators dealing with DEN-infected cultured cells of various kinds, such as Vero, BHK, LLCMK2, Raji and related human lymphoblastoid cells, as well as infected mouse brain cells. It was noted, at the same

time, that the changes revealed in J-111 cells were much more marked in amount and degree than those in the other cells. Regarded as particularly significant was visualization of "budding" particles from the cell surface membrane. The infected BHK-21 cells exhibited no budding picture. In the past literature the budding phenomenon as above-mentioned has been observed generally in cells infected with alphaviruses, but rarely in the same cells infected with flaviviruses. In our studies we found approximately 40% of the examined J-111 cells having particles budding from the cell surface membrane.

In hemadsorption experiments carried out in parallel, it was shown that numbers of erythrocytes adsorbed onto infected J-111 cells were significantly more than those onto similarly infected BHK cells.

The J-111 cells, when infected with DEN-1 virus, revealed the pictures generally seen in flavivirus-infected cells, on the one hand, and also some of those characteristic of alphavirus-infected cells, on the other. This culture system is perhaps a unique one for studying the biology of dengue viruses.

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(S.Hotta)

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE
RESEARCH INSTITUTE FOR MICROBIAL DISEASES, OSAKA UNIVERSITY

1. A rapid titration of dengue virus infectivity by immunoperoxidase technique

In our laboratory, the indirect fluorescent antibody (IFA) technique has been employed for the rapid titration of dengue viruses by counting fluorescent foci. We now apply the peroxidase-anti-peroxidase (PAP) technique which offers several advantages.

BHK21 cells on Lab-Tek 8 chamber tissue culture slides (Miles Ill. U.S.A.) were inoculated with serially diluted dengue viruses. After 2 hours adsorption cells were incubated for 2 days (type 2 and 4) or 3 days (type 1 and 3) in a CO₂-incubator at 37°C. The cells were fixed with acetone (4°C, 20 min) and were stained with three antisera: anti-dengue rabbit serum, anti-rabbit IgG sheep serum, and PAP complex (peroxidase combined with antiperoxidase rabbit IgG), in turn for 40 min each. Following peroxidase reaction, cells were dehydrated with ethanol series, cleared in xylene, and sealed with Canada balsam. Foci were counted under an ordinary microscope.

The focus counting by PAP technique in a bright field is easier and more accurate than the IFA method. Moreover, the PAP specimens can be preserved without change for delayed observation. This technique will be applicable for the serological survey of dengue virus neutralizing antibodies.

2. Development of dengue virus type 4 in BHK21 cells

The development of dengue virus type 4 (H241) within cultured BHK21 cells has been followed by electron microscopy. Virus morphogenesis was found to occur in close association with vacuolar structures which developed after virus infection. Matured virions appeared in these vacuoles after marked vacuolization of the cytoplasm. Budding of virions was observed in some of these vacuolar membranes. A new type of vesicle was observed which was massively associated with a marginal rosary-like arrangement of electron-dense particles. It is suggested that this type of vesicle is related to virus production, and was named "rosary body".

(Akira Igarashi)

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REPORT FROM THE DEPARTMENT OF VIROLOGY AND RICKETTSIOLOGY
NATIONAL INSTITUTE OF HEALTH, TOKYO, JAPAN

Human Japanese Encephalitis Cases in Japan in 1976

A surveillance study of human Japanese encephalitis (JE) cases was carried out in 1976 with the cooperation of the Ministry of Health and Welfare and the local government.

Thirteen cases including 9 deaths were diagnosed as JE by the survey team. Eight of the cases which included 4 of the deaths were confirmed serologically, and 5 deaths were thought to be presumptive JE cases considering clinical and epidemiological findings.

The first case was noted on 15 July and the last on 17 September. Among the above 13, 4 cases were male and 9 female. Age distribution of those cases are: 10-19 years, 1; 30-39 years, 3; 40-49 years, 2; 60-69 years, 3; and more than 70, 4.

All cases were from the western part of Japan, showing geographical deviation of the incidence. In fact, more than half of the cases, i.e., 8 (61.5%), were from Kyushu Island.

(Akira Oya)

REPORT FROM THE INSTITUTE OF POLIOMYELITIS
AND
VIRAL ENCEPHALITIS, MOSCOW

Immunodepressive effect of Tahina virus in mixed arbovirus infection

Tahina virus belonging to the Togoviridae family, when introduced extraneurally into adult mice, causes an inapparent infection accompanied by transitory disturbances in immune response to heterologous antibodies (Hannoun, 1972; Vargin, Semenov, 1976).

We studied the immunodepressive effect of Tahina virus in mixed arbovirus infection.

In our work we used white mice weighing 18-20 g. Tahina virus (adapted to tissue culture) was introduced intraperitoneally in a volume of 0.3 ml containing a dose of 10,000 LD₅₀. On the 4th day after receiving Tahina virus the mice were injected with Langat virus (strain TP-21), tick-borne encephalitis virus (strain Sofyin), or West Nile virus (strain B-956), as indicated in Table 1.

In all cases brain suspension obtained from suckling mice inoculated with the corresponding infective agent and showing the symptoms of the disease was used as virus-containing infective matter. The method of intracerebral inoculation of suckling mice was also used for the titration of viruses.

The data in Table 1 indicate that the mice inoculated with Tahina virus showed a change in sensitivity to the heterologous infective agents: Langat virus and tick-borne encephalitis virus.

The intraperitoneal injection of Langat virus into mice caused mostly asymptomatic infection. Morbidity rate was 8.5%. In the animals previously inoculated with Tahina virus the injection of Langat virus caused encephalitis with morbidity rate of 42.4%.

The outcome of infection caused by the intracerebral injection of Langat virus was the same both in the control mice and in the mice previously inoculated with Tahina virus. Morbidity rate in these groups was 85% and 88% respectively. Still the intensity of the infective process as manifested by the mean survival time was considerably lower in the presence of inapparent infection caused by Tahina virus.

In experiments with the causative agent of vernal tick-borne encephalitis the mice previously inoculated with Tahina virus also showed an increased sensitivity to heterologous virus. The subcutaneous injection of tick-borne encephalitis virus into mice in a dose of 5 LD₅₀ did not cause clinically pronounced disease. When tick-borne encephalitis virus was introduced following the inoculation with Tahina virus, 10% of the mice died.

It should be pointed out that in the presence of Tahina virus infection the immune response to West Nile virus in the mice remained unchanged as compared to the immune response in the control mice. Thus, the titre of antihemagglutinating antibodies to West Nile virus was the same in both groups of mice on day 8 from the date of the intraperitoneal injection of West Nile virus, as well as on day 20.

Thus, the mice inoculated with Tahina virus show an increased sensitivity to Langat virus or tick-borne encephalitis virus introduced extraneurally. Considering the wide spread of this virus in many regions of the U.S.S.R., Czechoslovakia, Austria and other European countries (Kunz, 1974) endemic for tick-borne encephalitis, the asymptomatic infection caused by Tahina virus may be supposed to induce a higher susceptibility of humans to the subsequent infection caused by tick-borne encephalitis virus.

TABLE 1

INFLUENCE OF TAHINA VIRUS ON IMMUNE RESPONSE TO WEST NILE VIRUS IN WHITE MICE AND ON THEIR SENSITIVITY TO LANGAT VIRUS AND TICK-BORNE ENCEPHALITIS VIRUS

Experiment No.	Viruses and route of inoculation*	Dose	Number of mice in the group	Mortality rate in %	MST**	Antihæmagglutinins***	
						Day 8	Day 20
1	Langat (i.p.)	10 ⁴ LD ₅₀	55	8.5			
	Tahina (i.p.) + Langat (i.p.)	10 ⁴ LD ₅₀	54	42.4****			
2	Langat (i.c.)	10 ⁴ LD ₅₀	28	85.2	8.5		
	Tahina (i.p.) + Langat (i.c.)	10 ⁴ LD ₅₀	36	88.4	11.1****		
3	Tick-borne encephalitis (s.c.)	5 LD ₅₀	20	0			
		50 LD ₅₀	20	10			
		500 LD ₅₀	20	45			
	Tahina (i.p.) + tick-borne encephalitis (s.c.)	5 LD ₅₀	20	10			
		50 LD ₅₀	20	15			
		500 LD ₅₀	20	45			
4	West Nile (i.p.)	10 ⁴ LD ₅₀	20			24.5	62
	Tahina (i.p.) + West Nile (i.p.)	10 ⁴ LD ₅₀	20			53	63

* Tahina virus was injected in a dose of 10⁴ LD₅₀ four days before the inoculation of the infective agents under test.

Routes inoculation: intraperitoneal (i.p.), intracerebral (i.c.), subcutaneous (s.c.).

** Mean survival time.

*** The reverse of the titre value.

**** $p < 0.05$

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Preparation of immune sera to Semliki Forest virus subcomponents.

The advances in research on arbovirus virion proteins open the possibilities of differentiated exploration of their antigenic properties. The difficulty of preparing sera to individual antigens for classification of arboviruses and identification of new strains restricts the wide spread of this method, for it requires large quantities of highly purified virus. The present study was aimed at the development of a method for preparation of antisera to envelope and nucleocapsid subunits of partly purified Semliki Forest virus (SFV) which would be available for routine studies. Two strains of SFV - Zaisan 260, isolated in the USSR, and R 16225, were used.

The virus which had undergone over 5 passages in chick embryo fibroblasts (CEF) culture with the growth medium containing rabbit serum was propagated in this culture in serum-free medium with the addition of ^3H -uridine and ^{14}C -protein hydrolysate, concentrated at 80,000 x g, disrupted with nonidet P-40, separated into subunits in 15-30% linear sucrose gradient. Fractions of the envelope and the nucleocapsid were collected on the basis of localization of ^3H and ^{14}C labels.

The rabbits were immunized with formaline inactivated material by injections into popliteal lymph nodes and re-immunized intravenously, each time with 75-100 μg of protein. Immunological methods were used to prevent the formation of antibodies in rabbits to virus host components. For this purpose the virus was propagated in CEF culture with rabbit serum and had been passaged before use at least 5 times in CEF culture. So, the material used for

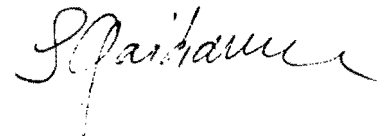
immunization of rabbits contained, in addition to the virus, only one extraneous component - chick fibroblasts. The antibody to the chick antigens were eliminated from the sera by adsorption with chick embryo cells.

The presence of specific antiviral antibody was measured by NT, HI and CF tests. All the tests included permanent controls for the absence of antibrain and antichicken antibody in the immune sera by the precipitation test.

In the HI and CF tests, sucrose-acetone mouse brain antigens, prepared from the appropriate strains and containing the whole set of virus antigens, were used. For titration of neutralizing antibodies plaque reduction test on CEF culture was employed.

As is seen in the table, sera from the rabbits immunized with virion envelopes contained all kinds of antibody in titres almost equal to those in antivirion sera. As a rule, nucleocapsid antisera did not neutralize the infectious virus, did not react in HI . . . Antinucleocapsid sera 122 and less so 123 had partial virus-neutralizing and weak hemagglutinating activity. Some authors also mentioned slight neutralizing activity of antinucleocapsid serum. We can conclude, that for routine purposes it is possible to prepare immune serum predominantly reacting with envelope or nucleocapsid components, avoiding the complex system of virus purification for immunization.

(S.Ya.Gaidamovich, E.E.Melnikova, Z.N.Krasnobajeva).



Serological tests with sera from rabbits immunized with
envelope and nucleocapsid antigens and intact virions
of Zaisan 260 and R 16225 strains

Antisera to strain	Serum No.	No. of re-immunization *)	Antigen for immunization	Serological tests		
				NT	HI	CFT
Zaisan 260	112	1	Virion	2560**)	2560	160
	114	1	Virion (no formalin)	> 5120	2560	640
	133	1	Virion	1280	40	40
	135	2	Envelope	320	640	160
	138	2	Envelope	> 2560	1280	80
	115	1	Nucleocapsid	8	0	40
	122	1	Nucleocapsid	32	10	80
	123	1	Nucleocapsid	8	10	160
	136	2	Nucleocapsid	< 8	0	320
	137	3	Nucleocapsid	< 8	0	80
R 16225	126	1	Virion	2560	320	80
	127	1	Virion	> 2560	640	320
	128	1	Virion	5120	160	80
	131	1	Envelope	> 2560	640	160
	132	2	Envelope	640	80	80
	129	1	Nucleocapsid	< 8	0	80
	130	3	Nucleocapsid	< 8	0	40

Notes: *) Number of booster injections after primary immunization into the popliteal lymph node.

**) Reciprocal of serum dilution.

Semliki Forest virus

1. Sites for the initiation of translation on SFV RNAs

In cells infected with Semliki Forest virus there are two major RNAs species, the genomic 42S RNA and the intracellular 26S RNA, which is a replica of the 3' third of the 42S RNA. Since both RNAs can serve as messengers it follows that there must be at least 2 sites for initiation of translation on the 42S RNA. To determine how many sites there are, we analyzed the polypeptides labelled with formyl (³⁵S)methionyl-tRNA_f^{Met} in a cell-free protein synthesising system derived from wheat germ. Formyl methionine can only be incorporated into the N-terminal of polypeptides, and, being nonphysiological in eukaryotic systems, is not cleaved from the polypeptide chain. Rounds of translation are not always completed in cell-free systems and so the reaction products were digested with trypsin and pronase. A single labelled tryptic peptide was detected in the 42S RNA programmed reaction and this was different from that in the reaction programmed with 26S RNA. Digestion of the 42S RNA directed product with pronase gave rise to the dipeptide fMetAla, whereas fMetAsN was detected in the 26S RNA directed reaction products.

The results indicate that, in the cell-free system, translation of the viral genomic 42S RNA starts from one initiation site only, presumably to give rise to the nonstructural proteins of the virus. Internal initiation, on the site equivalent to that on the 26S RNA (translation of which yields virion structural proteins) did not occur.

(N. Glanville, M. Ranki, J. Morser, L. Kääriäinen and A.E. Smith, Proceedings of the National Academy of Science, U.S.A., in press.)

2. The nonstructural proteins of SFV

Difficulties have been experienced in identifying the nonstructural proteins of Semliki Forest virus, since they are synthesised in only small

amounts late in infection, when host cell protein synthesis is shut off. Using a temperature sensitive mutant and the hypertonic initiation block to synchronise protein synthesis, two large proteins (ns 155 and ns 135) could be detected, as could four smaller, longer-lived proteins (ns 70, ns 86, ns 72 and ns 60). The kinetics of labelling of these proteins suggested that they were translated sequentially, and a precursor product relationship between them was proposed (Lachmi and Kääriäinen, Proc. Natl. Acad. Sci., U.S.A. 73,1936-1940).

Tryptic peptide mapping of these proteins showed that ns 70 and ns 86 contained different subsets of the peptides of ns 155, while ns 72 contained some of the peptides of ns 135. Ns 155 and ns 135 were also found to be distinct entities. These results confirm the hypothesis advanced previously. To prove that the proteins were virus coded, the 42 S RNA genome from the wild type virus was translated in a cell-free protein synthesising system. The product contained all the tryptic peptides of ns 155 and some of the peptides of ns 135.

Together the nonstructural proteins ns 155 and ns 135 and the structural proteins of the virion (total molecular weight about 420,000 daltons) most probably account for the full coding capacity of the 42 S RNA genome (molecular weight 4.5×10^6) of Semliki Forest virus.

(B. Lachmi, N. Glanville, A.E. Smith and L. Kääriäinen.)

3. Ribonucleoproteins in SFV infected cells

The virus-specific ribonucleoproteins in the cytoplasm of SFV infected HeLa cells have been studied. The capsid (C) proteins, labelled with short (^{35}S)methionine pulses, was found in three structures, sedimenting at 140 S, 100 S and 60 S, when the polysomes had been dissociated with EDTA and the membranes with Triton X-100. The 140 S structure corresponded to the nucleocapsid, and the 100 S was also a complex between the C-protein and the viral genome (42 S RNA). The 60 S material was found to be the large ribosomal subunit carrying the C-protein. At present we do not know whether this association affects the translational capacity of the ribosome, but pulse-chase experiments suggest that the C-protein is transferred to the nucleocapsid relatively fast.

The viral 26 S RNA sediments as a ribonucleoprotein at about 55 S but does not bind viral proteins. This suggests that the 42 S RNA contains a specific region which is recognized by the C-protein in the assembly of the nucleocapsid.

(I. Ulmanen, H. Söderlund and L. Kääriäinen, J. Virol., in press)

4. RNA synthesis of SFV ts-mutants.

RNA synthesis was studied in cells infected with temperature-sensitive mutants of Semliki Forest virus (SFV) at the restrictive temperature. The RNA synthesis of the mutant ts-4 (2 to 4 % compared to wild type) did not enter the typical exponential phase 2-3 h after the infection, and the viral 26 S RNA was not synthesized at all. The data suggest that the residual RNA synthesis of this mutant represents a very early transcriptional event while later steps are impaired by the mutation.

Another mutant, ts-11 also classified as RNA negative, interfered with the replication of wild type SFV. This mutant caused a transient inhibition of wild type RNA synthesis resulting in delayed and reduced production of infectious virus. The interfering activity was retained in the ts-11 preparation through 3 successive plaque to plaque clonings. The inhibition of RNA synthesis could be demonstrated by superinfecting the wild type infected cells with the mutant as late as 4 h postinfection. Neither the RNA nor protein synthesis of ts-11 are required for the interference, which might indicate that a structural component of the mutant virion is involved.

(S. Keränen and J. Saraste)

Uukuniemi virus

The RNA polymerase is a component of the virion ribonucleoproteins:

The Uukuniemi virus RNA polymerase is shown to be associated with the viral ribonucleoprotein (RNP). Purification of the RNP free of the envelope components resulted in altered properties of the polymerase: Mg^{++} was required for optimum activity as well as Mn^{++} . When using unfractionated

disrupted virus Mn^{++} alone was sufficient for polymerase activity. In spite of the apparent purification the RNA copied in vitro represented only a minor part of the template RNA.

Separation of the three virion RNPs (L-, M- and S-RNP) showed that each of them contained polymerase activity. Consequently each of the three RNA molecules in the virus functioned as templates for RNA transcription suggesting that the RNA pieces in the virion (L-, M- and S-RNA) are composed of non overlapping nucleotide sequences.

(M. Ranki and R. Pettersson)

(Received October 22, 1976 - Editor)

Isolation of Bhanja Virus from Haemaphysalis Ticks in Bulgaria

An investigation was undertaken by the Bulgarian Academy of Agriculture and Veterinary Science and the Institute of Parasitology of the Czechoslovak Academy of Sciences to elucidate if a disease of sheep with symptoms of CNS involvement is not a disease of natural focal character. Ticks were collected from sheep in the Ahtopol district, southern Bulgaria in October 1974. After being placed alive in polyethylene ampoules the ticks were sealed and transported in liquid nitrogen to Czechoslovakia.

Suspensions were prepared in PBS with 0,75% bovalbumine and antibiotics in a laboratory where no ticks were formerly virologically tested. Isolation experiments were performed on 1-3 days old suckling SPF white mice. 73 pools of ticks were tested (Table 1.) and two virus strains were isolated.

Table 1. Ticks collected in Bulgaria and virologically tested

Ticks species	Nymphs	Males	Females	Total	M.I.R. (Bhanja virus)
<i>Dermacentor marginatus</i>	-	-	1/1 *	1/1	0.0
<i>Ixodes ricinus</i>	-	-	2/5	2/5	0.0
<i>Haemaphysalis inermis</i>	3/44	-	2/3	5/47	0.0
<i>H. punctata</i>	5/27	14/253	35/167	54/447	2.2
<i>H. sulcata</i>	1/5	3/22	3/23	7/50	20.0
<i>Rhipicephalus bursa</i>	3/54	1/1	-	4/55	0.0
Totals	12/130	18/276	43/199	73/605	

*) Number of pools in numerator, number of individual specimens in denominator

**) Minimal infection rate per 1000 individuals

One strain (Bg 326) was obtained from a pool of 6 engorged females of *Haemaphysalis punctata*, another strain (Bg 335/336) from a suspension of 18 males of *Haemaphysalis sulcata*. These ticks were collected from sheep in the locality Achtos on the 21 October 1974. The suspensions (bacteriologically sterile) were prepared and inoculated on the 8th and 20th November 1974, respectively.

The original suspension Bg 326 killed 1 out of 10 suckling mice on the 14 th day after i.c. inoculation (0.02 ml). In the next i.c. passage 6 out of 9 inoculated suckling mice died 8-12 days after their inoculation. In the following passage, all 10 i.c. infected mice died on the 6 th day under marked signs of CNS involvement.

The original suspension Bg 335/336 killed 4 out of 9 mice between the 10th and 14th day after i.c.inoculation. In the next i.c. passage 7 mice out of 8 were dead between the 8th and 11th day. In the following passage all i.c. infected mice were dead between the 4th and 6th day.

Reisolation experiment from the original suspension Bg 326 was not performed since no virus isolation experiments were performed from ticks in this laboratory in the past. The original suspension Bg 335/336 killed in a reisolation experiment 2 out of 17 mice on the 16th day specifically.

Both isolated strains were ether sensitive, non pathogenic for adult white mice after i.pt. inoculation . Both strains were not neutralized with an immune canine resp.rabbit anti tick-borne encephalitis virus serum (supplied by Dr.M.Grešiková from the Institute of Virology, Bratislava) in an i.c. virus neutralization test in suckling white mice. Against these isolates, we have immediately prepared immune sera in rabbits.

Since in Italy and Yugoslavia Bhanja virus was isolated from Haemaphysalis ticks, we have requested Dr. P. Verani from Istituto Superiore di Sanità in Roma to send us this virus. The strain ISS.IR.205 of Bhanja virus was obtained on the 30th June, 1975 together with immune ascitic fluid. Our immune anti-Bg-326 and anti-Bg 335/336 rabbit sera inhibited in a HIT 4 haemagglutinating units of the Italian strain to titers 1:160 and 1:40, respectively, but the Italian antigen (Bhanja virus) was not inhibited by poly-A (Alfavirus) and poly-B (Flavivirus) immune mouse ascitic fluids (supplied by prof. S.Y.Gaidamovich, Ivanovský's Institute of Virology, Moskva) and anti-Ťahyňa immune mouse serum. On November 20th, 1975 we sent both Bulgarian strains to Dr. J. Casals from the Yale Arbovirus Research Unit, New Haven, U.S.A. with a request to identify both isolated strains. Dr.J.Casals informed us that "there is hardly any difference by complement-fixation test between both strains and prototype Bhanja virus".

The isolation of Bhanja virus in Bulgaria brought evidence that natural foci of this virus exist not only in Italy and Yugoslavia, but also in this south-eastern European country.

(P.Pavlov, B.Rosický, M.Daniel, Z.Hubálek, V.Bárdoš)

A mixed natural focus of tick-borne encephalitis and Uukuniemi viruses in southern Moravia (Czechoslovakia)

In the region of southern Moravia cases of tick-borne encephalitis were diagnosed in the past. Virus isolation experiments from ticks were undertaken in June 1976 to find out if a natural focus of tick-borne encephalitis is not present in

this part of the country.

A total of 1837 specimens (larvae, nymphs and adults) of the tick *Ixodes ricinus* was collected on grass. 86 pools were prepared (PBS with 0.75% of bovalbumin and antibiotics was used) and inoculated i.c. into 1-3 day old suckling white mice.(Table 2.).

Table 2. Ticks collected in southern Moravia and virologically tested.

I.ricinus	Specimens	Pools	Pools positive		M.I.R. ^{*)}	
			TBE	Uukuniemi	TBE	Uukuniemi
Larvae	100	2	0	0	0.00	0.00
Nymps	1500	60	1	3	0.67	2.00
Males	122	12	0	2	0.00	16.39
Females	115	12	1	1	8.70	8.70
Adults in total	237	24	1	3	4.22	12.66
T o t a l s	1837	86	2	6	1.09	3.27

*) Minimal infection rate per 1 000 individuals

Eight strains were isolated. Two were diagnosed as tick-borne encephalitis virus. Six other original suspensions killed 1 to 6 mice out of 6-10 i.c. inoculated suckling white mice in 8 to 17 days. In the next passages, all 6 strains killed the i.c. inoculated suckling mice between the 5th and 15th day after inoculation, and in the following passages, the suckling mice were killed in 5 to 11 days after infection. These six strains did not kill i.c. inoculated adult white mice. An identification complement-fixation test was performed with immune mouse ascitic fluids supplied by Prof.Dr.S.Y. Gaidemovich from the Ivanovsky's institute of Virology, Moskva - Table 3.

Table 3. CF identification tests of the non-TBE virus strain "417"
 (a crude antigen of the strain "417" was used)

Immune sera (mouse)	"417"					Ťahyňa "92"
Immune ascitic fluids (mouse)		TBE	Kemerovo	Uukuniemi -Sumakh	Tribeč	
CF titers (serum/antigen)	32/64	0/0	0/0	64/16	0/0	0/0

Only Uukuniemi-Sumakh immune ascitic fluid gave positive results.

Table 4. CF tests with crude antigen "417" and immune mouse
 sera of the other five isolated non TBE viruses.

Immune mouse sera	"356"	"379"	"393"	"408"	"409"	"417"
CF titers	64/128	512/128	64/32	128/64	256/32	32/64

All tested strains were identified as Uukuniemi viruses.

Both strains of TBE virus were reisolated from the original suspensions, and also all Uukuniemi virus strains (except the strain "356") were recovered from original suspensions. No work with Uukuniemi virus was performed in our laboratory in the past.

On the strength of the results of these isolation experiments it can be stated that the investigated area of southern Moravia is a mixed natural focus of tick-borne encephalitis and Uukuniemi viruses.

(Z.Hubálek, J. Ryba, V.Bárdoš, Z.Juřicová)

REPORT FROM THE DEPARTMENT OF ECOLOGY OF ARBOVIRUSES,
INSTITUTE OF VIROLOGY, SLOVAK ACADEMY OF SCIENCES,
BRATISLAVA, CZECHOSLOVAKIA.

Serological survey on birds sera with some arboviruses.

One hundred and seventy seven sera from 15 species of birds collected in Austria by Dr. W. Sixl from the Institute of Hygiene, University of Graz, were send to WHO Collaborating Centre for Arbovirus Reference and Research in Bratislava for serological examination. Experiments were carried out with following antigens: Sindbis virus /group A/, tick-borne encephalitis and West Nile /group B/, Bujaru and Karimabad /Phlebotomus fever group/, and Quaranfil /Quaranfil group/ of arboviruses. The sera extracted by acetone and adsorbed on the concentrated erythrocytes were centrifuged and diluted in borate buffer solution /pH 9,0/ containing 0,4 % bovine albumin. The haemagglutination-inhibition /HI/ tests were carried out by the method of Clarke and Casals using microtitrator.

The results of HI tests are presented in Table 1. No antibodies to Sindbis, tick-borne encephalitis and West Nile viruses could be detected.

HI antibodies to Phlebotomus fever group were detected in 10 sera belonging to 6 species of birds.

HI antibodies to Quaranfil virus were detected in 22 sera belonging to 7 species of birds. The titres generally

varied from 1:20 to 1:40 /Table 2/.

HI antibodies to A and B group of arboviruses in birds sera have been detected in 1962 in Bulgaria. The presence of Sindbis, tick-borne encephalitis and West Nile viruses in birds in Czechoslovakia have been demonstrated by virus isolation experiments. As follows from our serological survey, no antibodies to A and B group of arboviruses could be detected. Isolation of Sindbis and tick-borne encephalitis and West Nile viruses however confirmed that birds acts as hosts of these viruses in Central Europe.

Only little attention has been payed so far to the importance of the viruses of the Phlebotomus fever and Quaranfil groups of arboviruses in Central Europe. The occurrence of HI antibodies in birds sera against Phlebotomus fever and Quaranfil groups are therefore of interest. It is not clear yet whether those viruses are circulating in Central Europe or whether migrating birds may introduce these viruses from Africa to Central Europe. The circulation of these viruses depends on the presence of suitable vectors and hosts.

The isolation of arboviruses from the Phlebotomus and Quaranfil group have been not reported in Central Europe. Because the specificity of HI reactions could not be proved, the question concerning the presence of these viruses in Central Europe is still open.

/ M. Grešíková, W. Sixl, D. Štünzer., V. Bieliková./

Haemagglutination-inhibiting antibodies to some arboviruses
in sera from birds collected in Austria.

Species of bird	No of positive sera / No of sera tested with antigens					
	Sindbis	Tick-borne encephalitis	West Nile	Bujaru	Kari- mabad	Quaranfil
<i>Acrocephalus arundinaceus</i>	0/18	0/18	0/18	0/18	0/18	3/18
<i>Acrocephalus scirpaceus</i>	0/5	0/5	0/5	0/5	0/5	2/5
<i>Acrocephalus schoenebeuus</i>	0/11	0/11	0/11	0/11	0/11	1/11
<i>Carduelis spinus</i>	0/1	0/1	0/1	0/1	0/1	0/1
<i>Emberiza citrinella</i>	0/2	0/2	0/2	1/2	0/2	0/2
<i>Emberiza schoeniclus</i>	0/27	0/27	0/27	5/27	1/27	4/27
<i>Erythacus rubecula</i>	0/4	0/4	0/4	0/4	0/4	1/4
<i>Ixobrychus minutus</i>	0/8	0/8	0/8	1/8	0/8	0/8
<i>Lanius collurio</i>	0/5	0/5	0/5	0/5	0/5	0/5
<i>Motacilla alba</i>	0/1	0/1	0/1	0/1	0/1	0/1
<i>Motacilla cinerea</i>	0/5	0/5	0/5	1/5	0/5	0/5
<i>Parus major</i>	0/45	0/45	0/45	1/4	0/45	7/45
<i>Parus caerulus</i>	0/3	0/3	0/3	0/3	0/3	0/3
<i>Passer montanus</i>	0/37	0/37	0/37	0/37	0/37	4/37
<i>Sylvia atricapilla</i>	0/5	0/5	0/5	0/5	0/5	0/5

Haemagglutination - inhibiting /HI/ titres against Bujaru,
Karimabad and Quaranfil antigens in bird's sera.

Bird species	HI titres with antigens		
	Bujaru	Karimabad	Quaranfil
Acrocephalus arudinaceus	1 x 1:20		2 x 1:20 1 x 1:40
Acrocephalus scirpaceus			2 x 1:20
Acrocephalus schoenebenus			1 x 1:20
Emberiza citrinella	1 x 1:20		
Emberiza schoeniclus	5 x 1:20	1 x 1:40	4 x 1:20
Erythacus rubecula			1 x 1:20
Ixobrychus minutus	1 x 1:20		
Motacilla cinerea	1 x 1:40		
Parus major	1 x 1:20		7 x 1:20
Passer montanus			4 x 1:20

In October 1976, in collaboration with the Forest Research Institute, Budapest and the institute of Virology, Slovak Academy of Sciences, Bratislava, investigation of a TBE natural focus was performed in the Northern Intermediate Mountains of Hungary. 176 I.ricinus ticks were collected from the vegetation and 70 wild vertebrates /rodents and insectivores/ were trapped alive.

1. Virological studies

1.1. Ticks

Ticks were homogenized and processed in 11 pools. One TBE virus strain was isolated in intracerebrally inoculated suckling mice.

1.2. Wild vertebrates

1.2.1. Heparinized blood samples of the small mammals were inoculated into suckling mice. One TBE virus strain was isolated from an A.sylvaticus.

1.2.2. From the pooled organs of an A.flavicollis, an arbovirus strain lethal for suckling mice, was isolated. Identification of this strain is still in progress.

2. Serological studies

2.1. Wild vertebrates

Serum samples of two A.flavicollis inhibited the HA activity of the SIN and one of them, that of the TBE antigen.

2.2. Forest workers

Four serum samples of 300 forest workers in this area, taken in January 1976, showed HI antibodies against SIN and ten serum samples, against TBE antigen.

These data show alphavirus circulation and confirm TBE virus activity in the area.

/E.Molnár, M.Sztankay, K.Gerzsenyi, J.Kosek/

REPORT FROM ARBOVIRUS UNIT, VIRUS DEPARTMENT

ISTITUTO SUPERIORE DI SANITA', ROCCO, ITALY

Experimental infection of monkeys with a SFN-like virus (ISS.Phl.3 strain).

Preliminary studies have been conducted on the response of monkeys to primary infection with a SFN-like virus (see Arbo Info Exchange n°26 p.49, March 1974) originally isolated from Phlebotomus perniciosus collected in a central Italian region (Toscana) in 1971. The virus strain used, designated ISS.Phl.3, is representative of three isolates. By HI, CF, immunodiffusion and NT tests, these strains are closely related to SFN virus but differ in their biological characteristics. In particular, they are more pathogenic both for laboratory animals and for tissue cultures than the prototype SFN strain (Sabin).

In order to better define the pathogenicity of the SFN-like virus, experimental infection of cynomolgus (M.fascicularis) was performed. A virus suspension with a titre of $10^{6.6}$ LD₅₀/0.01 ml, as determined in suckling mice, was used. A total of six monkeys were inoculated. Two monkeys were inoculated intrathalamically (i.t.) with 0.5 ml per thalamus, one monkey intraspinally (i.s.) with 0.1 ml, two monkeys intraperitoneally (i.p.) with 2 ml and one monkey subcutaneously (s.c.) with 2 ml of virus suspension. For comparison a monkey was inoculated intrathalamically with 0.5 ml per thalamus of a SFN virus suspension (Sabin strain) with a titre of $10^{6.3}$ LD₅₀/0.01 ml, as determined in suckling mice.

Clinical signs, rectal temperature, viremia, antibodies in the blood, and virus in the CNS and in the viscera were followed. Signs of illness as mucal rigidity, severe ataxia, adynamia, tremors, spastic contractions and lethargy occurred in all monkeys inoculated with ISS.Phl.3 virus into the CNS. They were sacrificed when moribund (Table 1). One monkey inoculated i.p. developed signs of illness and was sacrificed on day 18 postinfection (p.i.) when moribund. The other monkey inoculated i.p. and the monkey inoculated s.c. remained normal during the duration of the experiment and were sacrificed on day 24 p.i.

The monkey inoculated i.t. with SFN virus (Sabin strain) showed no signs of illness except it developed a transient fever on day 15 p.i. which later subsided. It was

sacrificed on day 24 p.i. in apparently healthy conditions.

Transient viremia was detected only in two monkeys. Antibody response (detected by HI test) could be shown only in those animals that had received the virus by a peripheral route.

The levels of infectious virus in various parts of the CNS and in the viscera was determined in suckling mice (Table 2). The virus was recovered from all parts of the CNS of the monkeys inoculated both i.t. and i.s. with ISS.Ph1.3 virus. It seems that whatever the route of inoculation of the virus (thalamus or spinal cord), it could spread along the CNS reaching similar levels of infectious virus. Virus was found in the cerebellum of the i.p. inoculated monkey which showed also viremia and signs of illness. Only the i.t. inoculated monkey with detectable viremia had virus in the viscera. No virus was recovered from the monkey inoculated i.t. with SFN virus (Sabin strain).

The histopathologically data about these monkeys are not yet available.

These results again indicate a marked difference in pathogenicity between SFN-like virus (ISS.Ph1.3 strain) and SFN virus (Sabin strain), which is known to be non-pathogenic for the majority of laboratory animals.

P.Verani, M.C.Lopes and M.Baldacci

Table 1 - Response of monkeys to inoculation with a SFM-like virus (ISS.Phl.3).

Monkey examined	Route of inoculation	Fever	Viremia	Signs of disease	Onset of disease (day)	Day of killing	Antibody production
n° 1	i.t.	+	+	+	6	8	+
n° 2	i.t.	+	+	+	7	9	+
n° 3	i.s.	+	+	+	7	10	+
n° 4	i.p.	-	+	+	16	18	+
n° 5	i.p.	-	+	-	-	24	+
n° 6	s.c.	-	+	-	-	24	+
n° 7 (Sabin strain)	i.t.	+	-	-	-	24	-

Table 2 - Levels of infectious virus in tissues from monkeys after inoculation with a SFN-like virus (ISS.Ph1.3).

Monkey examined	Route of inoculation	Frontal lobe	Thalamus	Cerebellum	Medulla oblongata	Cervical cord	Lumbar cord	Liver	Spleen	Kidney	Lung
n° 1	i.t.	5.1 ⁺	n.d.	6.9	6.4	5.5	6.1	neg.	1.5	3.1	2.3
n° 2	i.t.	2.4	2.2	3.7	2.9	3.0	3.7	neg.	neg.	neg.	neg.
n° 3	i.s.	3.8	5.5	5.4	n.d.	4.5	4.5	neg.	neg.	neg.	neg.
n° 4	i.p.	neg.	n.d.	2.0	neg.	neg.	neg.	neg.	neg.	neg.	neg.
n° 5	i.p.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
n° 6	s.o.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
n° 7 (Sabin strain)	i.t.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.

+ log LD₅₀ per 0.01 ml 20% tissue suspension

n.d. not done

Isolation of a Marburg-like virus from a case of haemorrhagic fever
in Zaire

Patient E.L., 42 years old, started her disease on September 23, 1976 in Yambuku, Equateur Province, Zaire. She was transported by air on September 25, to Kinshasa, where gradually a haemorrhagic syndrome developed.

Clotted blood taken at the 5th day of illness of patient M.E. hospitalized in Kinshasa (Zaire) was sent on ice to the Institute of Tropical Medecin, Antwerp. The sample arrived in the evening of September 29th and was kept in the refrigerator.

The next morning serum was inoculated into 6 young adult mice by intracerebral and intraperitoneal routes, into 2 litters of newborn mice intracerebrally and into 10 tubes of VERO cell cultures (grown in medium 199 containing 7.5% calf serum).

The serum was tested in the complement fixation test for Lassa virus antibodies (result negative) and in a neutralization test on VERO cells for antibodies against yellow fever virus : antibodies were present at a 1/30 dilution.

Results of inoculations were as follow :

Mice : one animal was found dead on the 4th and a second on the fifth day. Brains were harvested from these and the remaining survivors on the fifth day.

Newborn_mice : on the fifth day of observation one animal was found dead and partially eaten, in each litter.

In one litter several mice had disappeared on days 6 and 7, leaving only one animal. In the second litter however, in which the animals had been very healthy looking during the whole observation period, only 3 young mice were left : one dead, one paralysed and one very sick.

The brains of these animals were harvested and sent to the Microbiological Research Establishment in Porton for further study.

VEPO_cells : during the first 4 days of observation some cells in the bottom of most tubes had loosened from the glass surface. This was first interpreted as a possible partial cytopathic effect. It did however not increase during the following days and was then considered as non specific. On day 5 the tissue culture medium was changed to the succinate-succinic acid buffered medium as described by Plaisner e.a. (1974) without serum. In our experience this medium allows the observation of VEPO cells for several weeks, while many arboviruses produce a cytopathic effect in these conditions. On day eleven a very marked cytopathic effect was observed in these tissue cultures, with most cells still attached to the glass. (Cytopathic effect was almost complete on the 12th day).

The supernatant fluid of 3 tubes was decanted and they were filled with glutaraldehyde 3% for 30 minutes. The cells were then scraped off in a small amount of glutar-

aldehyde, rinsed with cacodylate buffered sucrose (7.5%) and postfixed in 1% phosphate buffered osmiumtetroxyde, and prepared by the albumin coagulation method. Block staining was done with uranylacetate 0.5%, followed by dehydration and embedding in Spurr's low viscosity medium. The examination of ultrathin sections of this material revealed extracellular straight and cross sectioned virus particles morphologically similar to Marburg virus.

Intracellular nucleocapsids were also observed, some of them apparently originating in vesicles.

At the same time sections of the liver of the patient from which this virus had been isolated and who had died on October 1st became available. Although the ultrastructure of this tissue was very poorly preserved, rendering its examination quite difficult, similar virus particles were observed.

It was therefore concluded that the agent responsible for the epidemic of haemorrhagic fever in Central Africa was either Marburg virus or a virus serologically different from it, but belonging to the same virusgroup (either rhabdo- or togovirusgroup, Siegert et al. 1967, Almeida et al. 1971).

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(x) University of Antwerp, Belgium.

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(Editor's note: This report is essentially as it will appear in a March issue of Lancet.)

REPORT FROM THE ARBOVIRUS LABORATORY, VIRAL ECOLOGY UNIT
PASTEUR INSTITUTE, PARIS, FRANCE.

Comparative results of three different techniques for the evaluation of post-vaccination yellow fever antibodies.

On July 1976 the Virus Diseases Division of WHO sent to our laboratory 359 paired sera taken in Ethiopia before and two months after a mass vaccination campaign with 17D Yellow Fever vaccine. Without discussing the efficiency of the vaccination, we give in this brief report a comparative evaluation of the results of the various serological tests used to detect and titrate post-vaccination antibodies in the sera submitted to test.

For the detection and titration of hemagglutination-inhibition (HI) antibodies, two sucrose-acetone antigens prepared from baby-mouse brain were used: one prepared with the French Neurotropic Virus (FNV) and the other with the 17D vaccine strain. In the micro-titer technique, 4 hemagglutinating units (HA) were used with an incubation time of 18 hours at 4°C; sera were tested from 1:10 to 1:1280 dilutions and all sera giving HI at 1:10 and above were considered as "positive".

For 354 pairs of sera without detectable HI antibodies in the pre-vaccination sample, post-vaccination HI antibodies were detected in 77 sera with the FNV antigen (21,7 %) and in 115 sera with the 17D antigen (32,5 %). All sera positive with FNV were also positive with 17D, but among the 115 sera positive with 17D, 38 were negative with FNV.

HI TITER WITH FNV ANTIGEN	HI TITER WITH 17D ANTIGEN					TOTAL TESTED	TOTAL POSITIVE	GMT of POSITIVE WITH 17D
	-10	10	20	40	=/+80			
-10	239	16	13	7	2	277	38	18
10		22	23	5		50	50	16
20		4	6	5	2	17	17	24
40				2	5	7	7	72
=/+80					3	3	3	160
TOTAL TESTED	239	42	42	19	12	354	115	
TOTAL POSITIVE		26	29	12	10	77		
GMT of POSITIVE WITH FNV		11	11	16	46			

(GMT = geometric mean titer)

Using the same FNV antigen in the complement fixation test (LECF), 73 of the 77 sera HI/FNV positive were tested: no CF antibodies were detected (initial serum dilution 1:8).

Neutralizing antibodies against 17D virus were titrated by the Plaque Reduction Test (PRT) in PS cells, in micro-plates. The sera were diluted from 1:10 to 1:80; the serum dilution giving a 80 % reduction of the plaque number was considered positive. All sera with a HI/FNV reaction positive at 1:40 and above were also positive in the PRT; only 64 % of the positive at 1:20 and 51 % of the positive at 1:10 in the HI/FNV were positive in the PRT. Out of the 38 sera which were positive only in the HI/17D test (and negative in the HI/FNV), 27 were tested in the PRT: none of them was found to contain detectable neutralizing antibodies.

HI TITER WITH FNV	TOTAL TESTED HI/FNV	NUMBER POSITIVE HI/17D	NUMBER TESTED PRT	NUMBER POSITIVE PRT	%	PRT TITER				
						-10	10	20	40	=/+80
-10	277	38	27	0	0	27				
10	50	50	47	24	51	23	12	6	5	1
20	17	17	14	9	64	5	5	1	2	1
40	7	7	7	7	100			3	1	3
=/+80	3	3	3	3	100					3

Conclusion. To detect and titrate post-vaccination antibodies after vaccination with 17D vaccine, the CF test cannot be used. The HI test with an homologous 17D antigen may detect more sero-conversions than the HI test with a FNV antigen. But one might doubt if individuals with HI/17D positive only (and HI/FNV negative) are actually protected since none of these cases had detectable neutralizing antibodies in the PRT, at least using an initial dilution of 1:10 of serum; it could be advisable to start serum dilutions for the PRT at 1:2 or 1:4 to have a better correlation between HI tests and PRT.

(Dr. Pierre SUREAU).

Immunomodification and virulence expression

Recent reports (Journal of General Virology 1975; 26, 265-275; 28, 225-250) have indicated that when mice are infected with defined strains of Semliki forest virus or Venezuelan equine encephalomyelitis virus the course of virus invasion and the development of disease or immunity may be considered in terms of three essentially distinct phases or mechanisms:

- i) The events immediately following virus encounter when largely non-specific clearance mechanisms determine the efficiency of primary infection. This is conveniently expressed in terms of the number (or its reciprocal) of infective units (p.f.u. or SMic LD50) required for one ID50. This efficiency of primary infection is independent of the later outcome of infection which may range from death (when ID50 is LD50) to benign protection (when ID50 is PD50).

Strains of SFV and VEEV of extreme virulence or avirulence may have high (1 SMicLD50 per host ID50) or low ($\sim 10^4$ SMicLD50 per host ID50) efficiency of infection.

- ii) The events controlling the biphasic responses to primary infection and the expression of virulence or avirulence.
- iii) The events controlling the responses to a secondary or challenge infection by a virulent strain of virus. Here the status of the host in terms of immunity and protection is expressed.

The distinctions between these stages of host-response become particularly important when studies are made of the expression of virulence and immunogenicity through the use of immunomodifying agents. Single treatments by drug or radiation may act to enhance or suppress by distinct mechanisms at any one or more of the above three stages. Thus it has been shown that single-doses of interferon or double-stranded RNA act largely at stage i) (to be published), MYOCRISIN at stages i) and ii), cyclophosphamide at stages ii) and iii), and wholebody γ -irradiation by 250R at stage iii) (to be published).

A feature of the above treatments is the relatively short duration of the immunomodification which is then superimposed upon the in vivo kinetics of the ongoing infection itself. In order to clarify this point by the use of a known and time-stable immunological deficiency, tests were made on athymic nude mice which can mount no significant T-lymphocyte response.

In a typical experiment, male nude-mice and their normal litter mates at 50-60 days old received 10^4 p.f.u. i.p. of the TC83 vaccine strain of Venezuelan equine encephalomyelitis virus. At 8 and 20 days after infection all mice were bled individually for assay of the neutralizing activity of their sera. Also on the 20th day all mice were challenged with 10^4 p.f.u. i.p. of the P2023 virulent strain of VEE virus. The same treatment of primary

infection by avirulent virus and secondary challenge by virulent virus was also applied to a second group of nude mice which had received a transfer of about 10^6 spleen cells from their normal litter-mates on the day before avirulent infection. The results in Table 1 show the responses to primary and secondary infection and in Table 2 the antibody activities at days 8 and 20. An essentially similar pattern of results has been obtained in equivalent experiments using the virulent (L10) and avirulent (A774) strains of Semliki Forest Virus.

Although each group of mice shows a spread of responses, the following conclusions appear to express the response of the majority in Tables 1 and 2.

- a) No animals die before lethal challenge so that the avirulence of strains TC83 and A774 appears not to depend upon a dominant T-cell function.
- b) A normally rising (R) pattern of antibody synthesis requires the presence of 'memory' T-cells:
- c) The above function may be restored to nude mice by normal spleen cells and is associated with the capability to resist lethal challenge.

These results suggest that the expression of virulence or avirulence after primary infection (phase ii) is not strongly T-cell dependent but that the resistance to secondary lethal challenge (phase iii) is much more strongly T-cell dependent. These experiments continue in order to clarify the influences of virus strains and doses and the times of challenge after spleen cell transfer.

(R. Fitzgeorge and C. J. Bradish)

TABLE 1

GROUP	NUMBER OF MICE SHOWING		
	DEATH AFTER AVIRULENT INFECTION ON DAY 0	DEATH AFTER VIRULENT CHALLENGE ON DAY 20	PROTECTION AGAINST CHALLENGE
NUDE MICE	0	5(F)	5(I)
NUDE MICE + SPLEEN TRANSFER ON DAY-1	0	2(F)	7(R)
NORMAL LITTER MATES	0	2(R)	6(R)

132

TABLE 2

GROUP	Serum neutralization index (log. antibody activity) for individual antisera collected on	
	Day 8	Day 20
F : falling antibody activity	2-3	1.5-2.5
I : intermediate	2.5-3.8	2.2-2.6
R : rising antibody activity	3.2-4.2	3.5-4.5

REPORT FROM THE ARBOVIRUS UNIT AND ENTOMOLOGY
DEPARTMENT, LONDON SCHOOL OF HYGIENE &
TROPICAL MEDICINE.

The collaborative project established in 1969 between the British Medical Research Council and the Kenya National Irrigation Board is continuing with the aim of assessing the importance of arboviruses in changes occurring as a result of land development.

A single virus isolation has been obtained from the serum of a febrile child from the area of the proposed Kano Plain irrigation scheme. The virus (strain NY 45) is a member of the Bwamba group (Table 1). Cross-neutralization tests against Bwamba, Pongola and 3 strains of Pongola isolated from mosquitoes in the area suggest that the virus may be distinct from the other members of the group. A similar or identical virus has been recovered from a pool of 8 Rhipicephalus species ticks taken from a goat in the same area.

Isolation attempts from approximately 218,000 mosquitoes have produced 4 isolates related to the group. All have been identified as Pongola and all came from pools of Mansonia uniformis. Additional isolations of Ilesha and Barur were made from M. uniformis in the area. Virus strains were also recovered from Anopheles gambiae (Ilesha and Sindbis), An. funestus (similar or identical to Tataguine) and one virus strain, as yet unidentified, from Culex antennatus.

Organ suspensions from 2428 mammals and from 1202 birds were processed for virus isolation. Arumowot virus was recovered from mammal spleens on 4 occasions and Germiston virus twice. Identification is not complete on viruses isolated from 2 birds and an agent isolated on 3 occasions from small mammals.

Virus has been recovered from 10 of 901 pools of ticks (12, 253 individuals) from the Kano Plain. At this time only 1 strain, mentioned previously, has been partially identified and it appears closely related to Bwamba virus. The other 9 isolates came from Amblyomma variegatum pools.

Preliminary investigations of arboviruses in the Tana River Basin have also been conducted in conjunction with the Kenya National Irrigation Board. Isolation attempts were made on 214 pools of mosquitoes (11,240 individuals) from the area. Virus was isolated from 1 pool of M. africana and 1 pool of M. uniformis. Neither virus has been identified but it has been ascertained that they do not react with NIH grouping fluids to approximately 160 known viruses, many of which occur in Africa. Virus was isolated from 3 pools of Amblyomma lepidum out of 194 tick pools tested (2,840 individuals). None of these react with the grouping fluids or specific antisera available.

In the course of this study it has been found that the cell line (XTC-2) established from the South African clawed toad Xenopus laevis (Pudney, Varma and Leake 1973, Experientia 29: 466) will show c.p.e. and/or plaque formation under carboxymethylcellulose overlay at 28°C on infection with a wide range of arboviruses. Of 38 arboviruses tested, 28 produced clear signs of c.p.e., and of the 10 viruses failing to produce c.p.e., 9 were flaviviruses (see table 2). Plaque assay experiments have indicated the usefulness of this cell line for the study of tick-borne arboviruses, other than the flaviviruses. This has been confirmed in work on tick isolates from the Kano Plain.

All of the tick isolates to date form clear plaques in Xenopus cells. Some of the isolates fail to cause c.p.e.

in any other cell line which we use (Vero, LLCMK₂, BHK-21 or PS cells). All of the members of the Hughes and Congo groups form plaques in these cells although results with Congo virus have not been consistent.

A micro-neutralization test using flat bottomed 96 well tissue culture plates with BHK cells has been employed to carry out a cross-relationship study of Alpha-viruses. The results (table 3) were essentially the same as those of Karabatsos (1975. Am. J. Trop. Med. Hyg. 24: 527). The micro-test has proven to be sensitive and economical and is now used routinely in our laboratory.

The cytopathic response of the cell line Aedes pseudoscutellaris (LSTM-AP-61) when infected with some arboviruses has been studied further (M.G.R. Varma, Pudney, M. and C.J. Leake. Trans. Roy. Soc. Trop. Med. Hyg. 68: 374-382, 1974). It has been shown to provide a simple, rapid and inexpensive method of isolating yellow fever virus from naturally infected mosquitoes, human liver and the serum of a sentinel monkey (Varma, M.G.R., Pudney, M. Leake, C.J. and Peralta, P.H. Intervirolog. 6: 50-56, 1975/6). Primary isolations from mosquito pools have been made in these cells, of Sindbis, Japanese encephalitis, Tembusu and Getah viruses.

The AP-61 cells are also being used for plaque studies using a low temperature agarose. To date, plaques have been produced by Japanese encephalitis, Tembusu, Dengue 1 & 2, Ntaya, Zika, and all the wild strains of yellow fever virus isolated in mosquito cells. This is similar to the results of Yunker and Cory who found that they got a greater degree of plaquing with group B mosquito-borne viruses in the Aedes albopictus cell line. They were unable to plaque Zika virus which was possibly due to its high mouse brain passage.

A mosquito picornavirus, Kawino virus, has also been isolated in these cells from a pool of Mansonia uniformis mosquitoes from the Kano Plain, Kenya. Further studies are in progress.

(D.I.H. SIMPSON, M.G.R. VARMA, MARY PUDNEY,
B.K. JOHNSON AND C.J. LEAKE)

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TABLE I. LOG NEUTRALIZATION INDEX OF BWAMBA GROUP VIRUSES COMPARED TO NY-45 ISOLATE.

Ab 1/10	NY-45	BWAM	PONG	NYANDO
VIRUS				
NY 45	≥7.0	3.0	0.5	0
BWAM	3.0	3.5	0.5	0
PONG	2.5	0.2	2.8	0
NYANDO	0.5	0	0	1.0

TABLE 2. PRODUCTION OF CPE BY ARBOVIRUSES IN XTC-2 CELLS

VIRUS	ANTIGENIC GROUP	EXTENT OF C.P.E.	DAY P.I.CPE COMMENCED	DAY P.I.CPE ENDPOINT	TITRE DEX CPD ₅₀ /O.1ML
BEBARU	A	4	2	4-5	7.7
CHIKUNGUNYA	A	1-2	2	5-6	6.0
GETAH	A	4	2	4-5	8.5
MAYARO	A	4	2	5-6	8.5
MIDDLEBURG	A	2-3	4	5-6	6.8
NDUMU	A	2-3	3	5	7.5
O' NYONG-NYONG	A	2-3	2	4-5	6.4
SEMLIKI FOREST	A	3-4	2	4-5	7.5
SINDBIS	A	3-4	2	3-4	6.5
WHATAROA	A	4	2	5	7.5
DENGUE-2	B	NO	-	-	-
ENTEBBE BAT	B	NO	-	-	-
JAPANESE ENCEPHALITIS	B	NO	-	-	-
LANGAT	B	NO	-	-	-
LOUPING ILL	B	NO	-	-	-
MODOC	B	NO	-	-	-
NTAYA	B	2-3	4	5-6	5.8
TEMBUSU	B	NO	-	-	-
YELLOW FEVER	B	NO	-	-	-
WEST NILE	B	2-3	4	6-8	6.5
ZIKA	B	NO	-	-	-
GERMISTON	BUN	3-4	4	5-7	8.2
PONGOLA	BWA	NO	-	-	-
CALIFORNIA ENCEPHALITIS	CAL	+	4	4-6	4.4
TAHYNA	CAL	2	2	5-7	5.2
PIRY	VSV	4	1	3-5	6.5
CHANDIPURA	VSV	4	1	3-5	7.9
SANDFLY FEVER	PHL	2-3	2	5-6	5.9
ARUMOWOT	PHL	3-4	4	4-5	7.2
ZIRQA	HUG	1-2	4	5-8	5.8
GANJAM	GAN	1-2	4	5-6	4.2
DUGBE	GAN	2-3	4	5-6	5.5
LANJAN	KSO	1-2	4	5-6	2.5
QUARANFIL	QRF	1-2	4	5-7	7.0
KERN CANYON	UNG*	2-3	3	5-7	6.7
LAGOS BAT	UNG*	2-3	5	6-9	6.5
MOUNT ELGON BAT	UNG*	+	5	6-8	3.9
KETERAH	UNG*	2-3	4	5-8	6.7

* Ungrouped viruses.

REPORT FROM THE ARBOVIRUS RESEARCH UNIT,
 EGYPTIAN ORGANIZATION FOR BIOLOGICAL & VACCINE PRODUCTION,
 AGOUZA, CAIRO, EGYPT

I. STUDIES ON ANTIBODIES AGAINST WEST NILE (WN)
 AND SINDBIS (SIN) VIRUSES

A. Comparison of Hemagglutination-Inhibiting (HI) and Complement-Fixing (CF) Antibody Prevalence.

One hundred and eighty human sera were randomly selected and tested for HI and CF antibodies against both WN and SIN viruses. The selection was made from sera collected from Upper Egypt where WN and SIN are known to exhibit higher prevalence rates. The following table shows that 80.5 percent of the specimens exhibited HI antibodies for WN virus and 30.0 percent for SIN virus; 10.5 percent had CF antibodies for WN and none for Sindbis. This shows that the prevalence of CF antibodies was much lower than HI rates for WN (about 8 times) and CF antibodies were not detected at all for SIN virus.

	No. of sera tested	HI		CF	
		No.	%	No.	%
West Nile	180	145	80.5	19	10.5
Sindbis	180	54	30.0	0	0

For WN virus, no person had CF antibodies without accompanying HI antibodies. When the group was examined by age (see next table) for

the pattern of antibody prevalence, it was noticed that CF antibodies reached the peak at age 20-29 years and were not detected after 40 years. HI antibodies on the other hand almost maintained their high rate (95% to 100%) from age of 20 years till above 50 years. Although the HI antibodies for WN ranged from 1:20 to 1:640, the CF titers were only 1:4 or 1:8. HI antibodies to SIN virus ranged from 1:20 to 1:320.

Age group	No. tested	WN - HI		WN - CF	
		No.	%	No.	%
10 - 19	104	71	68.27	8	7.69
20 - 29	21	20	95.24	6	28.57
30 - 39	28	27	96.43	5	17.86
40 - 49	20	20	100.00	0	0
50 & above	7	7	100.00	0	0
TOTAL	180	145	80.50	19	10.50

One can conclude from this study the following:

1. On evaluation of the prevalence of WN and SIN virus infection in a population, estimates varies according to the type of test used. With WN infection, an 8-fold difference may be noticed between HI and CF tests and similar results were found by Schmidt and El-Mansoury 1963 on using equine sera. For Sindbis virus prevalence, CF test does not seem to be a feasible one.

2. It appears that WN CF antibodies may have a shorter duration than HI antibodies. They exhibited low titers and were not detected after the age of 40 years. The non-detection of SIN CF antibodies may indicate that the SIN CF test is not as sensitive as the WN CF test in demonstrating antibodies at the level associated with inapparent infection, or it may reflect a more transient nature of SIN CF antibodies, or a repeated exposure to WN infection.

B. Prevalence of WN and Sindbis viruses in School Children.

Sera from school children of age 10-18 years from Cairo, Alexandria, and Assiut Governorates were tested for HI antibodies against WN and SIN viruses. The following table represents the distribution of positive reactions for the 1170 sera tested.

Governorate	No. of sera tested	WN		Sindbis	
		No.	%	No.	%
Alexandria	354	127	35.8	0	0
Cairo	605	337	55.7	109	18.0
Assiut	211	120	57.0	48	22.7
TOTAL	1170	584	49.9	157	13.4

This result shows that the overall positivity rates for WN and SIN viruses are 49.9% and 13.4% respectively. The high rate at that age

group indicates that infection by these 2 endemic viruses starts at a relatively young age. We are now planning to test sera from primary school and pre-school children to determine the infection rates at that age.

Comparing the geographical distribution of HI antibodies against the 2 viruses, it is clear that the coastal Alexandria Governorate exhibited a lower rate for WN infection than the more inland Cairo and Assiut Governorates. For SIN virus which generally shows a lower overall prevalence than WN, Alexandria was free from reactive sera as compared to Cairo and Assiut. This north-to-south increase was previously reported and attributed to decreased mosquito density.

The next table shows comparison of HI titers for WN and Sindbis viruses. WN virus exhibits wider range (up to 1:640), 25.9% of the specimen presented titer of 1:640 and only 27.6% had a low titer of 1:20. Sindbis virus which is less prevalent than WN, reacted maximally with titer of 1:320, and 45.9% of the specimen had a low titer of 1:20.

HI Titer [*]	WN		Sindbis	
	No.	%	No.	%
20	161	27.6	72	45.9
40	76	13.0	30	19.1
80	61	10.4	32	20.4
160	60	10.3	15	9.5
320	75	12.8	8	5.1
640	151	25.9	0	0
TOTAL	584	100.0	157	100.0

II. COLLECTION OF TICKS FOR VIRUS ISOLATION

Several viruses have been isolated in Egypt from different ticks. Quarafil, Chenuda, Nyamanini, Qalyub, Abu Hammad, and Abu Mina viruses were isolated from soft ticks (Family Argasidae), whereas Dhori, Wanowrie, Thogoto, and probably also Bahig and Matruh viruses were collected from hard ticks (Family Ixodidae).

The activity of these viruses, or at least some of them, in the community has been shown by the isolation of Quarafil virus from man, antibodies to Quarafil and Qalyub in man, and also antibodies against certain of these viruses in other mammalian sera.

Different ticks have been collected from various places and geographical localities in Egypt. The soft ticks included certain species

* Reciprocal of serum end-point dilution.

of the genera Argas and Ornithodoros, while the hard ticks represented the genera Hyalomma and Rhipicephalus. In fact most of the viruses isolated from ticks in Egypt were from the first 3 genera.

The ticks so far tested for its viral content are listed in the following table. The results showed that all, but collection No. (5), did not furnish any virus isolate.

The isolate (Collection No.5) was from a male pool of Ornithodoros erraticus collected from Abu Ghalib, Imbaba, Giza governorate at November 1975. It was identified by CFT & NT as Qalyub Virus. The previous isolation of Qalyub Virus from the same tick species in 1952 and 1968 in Egypt, besides this reported one of 1975, may be suggestive of its widespread and/or cyclic circulation in the community.

(Medhat A. Darwish and Imam Z. Imam)

Collection No.	Tick species	Date of collection	Place of collection	Animal collected from	Results
1	<u>Hyalomma dromedarii</u> , 45 ♀	Sept. 75	Abu Rawash, Imbaba, Giza	Domestic camel	Negative
2	<u>Hyalomma dromedarii</u> , 53 ♀	Oct. 75	Bashtil, Imbaba, Giza	Camel	Negative
3	<u>Rhipicephalus sanguineus</u> , 10 ♂, 5 ♀, 3 N	Oct. 75	Abu Rawash, Imbaba, Giza	Dog	Negative
4	<u>Hyalomma dromedarii</u> , 1 ♂, 40 ♀	Nov. 75	Abu Rawash, Imbaba, Giza	Camel	Negative
5	<u>Ornithodoros erraticus</u> , 29 ♂, 24 ♀, 62 N	Nov. 75	Abu Ghalib, Imbaba, Giza	<u>Arvicanthis n. niloticus</u> burrow	Positive
6	<u>Ornithodoros erraticus</u> , 1 ♂, 11 ♀, 40 N	Dec. 75	Minshat El-Bakkari, Imbaba, Giza	<u>Arvicanthis n. niloticus</u> burrow	Negative
7	<u>Argas (Persicargas) persicus</u> , 15 ♂, 15 ♀, 15 N	Dec. 75	Naga Hammadi, Qena	Domestic chicken house	Negative
8	<u>Argas (Persicargas) persicus</u> , 50 ♂, 50 ♀, 50 N	Dec. 75	Kom Ombo, Aswan	Domestic chicken house	Negative

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUT PASTEUR AND ORSTOM

DAKAR - SENEGAL

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During the second half of 1976, joint studies were continued at our field station in Kedougou.

In addition, serological studies were carried out on sera from Senegal, Madagascar and Upper Volta.

1. VIROLOGICAL STUDIES

1.1. Human blood samples :

48 blood specimens collected from febrile children at the clinic established in Bandia were processed for virus isolation without success. There has been no virus isolation in Bandia since 1974. This may be due to the fact that Bandia forest has been ravaged by droughts and charcoal-burners. From the blood of a laboratory technician working once a week at the clinic in Bandia, a strain of Orungo virus (Ug MP359) was isolated. This is the first isolation of this virus in Senegal.

Blood samples were taken from 24 patients in the Kedougou Hospital and inoculated into suckling mice. No isolate was recovered.

From the blood of 5 mosquito-catchers working in Kedougou, 1 strain of Zika virus was isolated.

1.2. Wild vertebrate samples :

22 blood samples collected from monkeys caught in Kedougou were inoculated into suckling mice. One strain of chikungunya virus was isolated from the blood of a baboon (*Papio papio*) collected in november 1975.

1.3. Arthropods :

All mosquitoes were caught in Kedougou area.

.../...

11.554 mosquitoes were processed in 577 pools for virus isolation. Some of the mosquitoes were caught during the 1975 rainy season the others in 1976.

From the mosquitoes caught in 1975 the following results were obtained :

- Semliki forest virus was isolated from *Aedes vittatus*: this is the first isolation of SFV in Senegal (june 1975).
- 10 strains of chikungunya virus were isolated from *Aedes furcifer taylori* (3) *Aedes luteocephalus* (5) *Aedes dalzieli* (1) and *Anopheles coustani* (1) (oct-nov.1975).
- 3 strains of Ndumu virus were isolated from *Aedes dalzieli* (2) and *Aedes minutus* (1). Two isolates have been recovered only after intrathoracic inoculation of *Aedes aegypti* mosquitoes.

Kedougou virus (Ar D14701) a new flavivirus was isolated for the second time from *Aedes minutus* collected at the begining of the rainy season (june 1975).

Eret 147 virus, of the Nyando group, was isolated from *Aedes dalzieli*. Isolates described as Nyando viruses in the last report are, in fact, strains of Eret 147.

From the mosquitoes caught in 1976, the following isolations were made :

- 10 strains of Zika virus from *Aedes luteocephalus* (7) and *Aedes furcifer taylori* (3).
- Bwamba virus from a pool of *Anopheles gambiae*. This is the first isolation from an arthropod identified at the Dakar Reference Center. There was a mistake in the last report (A.B.V. I.E. N°31, september 1976 p 129, 1st line) : one must read Pongola virus instead of Bwamba.
- Pongola virus from *Aedes vittatus*

As we can see, there is a wide variety of viruses in Kedougou where chikungunya virus has been very active in 1975 and Zika virus in 1976.

2. SEROLOGICAL STUDIES

2.1 Human sera :

2.1.1. From Senegal

62 serum specimens from Bandia and Dakar were examined for arbovirus antibodies. In no case the pattern of antibodies allowed a specific diagnosis.

2.1.2. From Madagascar

86 serum samples from Bezaha, south part of the island were tested for HI and N antibodies. Very few sera reacted with some group B antigens and always at a low level 1/10-1/20. All sera were negative for yellow fever neutralizing antibodies.

2.1.3. From Upper Volta

As part of the yellow fever surveillance system, serum samples from 11 patients with jaundice were sent to the laboratory.

3 samples showed very high titer for group B antigens in HI as well as in CF test. One or more flavivirus seem to be very active in the area but specific diagnosis is impossible.

2.2 Wild vertebrate samples

33 sera from monkeys caught in Kedougou were tested for HI, CF and N antibodies.

29 (88%) have HI antibodies for chikungunya virus and most have CF antibodies also indicating a rather recent infection which is in agreement with the numerous chikungunya virus isolations from mosquitoes in the area. Zika virus is also very active among the monkey populations.

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

REPORT FROM THE WORLD HEALTH ORGANIZATION, ARBOVIRUS VECTOR
RESEARCH UNIT, ENUGU, NIGERIA.

The WHO, Arbovirus Vector Research Unit(AVRU), a field project of Vector Biology and Control in Geneva, was established in Enugu, Nigeria in 1973 to investigate the ecology of the potential vectors of yellow fever and other arboviruses in Nigeria. Yellow fever epidemics have occurred in Nigeria in 1969-70 on the Jos Plateau (Northern guinea savannah) and in 1974-75 in the Cross River State(rain forest zone). In 1975 a collaborative programme was established between AVRU and the Virus Research Laboratory, University of Ibadan(Prof. I. Fabiyi, Director). During the first year(November 1975-November 1976) several hundred pools of Aedes mosquitoes and other species have been processed for virus isolation attempts. Preliminary results indicate the presence of several group B arboviruses in pools of Aedes africanus collected in a fresh water swamp forest at the Mamu River Forest Reserve, located about 75 km west of Enugu. Characterization of these agents is pending.

Y. H. Bang, D.N. Bown, and A.B. Knudsen.

ARBOVIRUS UNIT : NATIONAL INSTITUTE FOR VIROLOGY,
JOHANNESBURG, SOUTH AFRICA.

Chikungunya.

In our previous contribution (No. 31, Sept. 1976) we described a small rural epidemic of chikungunya in the wooded savanna in South Africa in 1976, in which virus had been isolated from man and Aedes furcifer/taylori and it was suggested that baboons (Papio ursinus) had probably acted as amplifying hosts, since this species is known to develop a high level of viraemia and was present in some numbers in the area.

Since that report immune rates have been determined among samples of the human and baboon populations in the affected area and the table shows the results:

<u>Locality (farms)</u>	<u>Immune rates (% positive)</u>	
	<u>Man</u>	<u>Baboon</u>
Grietjie	40 (5) ^a	
Morelag		0 (15)
Tillie	100 (19)	100 (21)
Hope	87 (46)	100 (71)
Marble Bath	19 (34)	
Oxford	38 (34)	
Cambridge	-----	<u>81 (16)</u>
	<u>62 (125)</u>	<u>85 (123)</u>

a (5) = total number tested.

It can be seen that high immune rates were recorded from most farms in both hosts. Particularly noteworthy are the high immune rates on Tillie and Hope. On these adjoining farms is a cluster of granite hills which provide a haven for large numbers of baboons which utilize the summits of the hills as a safe refuge and as dormitories and from where infected furcifer

had been collected during the epidemic. Also of interest to record is that while Morelag and Hope are only 5 km apart, the baboons on Morelag remained free from infection suggesting that viral transmission can be extremely localized. The farms Oxford and Cambridge adjoin each other and lie along the large Olifants River, with its distinctive riverine flora, dominated by very large trees. These trees also provide dormitory sites for other baboon troops and breeding holes for furcifer.

It seems likely that foci of infection along this riverine habitat might have arisen independantly and remained isolated from the Tillie/Hope focus. The territorial habits of the baboons would encourage this localization.

(B.M. McINTOSH.)

REPORT FROM THE VIRUS UNIT, INSTITUTO NACIONAL
DE MICROBIOLOGIA "DR. CARLOS R. MALBRAN", BUENOS AIRES, ARGENTINA

In the last 6 months of 1976, 42 suspected cases of Argentine Hemorrhagic Fever were studied by CF test with Junin and LCM virus. A breakdown of the results by month, location, age and sex is shown in the following tables.

The number of studied cases was lower than that of the first semester reported before (212 cases). This was expected since the peake of the epidemic is regularly observed in May and June.

(Drs. Maria Eugenia Grela and Angelica R. Teyssie')

Table 1 MONTHLY DISTRIBUTION OF AHF SUSPECT CASES OF 1976 (last 6 months)
BY CF TESTS

Onset of illness	Positive cases with Jun virus antigen/number of suspects	
	With paired sera (4-fold rise)	With single sera (1/4)
Jul.	21/27 (3)	0/0
Aug.	6/9	0/0
Sept.	3/3	0/0
Oct.	0/0	0/0
Nov.	2/3	1/1
Dic.	0/0	0/0
Total	32/42 (3)^a	1/1

Table 2 GEOGRAPHIC DISTRIBUTION

Province	Positive cases with Jun virus antigen/number of suspects	
	With paired sera	With single sera
Buenos Aires	22/29 (2)	1/1
Santa Fe	10/12 (1)	0/0
Cordoba	0/1	0/0
Total	32/42 (3)	1/1

Table 3 AGE DISTRIBUTION

Age (years)	Positive cases with Jun virus antigen/number of suspects	
	With paired sera	With single sera
0-5	0/0	0/0
10-14	1/2	0/0
15-24	3/4	0/0
25-40	15/20 (2)	1/1
41-59	8/10 (1)	0/0
+ de 59	5/6	0/0
Unknown	0/0	0/0
Total	32/42 (3)	1/1

Table 4 AGE DISTRIBUTION

Sex	Positive cases with Jun virus antigen/number of suspects	
	With paired sera	With single sera
Male	24/31 (3)	1/1
Female	8/11	0/0
Total	32/42 (3)	1/1

REPORT FROM THE
DEPARTMENT OF EPIDEMIOLOGY
SCHOOL OF PUBLIC HEALTH
UNIVERSITY OF SAO PAULO
SAO PAULO, BRAZIL

In 1976 investigations were initiated on the Ribeira Valley of the State of São Paulo, where an outbreak of encephalitis appeared. In a first stage, the aim was to ascertain the local mosquito fauna composition and the main species activities. Series of collections were made on three places established in the Iguape, Pariquera-Açú and Sete Barras counties. The choice of these places was guided by particular ecological aspects and the occurrence of human cases. So, Iguape was representative of the local coastland and Pariquera-Açú of the inland valley lowland, where disease occurred. The Sete Barras Station was placed at the mountain region with no recorded cases of that infection. Series of collections were made and a total of 39.945 adults mosquitos caught. The more dense species are presented in Table 1, as the rates of total catches. The high numbers of Aedes scapularis, Ae. serratus, Culex taeniopus and several Culex (Melanoconion) sp. in Iguape and Pariquera-Açú, were contrasting with the low rates of these mosquitos in Sete Barras. Otherwise, Mansonia chrysonotum showed high rates in all the three places, including the Sete Barras one, where no human cases of encephalitis appeared until now.

Mosquito activity was investigated by serial domiciliary, open ground and sylvatic collections. Due to unexpected factors occurred at the work on the Iguape Station, their data were lost. Nevertheless the Paríquera-Açú and Sete Barras ones were obtained and so in Table 2 results of domiciliary catches are presented. It was possible to observe the absence of Aedes scapularis and Ae. serratus in Sete Barras, and otherwise their occurrence of the first one in Paríquera-Açú, beside Culex (Melanoconion) species, including C. taeniopus. The presence of Mansonia mosquitoes was almost the same in the two Stations, and Anophelis cruzii was nearly restricted to Sete Barras one.

In the others outdoor catches , the seasonal activities of these two Aedes mosquitos were calculated for the July to December 1976 period, as presented in Figure 1. The fall of activity maybe will be due to the rainy season who in this region start at October-November period.

(Oswaldo Paulo Forattini)

Table 1 - Mosquito fauna at the three stations on Ribeira Valley, São Paulo State, Brazil. Total and percentage rates obtained at serial catches during 1976.

Species	Iguape		Pariquera-Açú		Sete Barras	
	N	%	N	%	N	%
<u>Aedes scapularis</u>	1429	6,17	909	8,98	8	0,12
<u>Aedes serratus</u>	2228	9,63	2595	25,64	7	0,10
<u>Anopheles cruzii</u>	429	1,85	79	0,78	1635	24,47
<u>Culex (Melanoconion) sp.</u>	2389	10,32	321	3,17	33	0,49
<u>Culex taeniopus</u>	200	0,86	295	2,91	-	-
<u>Mansonia chrysonotum</u>	4733	20,45	739	7,30	1298	19,42
<u>Mansonia venezuelensis</u>	34	0,15	450	4,44	107	1,60
<u>Psorophora lutzii</u>	578	2,50	4	0,04	-	-
<u>Phoniomyia davisii</u>	1395	6,03	493	4,87	749	11,21
<u>Phoniomyia neivai</u>	558	2,41	68	0,67	49	0,73
<u>Phoniomyia quasilongirostris</u>	1309	5,66	423	4,18	232	3,47
<u>Phoniomyia theobaldi</u>	881	3,81	474	4,68	164	2,45
<u>Wyeomyia confusa</u>	1794	7,75	124	1,22	2194	19,36
<u>Wyeomyia mystes</u>	739	3,19	14	0,14	81	1,21
Others	4445	19,22	3134	30,98	125	15,37
Total	23141	100,00	10122	100,00	6682	100,00

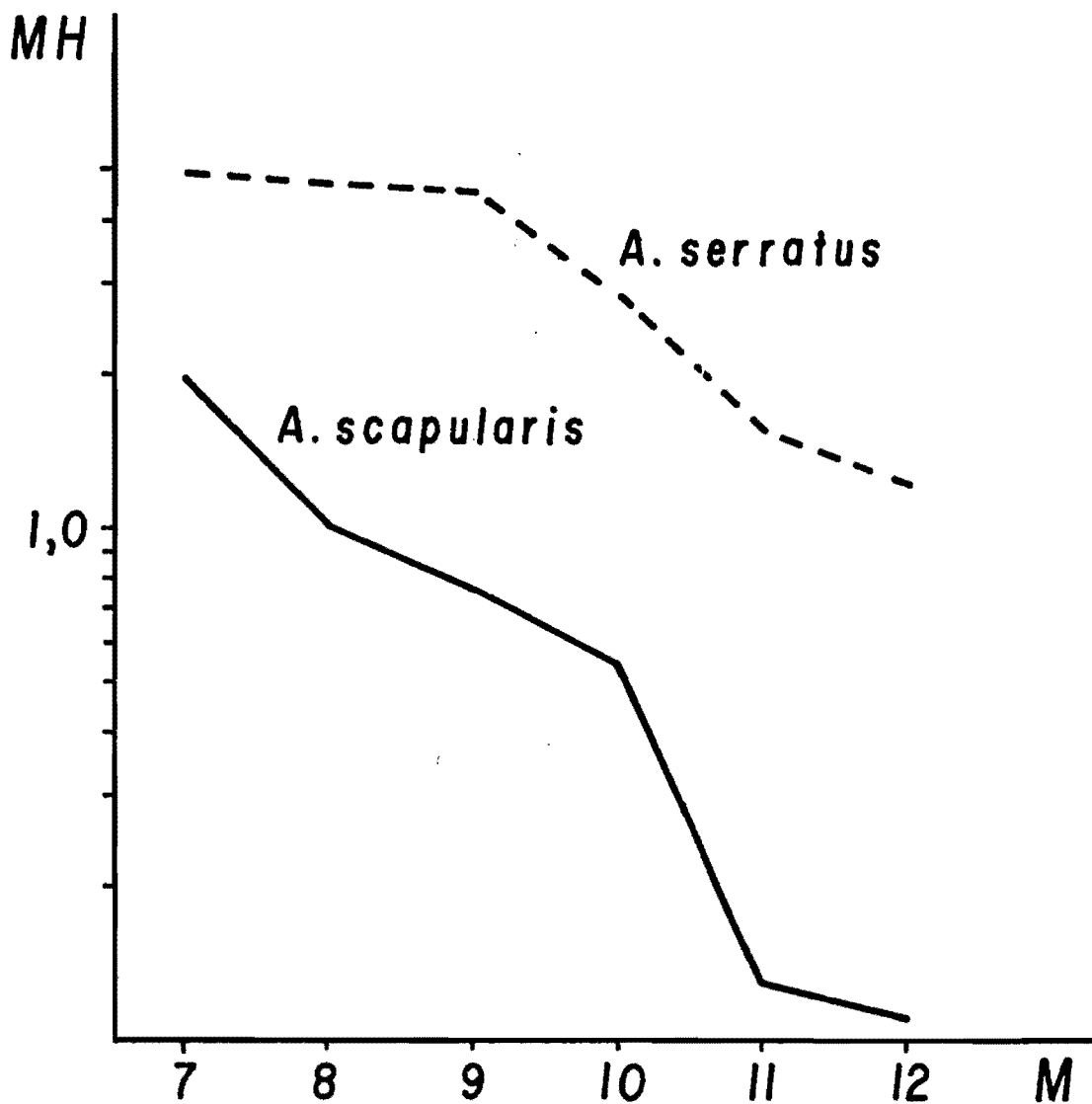
Table 2 - Domiciliary catches in Ribeira Valley, São Paulo State, Brazil
 July to December 1976 (including indoor and surrounding
 buildings collections).

Species	Pariquera-Agú		Sete Barras	
	N	%	N	%
<u>Aedes scapularis</u>	22	3,91	-	-
<u>Anopheles cruzii</u>	1	0,18	188	13,50
<u>C. (Melanoconion) sp.</u>	55	9,79	-	-
<u>Culex taeniopus</u>	2	0,35	-	-
<u>Mansonia chrysonotum</u>	458	81,50	1173	84,21
<u>Mansonia venezuelensis</u>	24	4,27	15	1,08
<u>Phoniomyia davisi</u>	-	-	8	0,57
<u>Phoniomyia quasilongirostris</u>	-	-	2	0,14
<u>Wyeomyia confusa</u>	-	-	7	0,50
Total	562	100,00	1.393	100,00

Figure 1 - Aedes scapularis and Ae. serratus activities in Parquera-Açú Station, Ribeira Valley, São Paulo, Brazil, during July to December 1976 period.

M - Months

MH - Number of mosquitoes/man-hour



BRAZILIAN MINISTRY OF HEALTH

BELEM, BRAZIL

1. SEROLOGICAL STUDIES WITH VIRUS STRAINS ISOLATED IN THE AMAZON REGION.

During a 3 month period (Dec.1975-Feb.1976) two of us (APATR and JFTR) stayed at the YARU carrying out studies on the serological relationships of 16 agents isolated in the Amazon region of Brazil. These agents have been isolated from a variety of wild animals and arthropods and they have been shown in Belém to be different from other arboviruses which occur in Brazil. Therefore at the YARU they were compared with arboviruses from other parts of the world. As result of such studies, 12 new viruses to the world were uncovered, plus 4 new agents to Brazil.

New viruses to the world

1. Guamá group. Two agents, Be An 109303 and Be An 116382, named Ananindeua and Timboteua, respectively. The Be An 109303 strain has been isolated 193 times (up to the first semester of 1975), as follows: 152 from sentinel mouse, 8 from marsupials (7 from Caluromys and 1 from Didelphis) 1 from sentinel monkey, 1 from sentinel chicken, 1 from wild bird and 30 from arthropods (24 from several species of Culex, 1 from Mansonia venezuelensis and 5 from Culicoides). The Be An 116382 strain has been isolated only in 3 occasions, from sentinel mice.
2. Capim group. Two new viruses: Be An 84381 (Benfica) and Be An 153564 (Benevides). Together with Acará virus they comprise a complex within the Capim group. Thus, although the 3 agents are practically indistinguishable by the CF test, they can be readily separated by the HI and N tests. They have been isolated from sentinel mice and, in addition, Acara and Benfica viruses have also been recovered from the Nectomys rodents.

3. Phlebotomus group. Also two new agents: Be An 100049 (Urucuri), isolated from a Proechimys caught near Belém, and Be An213452 (Itaituba) recovered from blood of a Didelphis captured in the Itaituba area. Urucuri and Icoaracy viruses are antigenically close related in the HI test however, they are readily separated by the CF test. Itaituba and candiru viruses are practically identical in the CF test but they do not cross react in the N test.

4. California group. One strain, Be Ar 103645 (Serra do Navio) which was isolated from a pool of Aedes fulvus collected in the Amapá Territory. At YARU this strain was readily distinguished from other California viruses by the CF test, using hamster immune sera.

5. Supergroup Bunyamwera unassigned. At the YARU the strain Be An 141106 (Belém) was tested by CF, HI and N against all the grouping sera and ungrouped sera available. The only reaction found was a 1:20 titer in the HI test with a hyperimmune group C antiserum. This suggests that the virus may belong to the unassigned viruses of the Bunyamwera supergroup.

6. Tacaribe group. As reported in the Information Exchange nº 31, Be An 293022 (Flexal) is a possible new member of this group.

7. Ungrouped viruses. Three new members to the world were recognized: Be Ar 177325 (Inhangapi), Be An 174214 (Araguari) and Be An 238758 (Santarém) viruses. Be Ar 177325 was isolated from a pool of Lutzomyia flaviscutellata caught by Disney trap, near Belem. No significant reaction was found in the CF test between its antigen (1:4 and 1:40) with the following grouping sera: A, B, C, Guamá, Bunyamwera, Capim, Simbu, VSV, Phlebotomus, Tacaribe, California, Sakhalin, Kemerovo, Palyam, and with the following polyvalent sera: Quarantil, Patois, LCM, Rabies, Anopheles A, Congo and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, and with antisera to Be Ar 103645, Be An 238758 and mouse hepatitis. In addition, a hyperimmune mouse serum to the agent failed to react in the CF test with 193 antigens of nearly

all arboviruses of the YARU collection. Similar results were found upon testing Be An 174214 and Be An 238758 viruses, with the exception that the homologous antiserum to the last agent reacted at a 1=4 titer with two Congo strains (Ib Ar 10200 and Semunya), in the CF Test.

New viruses to Brazil

1. Group Kwatta. The strain Be An 157575 differs slightly from kwatta virus by CF and N, being considered, by now, a kwatta subtype. Upto now four strains of kwatta virus have been recovered in the Amazon region, all of them from wild birds: 2 near Belém, in 1969 (Pyriqlena leucoptera and Thamnophilus aethiops) and two at the Km 212 of the Itaituba-Jacareacanga section of the Transamazon highway, both in 1973 (Phlegopsis nigromaculata and Myrmoborus myotherinus)

2. Ungrouped viruses. (3 agents) Be Ar 202527 was considered as a strain of Aruac virus and Be An 235467 as a strain of Trinita virus. The former agent was isolated in 1971 from a pool Culex(m) portesi collected near Belém and Be An 235467 was isolated in 1973 from blood of an Agouti (Ro 13180-cotia) originated from Barcarena, Pará State. The Be Ar 185559 strain isolated from Culex (m) portesi collected near Belém, was found indistinguishable by cross CF from Co Ar 1279, and showed a slight relationship by CF with two Rhabdoviruses : Hart Park and Kamese. EM studies of this strain carried out by Dr Ronaldo Araujo at the University of Pará confirmed that the agent is a Rhabdovirus.
In addition, 3 strains obtained from frogs were taken to YAPU, but two of them (Be An 227841 and Be An 238209) were not viable.
• The third one, Be An 228209 is not an arbovirus and more studies are needed for its characterization.

2. ARBOVIRUS STUDIES ALONG NEW HIGHWAYS OF THE AMAZON REGION

Previous studies have been described at the Information Exchange nº 28. The present report deals with 2 areas: 1. Km 212 of the Itaituba-Jacareacanga section of the TransAmazon highway (Flexal area), Nov.-Dec. 1975 2. Km 446 of the Santarém-Cuiabá highway, rio Jamanxizinho area, April-May 1976.

Human studies. Two person were bled at the Flexal area for attempted virus isolation with negative results, but 3 out 6 members of our field team got infected with P. falciparum. In addition, a German physician who spent 2 days in our camp developed a febrile illness which begun 3 days after he left the area. The fever lasted about 5 days and it was accompanied by headache and asthenia . He was treated with antipyretic drugs only. No virus could be isolated from a blood specimen taken from him 6 days after the onset of illness. A rise from <1:10 to 1:20 in HI antibody titer to Caraparu virus was demonstrated in his paired sera (taken 3 months apart).

289 people living along the Santarém-Cuiabá highway, between the intersection of this road with the TransAmazon highway and the Jamanxizinho area, were bled during April-May 1976. 259 of these persons were originated from other States and they were living in the area for several months. Of the remaining individuals, 15 were natives from Itaituba county and 15 were born in other counties of Pará State. Results of HI tests with these sera and 18 arbovirus antigens are summarized in Table 1.

It is noteworthy that 2 immigrant boys, 6 and 2 y.o., had HI titers of \geq 1:160 and 1:160 to EEE virus in their sera. The boys were living for 8 months in the area, and showed no signs of neurologic disease. N tests should be performed with these two sera against EEE virus.

Wild animals. Table 2 shows the mammals , birds and reptiles captured in the two areas, for virological and serological studies.

Virus isolations: Five strains of a possible new arenavirus were isolated from 3 Oryzomys captured in the Flexal area (see Information

Exchange nº 31). The only other agents isolated were 1 strain of Pacora (?) virus and 2 strains of WEE virus, all from wild birds:

<u>Date</u>	<u>Virus</u>	<u>Nº An</u>	<u>Nº Av</u>	<u>Specimen</u>	<u>Area</u>
Nov. 10,75	Pacora (?)	292426	5355	Blood	Flexal
April 13,76	WEE	299377	6868	"	Rio Jamanxizinho
" 20,76	WEE	299488	6979	"	" "

Serology. Up to now results of HI testing are available only for the 378 birds captured at the rio Jananxizinho area, as shown bellow, according to the % of positivity:

Group A : EEE-0;WEE-2,9%; Mayaro-12,9%; Mucambo-0; Cross-2,1%
 Group B : YF-0; Ilhéus-0; SLE-1,0%; SP H 34675-0,36%; Cross-0,79%
 Group C : Marituba -3,4%
 Group Guamá : Moju-0,5%; An 109303-1,8%; Cross-1,8%
 Bunyamwera group : Guaroa -1,58%
 Simbu group - Oropouche -5,8%; Utinga-0,26%; Cross-0,26%
 Turlock group - Turlock -1,0%
 Anopheles A group : Tacaiuma -1,58%
 Phlebotomus group - Itaporanga -5,8%
 Supergroup Bunyamwera (unassigned): An 141106 (Belém)-0,79%

The majority of positive birds belonged to the Formicariidae family.

Haematophagous insects. A total of 15,968 mosquitoes and phlebotomine flies collected at the Flexal area were inoculated in suckling mice, as 701 pools. In addition, 2.066 ectoparasites were also inoculated (36 pools). Most of these insects were combed from Oryzomys rodents: Gigantolaelaps oudemansi -1500; G. intermedia -33; G. gilmorei -5; Laelaps sp. -24; Haemolaelaps sp -180 and Laelaps pilifer -10.

At the rio Jamanxizinho area a total of 3070 insects were collected and inoculated in mice as 165 pools.

Not a single virus isolation was obtained as result of these inoculations.

Three methods of collection were used: Human bait (day time: ground & tree and night time, ground), Trinidad n° 17 baited with mice, and suction.

The suction method was the most productive, accounting for more than 90% of collections at the Flexal area and for 51% at the rio Jamanxizinho area. 80-90% of these collections were made up by Culex (melanoconium) sp. More than 90% of the insects caught in the Trinidad n° 17 were also Culex (M) sp. The same insect was present in the human bait collection done at ground level during day and night time, but in the last period only a few specimens were collected. Haemagogus mosquitoes predominated in the tree captures. The species Trichoprosopon (T) digitatum and Psorophora (J) albipes were the predominant ones in the day time collections at ground level, in the Flexal and rio Jamanxizinho areas, respectively. Several species of Aedes, Sabethes, Wyeomyia, Anopheles were also present in human bait collections, but in small numbers.

The average of insects collected by human bait varied from 3,1 to 10,3 man/hour, according to the area, timetable and height. The average of insects captured per hour by suction was 193 and 32,5 at the Flexal and rio Jamanxizinho areas, respectively.

3. VIRUS OF THE PHLEBOTOMUS GROUP NEW TO BRAZIL

During the course of a field investigation in the Alenquer county, Para State, due to the occurrence of a fatal case of yellow fever, we bled a 27 y.o. male who was in the first day of a febrile illness, and from his blood a virus belonging to the Phlebotomus group was isolated (see Information Exchange n° 31, pages 57 and 58). The patient could not be relocated until September 20, 1976, at which time a second blood sample was obtained from him (first blood collected May 31). He claimed that his illness lasted two days only and that besides the fever he had headache and myalgia.

The comparison of antibody titres in his two serum samples revealed the following results:

	<u>CF test</u>	<u>N test</u>
Acute serum (May,31)	8/8	0
Convalescent serum (Sept.,20)	16/32	1.4 (N.index)

The isolation was obtained in 3 day old swiss mice. One animal sickened on the 12 th day and two others were found dead on the 14 th day p.i. Virus reisolation was not tried yet. The A.S.T. of baby mice inoculated with a 3rd mouse passage material was 4.7 and 11.8 days by the i.c. and i.p. routes, respectively. Adult mice survived after i.c. and i.p. inoculation.

The virus strain (Be H 301101) reacted in the CF test with a Phlebotomus group mouse hyperimmune ascitic fluid. Further comparison by the CF test of the strain and its homologous ascitic fluid with six others Phlebotomus viruses found in Brazil, revealed that the agent is antigenically different from them as shown in Table 3 (which includes results obtained with antisera to Sicilian, Punta Toro and JW10 viruses).

No HA could be obtained from infected mouse brain and liver treated with sucrose - acetone, then sonified.

The agent multiplies in Vero cell kept under fluid medium, causing a CPE easily recognizable, reaching titers up to $6.5 \log_{10} \text{TCD}_{50} / 0.1 \text{ ml}$. No plaques however could be seen in the same cell line maintained under overlay medium (Bacto agar), during a 7 day period of observation. A $4.7 \log_{10} \text{LD}_{50} / 0.02 \text{ ml}$ titer was obtained in suckling mice inoculated by the i.c. route.

DCA sensitivity tests carried out in VERO cells indicated that the agent is inactivated by this chemical compound, as shown below:

Titer of control	—————	$3.75 \log \text{TCD}_{50} / 0.1 \text{ ml}$
" after DCA treatment	—————	$\leq 1.5 \log \text{TCD}_{50} / 0.1 \text{ ml}$
Amount of virus inactivated	—————	$\geq 2.25 \log \text{TCD}_{50} / 0.1 \text{ ml}$

(Francisco P. Pinheiro, Amélia P.A.T.Rosa and Jorge F.T. Rosa)

Table 1- HI antibodies to arboviruses among immigrants and natives of Para State. Santarém Cuiabá area, April- May 1976.

Group	Viruses	% of Positives		
		Immigrants		Natives
		M (134)	F (125)	M & F (30)
A	EEE	2.2	0	0
	WEE	2.2	1.6	0
	Mayaro	8.9	4.8	16.6
	Mucambo	0.7	0.8	0
	Cross	11.2	4.0	3.3
B	YF (wild)	5.2	3.2	3.3
	YF (17 D)	5.2	3.2	6.6
	Ilhéus	7.5	5.6	3.3
	SLF	0	3.2	3.3
	Cross	30	12.8	33.3
C	Caraparú	9.7	3.2	13.3
	Guamá	Catú	8.9	5.6
Bunyamwera	Guaroa	15.7	4.8	23.3
Simbu	Oropouche	2.9	3.2	3.3
	Utinga	0.7	0	0
	Cross	2.9	0.8	0
Phlebotomus	Itaporanga	6.0	0.8	10
Anopheles A	Tacaiuma	3.7	1.6	0
Buny unassigned	Belem	2.2	0.8	0
Ungrouped	Tinití	0.7	0.8	0
	Araquari	0	0	0

Table 2. Vertebrates captured at two areas of the newly opened highways of the Amazon region. 1975-76

A N I M A L S	Km 212 of Itaituba- Jacareacanga, Section of Transamazon highway, Flexal area (Nov.-Dez. 1975)	Km 446 of Santarém- Cuiabá highway, Rio Jamanxizinho area (April-May 1976)
<u>MARSUPIALS</u>		
<u>DIDELPHIS</u>	22	9
<u>MARMOSA</u>	31	-
<u>MARMOSA CINEREA</u>	7	1
<u>MARMOSA MURINA</u>	-	3
<u>MONODFLPHIS</u>	1	38
<u>METACHIRUS MUDICAUDATUS</u>	2	-
<u>PHILANDER O. OPOSSUM</u>	-	16
<u>RODENTS</u>		
<u>PROECHIMYS</u>	59	45
<u>NECTOMYS</u>	29	15
<u>RATTUS RATTUS RATTUS</u>	2	-
<u>RATTUS ALEXANDRINUS</u>	-	1
<u>ORIZOMYS</u>	47	3
<u>ORIZOMYS MACCONNELLI</u>	3	-
<u>ORIZOMYS OECOMYS</u>	8	8
<u>NEACOMYS</u>	19	2
<u>ACOUTI PACA</u>	1	-
<u>SCIURUS</u>	4	1
<u>DASYPROCTA AGUTI</u>	4	4
<u>PRIMATA</u>		
<u>ALOUATTA P. BELZERUL</u>	-	6
<u>ATELES</u>	1	-
<u>CEBUS</u>	6	7
<u>CALLITHRIX</u>	1	-
<u>CALLICEBUS MOLOCH</u>	-	2
<u>CHIROPOTES ALBINASUS</u>	-	6
<u>PHITECIA</u>	2	-
<u>LACOTHRIX</u>	3	-
<u>OTHER ANIMALS</u>		
<u>TAMANDUÁ TETRADACTILUS</u>	-	2
<u>JACURARÚ</u>	2	-
<u>CALANGO</u>	1	3
<u>MAZAMA SIMPLISICORNIS</u>	1	1
<u>NASUA NASUA</u>	1	-
<u>COBRA</u>	1	-
<u>JABOTI</u>	1	3
<u>TAYASSU PECARI</u>	-	1
<u>BIRDS</u>	559	460

Table 3. Cross HI between Be An 301101 virus and members of the Phlebotomus group

Antigens	Sera or ascitic fluids										
	Ico.	Buj.	Itap.	Anh.	Uruc.	Cand.	An301101	Sicilian	Punta Toro	JW10	Phleb. group
Icoaracy An 24262 *	16 ⁺ /8 ⁺						0				
Bujarú An 47693 *		8/8					0				
Itaporanga An 64582 ***			16/4				4/4				
Anhanga An 46825 *				8/8			0				
Urucurí An 100049 ***					16 ⁺ /8 ⁺		4/8				
Candirú H 22511 **						16 ⁺ /8 ⁺	0				
An 301101 *	0	0	0	0	0	0	16 ⁺ /8 ⁺	0	0	0	8/8

* mouse brain sucrose-acetone antigens

** mouse liver sucrose-acetone antigen

*** mouse brain crude antigen

DEPARTAMENTO DE VIRUS
 INSTITUTO NACIONAL DE HIGIENE
 "LEOPOLDO IZQUIETA PEREZ"
 GUAYAQUIL – ECUADOR

VEE has been isolated from sentinel hamsters exposed near Vinces, Los Ríos province, in February and March/76, in an area where this virus has produced outbreaks in 1.969 and in 1.972. VEE was isolated from brain, spleen, heart and skeletal muscle from three hamsters (over six exposed) in February and from the same visceras from two hamsters (over three exposed) in May. No human or equine cases have been detected in that area during the last three years. A group of 4 sentinel horses are under study and we found a HI titers of 1 :640 for VEE in one of them after 9 months living in the area. In equines three years old or younger than three years old HI for VEE titers have been $\geq 1 : 320$. In cows, serologically studied through year and a half, we have observed increasing titers of VEE HI antibodies. Mosquitoes collected in diferent months since February/75 are under study.

The results of a national equine survey have shown the following results in HI test with titers $\geq 1 : 80$.

Total tested	VEE	°/o	EEE	°/o	WEE	°/o	SLE	°/o
422	191	45.26	51	12.85	38	9.0	6	1.42

EEE has been isolated in Ecuador from mosquitoes and from the blood of cormorant in 1.975.

DR. ERNESTO GUTIERREZ V.

(Received October 26, 1976 – Editor)

Venezuelan equine encephalitis surveillance

VEE virus has been isolated from sentinel hamsters by personnel of the Instituto Venezolano de Investigaciones Cientificas (IVIC) in the Cata-tumbo region, southeast of Maracaibo Lake. In May, 1976 two young donkeys, free of antibodies against VE, EE and WE viruses (tested by HI), were placed in two different farms in a swampy area bordering Maracaibo Lake (Distrito Colon, Estado Zulia). A month later they were bled and HI titers of 1:80 and 1:160 were encountered for VEE only. No signs of disease was reported. Sentinel hamsters were exposed in the area with negative results.

To investigate further whether or not VEE virus is cyclic or endemic in the area, another young donkey free of antibodies for VE, EE and WE viruses has been placed in the area. Results are pending.

Eastern equine encephalitis

As reported in the September issue of the Information Exchange (p 52), eastern equine encephalitis virus was isolated for the first time in Venezuela in February 1976, from horses in an area close to that where we reported VEE conversion. No isolation of the EE virus or any other virus were obtained in our laboratory from 12 sera of equidae apparently healthy or showing signs of disease. HI tests performed on 42 human sera (13 months to 62 years) resulted in 4 VEE positive only. From 149 equidae sera, 29 were positive for VEE only, 20 for EE only, and 60 had antibodies for both VEE and EE.

(Slavia Ryder)

REPORT FROM THE MINISTRY OF AGRICULTURE,
GUYANA AND THE CARIBBEAN EPIDEMIOLOGY CENTRE
(CAREC), TRINIDAD.

SEROLOGICAL SURVEY OF EASTERN EQUINE ENCEPHALITIS
AND OTHER ARBOVIRUSES IN GUYANA

There have been periodic outbreaks of Eastern Equine Encephalitis (EEE) amongst equines in Guyana. Concerned with this problem the Ministry of Agriculture (Guyana) jointly with CAREC undertook a programme to study the distribution of EEE and other arbovirus activity by antibody determinations in equines. It was hoped that such a programme might indicate areas of activity leading to a programme to determine the vector, reservoir host(s), and human involvement, if any.

For sampling purposes the country was divided into six (6) blocks or regions as follows:

Block 1: Corentyne Coast

Block 2: West Berbice and Upper Demerara (Mahaica to Berbice River)

Block 3: Georgetown and East Bank Demerara

Block 4: West Demerara, Essequibo Coast and Mathews Ridge

Block 5: North Rupununi

Block 6: South Rupununi

It was planned that 100 sera would be collected from equines from each Block, but by the end of the year 367 samples were received at CAREC (Table 1). Sera were tested in the standard haemagglutination inhibition (HI) test using in addition to EEE, the following antigens: Venezuelan Equine Encephalitis (VEE), Western Equine Encephalitis (WEE), St. Louis Encephalitis (SLE), and Maguari (MAG).

Results of the tests are shown in the accompanying Table. A

total of 58 sera (15.8%) had detectable antibodies to one or more of the antigens. Positive reactions were more common to EEE (32/367; 8.7%) than to the other viruses with a preponderance of positives on the Corentyne Coast (9/46; 19.6%) and South Rupununi (7/29; 24.1%). Overall the latter area seemed to have the highest rate of positive reaction (41.4%).

Although VEE would be expected to occur in Guyana on geographic and ecological grounds, it has never been isolated from Guyana. It is interesting to note that 10 sera reacted to VEE antigen. Of the 10 positive sera, 8 were found on the Essequibo Coast. Only 1 serum reacted to WEE antigen. There were 6 positive sera to SLE, but these may represent generalized Group B cross reactions. None of the sera was positive to Maguari antigen. This survey is to be completed in 1977 when a detailed analysis will be made.

RESULTS OF HAEMAGGLUTINATION INHIBITION TESTS USING 5 ANTIGENS
ON EQUINE SERA COLLECTED FROM 6 REGIONS OF GUYANA

(Percent positive is given in brackets)

REGION	NO. TESTED	VEE	WEE	EEE	SLE	VEE EEE	WEE EEE	SLE EEE	TOTALS
Corentyne	46			9 (19.6)	2 (4.4)			2 (4.4)	13 (28.3)
West Coast Berbice	59	1 (1.7)		5 (8.5)	1 (1.7)	1 (1.7)			8 (13.6)
East Coast Demerara	86		1 (1.2)	5 (5.8)		1 (1.2)			7 (8.1)
West Demerara and Essequibo Coast	106	8 (7.6)		3 (2.8)	3 (2.8)				14 (13.2)
North Rupununi	41			3 (7.3)				1 (2.4)	4 (9.8)
South Rupununi	29	1 (3.5)		7 (24.1)		1 (3.5)	2 (6.9)	1 (3.5)	12 (41.4)
TOTAL	367	10	1	32	6	3	2	1	58
% POSITIVE		(2.7)	(0.3)	(8.7)	(1.6)	(0.8)	(0.5)	(0.3)	(15.8)

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

Dengue in the U.S. Virgin Islands, 1976-77

In December 1976, sera from several suspect cases of dengue were received from St. Thomas, U.S. Virgin Islands. In January 1977, convalescent sera from these cases and acute sera from further cases were received. Several of the paired specimens showed diagnostic rises in hemagglutination inhibition (HI) antibody titers for dengue, and four strains of flavivirus were isolated by the inoculation of acute sera into male Aedes aegypti and fluorescent antibody (FA) staining of the heads. Two of these isolates were of sufficient titer after one mouse passage to identify by complement fixation (CF) as dengue type 2. They were:

<u>Lab No.</u>	<u>Residence</u>	<u>Sex</u>	<u>Age</u>	<u>Onset</u>
H-11839	St. Thomas	F	40	1-10-77
H-11930	St. John	M	22	1-16-77

The resident of St. John works in St. Thomas.

As a result of an invitation by the Commissioner of the Virgin Islands Health Department, personnel from the San Juan Laboratories and three EIS officers, together with Virgin Islands Health Department personnel, carried out a house-to-house survey in St. Thomas beginning on Saturday, January 29. The results, together with data on specimens collected for surveillance, and laboratory confirmation are shown below.

	<u>Week Ending</u>	<u>House-to-House Survey</u>		<u>Surveil- lance</u>	<u>Total</u>	<u>Confirmed</u>
		<u>Rural</u>	<u>C.A.*</u>			
November 1976	6	3			3	
	13			1	1	
	20	6	2		8	
	27	1			1	
December 1976	4	2		2	4	1
	11			2	2	
	18	15	2	6	23	3
	25	5	2	10	17	6
January 1977	1	8	3	2	13	
	8	5	5	4	14	1
	15	8	3	3	14	2
	22	5	1	4	10	1
	29	16	6	8	30	
February 1977	5	1	2	10	13	
	12			3	3	
Total		75**	26	55	156	14

*Charlotte Amalie, St. Thomas

** +3 cases with no date of onset = 78

The total for the week ending January 29 may have been influenced by the fact that the survey was begun then.

Confirmed cases total 35% of those surveillance cases for which we have so far received second blood specimens. The overall results of the survey, on preliminary analysis, were:

	Charlotte Amalie		Rural		Total	
	No.	%	No.	%	No.	%
Occupied households	106	-	239	-	345	2.5*
Clinical attack rate	26/340	7.6	78/757	10.3	104/1097	9.4
Samples collected	194	57.0	391	52.0	585	53.0
Households with dengue	14	13.2	50	21.0	64	18.6

*Based on Virgin Islands Planning Office estimate of 14,000 dwellings in mid-1976.

Serological testing of the samples obtained is underway.

Continuing Dengue in Puerto Rico

Cases reported to San Juan Laboratories:

<u>Onset</u>	<u>1976</u>	<u>1975</u>
July	9 (0)*	8 (1)
August	13 (0)	27 (4)
September	22 (1)	64 (33)
October	35 (2)	60 (36)
November	36 (6)	177 (63)
December	42 (7)	194 (57)
January 1977	29 (-)	48 (10) 1976

*() Laboratory confirmed by serology

Geographical distribution: Mainly south coastal (Peñuelas, Salinas) and hill districts (Villalba, Adjuntas). First confirmed case from San Juan metropolitan area since May 1976 had onset November 19, 1976. Most recent suspect case from San Juan had onset February 13, 1977.

Isolations: One isolate from Salinas from a case which had onset on December 13, 1976, has been identified as dengue type 2.

Vector situation: Rainfall was only 75% of average during 1976.
Aedes aegypti ovitrap positivity rates in metropolitan San Juan were:

March	7.4%	June	24.3%	September	32.8%
April	10.1%	July	32.3%	October	34.0%
May	23.5%	August	30.7%	November	28.7%

South coast A. aegypti populations have declined from a peak in October. The affected southern districts have been sprayed by Vector Control.

(J. P. Woodall, R. H. López-Correa, C. G. Moore, G. E. Sather, G. Kuno, E. Ruiz-Tibén)

REPORT FROM THE DEPARTAMENTO DE VIROLOGIA

HOSPITAL GENERAL DE MEXICO, S.S.A.

AND

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

VENEZUELAN EQUINE ENCEPHALITIS HEMAGGLUTINATION-INHIBITION
ANTIBODY SURVEY; HUMAN SERA FROM SOUTHEAST MEXICO COLLECTED
IN 1974

Venezuelan equine encephalitis virus (VEE) was the etiological agent of the epidemic that occurred in Mexico in 1971.

A serological survey to investigate the VEE virus activity was carried out in the sera of humans collected during the months of February, March and April of 1974. The specimens came from different places of the southeastern states of Mexico: Veracruz, Campeche, Tabasco, Chiapas, Yucatán and Quintana Roo.

The hemagglutination-inhibition test was used and the results are presented in the following table:

Dr. César Wong-Chía
Apartado 70-434
Ciudad Universitaria
México 20, D. F.

VENEZUELAN EQUINE ENCEPHALITIS HI ANTIBODIES
IN HUMAN SERA FROM SOUTHEASTERN MEXICO*, COLLECTED IN 1974

AGE GROUP	NUMBER OF CASES	RESULTS				TOTAL OF POSITIVES
		POSITIVE**		NEGATIVE		
		MALE	FEMALE	MALE	FEMALE	
0 - 4	4	1	0	1	2	1
5 -14	36	2	4	17	13	6
15 -64	88	12	18	18	40	30
65 +	2	0	1	0	1	1
TOTAL	130	15	23	36	56	38

* The states represented are Veracruz, Campeche, Tabasco, Chiapas, Yucatán and Quintana Roo.

** HI positive titers ranged from 10 to 1280 against 4 HA units of VEE antigen.

Incidence of antibodies against VEE in certain animal species in Huixtla,
Chiapas, México.

At the end of 1974 and at the beginning of 1975, an outbreak of what was diagnosed as Venezuelan equine encephalitis occurred in Guatemala near the border with México. The ecologic conditions in the zone where the disease appeared are similar to those in the coast of Chiapas State, in México. However, in this zone there were no cases of encephalitis in human beings or equines.

The present study was done to determine if there is viral activity in the zone of Huixtla, Chiapas and if there has been a recent increase of viral activity which has not been manifested as disease in human beings or in equines.

In April and May of 1975, sera from different animals were obtained. Seventy eight bovines were divided in two age groups, 51 animals under 2 years of age and 27 animals above 2 years of age. Sera were also collected from 27 hens, 9 ducks, 4 turkeys, 7 dogs, 2 toads (Bufo spp), and 1 coatete (Basiliscus spp)

All the sera were tested with the hemagglutination inhibition test using as antigens, VEE virus TC-83 strain and 63U2 strain, Eastern Equine Encephalitis (EEE) virus, and Western Equine Encephalitis (WEE) virus. Control sera were

collected from 21 cattle 1 to 2 years of age from Tepetzotlán in the State of México. This area is considered free of VEE.

Of 51 bovines under 2 years of age, 27 had HI antibodies against VEE virus TC-83 strain. With titers reaching 1:320 in sera of two animals. In the group of 27 animals over 2 years of age, 16 had HI antibodies reaching a titer of 1:640 in one animal, and 1:320 in two animals. The HI titers were higher and more sera were positive when the 63U2 strain was used as antigen. This suggests that the antibodies were probably formed in response to an enzootic or epizootic virus in the area which recently has increased its activity.

Only sera of two dogs had antibodies against VEE (titer 1:40). The rest of the animals did not have antibodies against VEE.

None of the sera had HI antibodies to EEE or WEE above a titer 1:40.

The results show that the VEE virus is present in the area as animals under 2 years of age had antibodies, and the results also indicate that the infection was recent as high antibody titers were found. The fact that VEE is not present clinically in human beings or equines might be due to the fact that the equines are vaccinated every year or that the VEE strain has a low pathogenicity for man or animals.

(M. Gallo de la Torre, M.L. Zárate, C.R. Bautista G., C. Rosales, A. Morilla González)

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

August 1, 1976, through December 31, 1976.

Mosquitoes, Isolation

2293 pools of mosquitoes were tested, numbering, 21,513 mosquitoes. There were 11 isolates. Following is a list of isolates:

State #	Location	Species of Mosquito	Collection Date	Isolate
11200	Wichita Falls	C. quinquefasciatus	July 20, 1976	Hartpark
11202	Wichita Falls	C. quinquefasciatus	July 20, 1976	Hartpark
11591	El Paso	C. tarsalis	August 10, 1976	Wee
11663	Dallas	C. quinquefasciatus	August 3, 1976	Hartpark
11666	Dallas	C. quinquefasciatus	August 3, 1976	Hartpark
11668	Lubbock	C. tarsalis	August 11, 1976	Wee
11670	Lubbock	C. tarsalis	August 11, 1976	Hartpark
11911	Dallas	C. quinquefasciatus	August 18, 1976	SLE
11925	Dallas	C. quinquefasciatus	August 18, 1976	SLE
12019	Dallas	C. quinquefasciatus	August 26, 1976	SLE
12020	Dallas	C. quinquefasciatus	August 26, 1976	SLE and Hartpark

Wild Birds, Serology HI L:10>

Location	Collection Date	Negatives	New Positives	Antibody Detected
Tyler	September 16, 1976	3	0	None
Tyler	9-21-76	8	0	None

SENTINEL FLOCKS

Harlingen	August 31, 1976	73	0	None
"	September 30, 1976	68	0	None
"	November 4, 1976	65	2	SLE 1 WEE 1
"	December 2, 1976	64	0	None
"	December 16, 1976	61	0	None
Lubbock	August 1976	43	2	Wee
"	September 15, 1976	24	23	Wee 21 Vee 1 SLE 1
"	October 18, 1976	40	4	Wee 3 SLE 1
"	November 3, 1976	41	3	WEE
Dallas	August 2, 1976	115	0	None
"	August 25, 1976	94	10	SLE
"	September 7, 1976	99	8	SLE
"	October 4, 1976	72	9	SLE
"	November 9, 1976	142	17	SLE 15 VEE 2

Wild Birds, Isolation

<u>Location</u>	<u>Species</u>	<u>Number</u>	<u>Isolate</u>	<u>Date Collection</u>
Abilene	Nestling Sparrows	4	None	August 1976
Harlingen	House Sparrows	38	None	October 1976
Dallas	Cootie Bird	2	None	November 1976
	Pigeons	8	None	December 1976
	Grakyls	2	None	November 1976
	Blackbird	1	None	November 1976
	White Dove	1	None	November 1976
Lubbock	English Sparrows	10	WEE 1 Hartpark 1	August 1976
(Charles E. Sweet)				

DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES
 OFFICE OF LABORATORY SERVICES
 POST OFFICE BOX 210
 JACKSONVILLE, FLORIDA 32201

With the increased number of confirmed human SLE cases reported in neighboring states in 1976, our increased surveillance system did not detect any confirmed human cases of SLE in Florida. Where histories could be obtained, the patients with constant Group B titers had previously resided in areas of the United States where SLE had been reported, or in known endemic areas of the Caribbean. As in the past, as part of the general virus diagnostic services provided to the medical community, we have tested the sera of 570 patients against a battery of antigens associated with central nervous system diseases during the period from July 1976 to December 1976. The EEE reactor is a nine-month female infant from Georgia, who was hospitalized in Florida in September 1976. At this time, the patient appears to have made a complete recovery.

(E. E. Buff)

Human and Animal Sera Screened
 by the HI Technique with Arbovirus Antigens*

July 1976 - December 1976

Species	Number of Sera	Reactors
Human	570	10 Group B** 1 EEE
Horses	22	4 EEE 4 WEE
FIELD SPECIMENS Avian Sera	1442	3 SLE***
TOTAL	2034	22

* Arbovirus Antigens:

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

** Patients paired sera had constant low level HI antibodies to SLE and Dengue antigens.

*** North Florida Counties

REPORT FROM THE SPECIAL PATHOGENS BRANCH, VIROLOGY DIVISION, BUREAU
OF LABORATORIES, CENTER FOR DISEASE CONTROL, ATLANTA, GA

An outbreak of a severe febrile disease with hemorrhagic manifestations and an alarmingly high fatality rate occurred in the Equateur Region, Bumba Zone of northern Zaire during September–November 1976. A similar outbreak in Southern Sudan had preceded the one in Zaire by at least one month, the probable first case having been traced as far back as 27 June with death ensuing on 6 July 76.

Data collected by the epidemiological field team in Zaire gave a provisional total of approximately 262 cases with 245 deaths. The epicenter was located in the Yambuku Mission Hospital run by a Belgian staff. The probable first case was admitted on 5 September and died on 7 September 1976. In the subsequent five weeks 13 members of the hospital staff of 17 became sick. Tragically only 2 of the 13 cases survived. What appeared during the height of outbreak to be a highly contagious disease was found later to be far less infectious by person-to-person spread, and approximately one third of the cases were traced to contaminated syringe-needle transmission.

The causative agent, Ebola virus, is morphologically identical to Marburg virus by electron microscopy but immunologically distinct by the indirect FA test (1, 2, 3). The virus causes cytopathic effect in Vero cell cultures and death in guinea pigs. Acute blood specimens from 8 of 10 cases yielded virus with titers ranging from 3.5 – 5.5 TCID₅₀/ml. At this time, 850 sera from convalescents, asymptomatic contacts or controls have been tested for presence of FA antibodies using the indirect test. 25 sera with FA titers of 1:64 or > were found. Further serologic testing of sera from "negative" control villages is in progress.

The most encouraging outcome of this outbreak has been the collection of 204 units of plasma from 24 donors in Zaire. Most of the units have FA antibody titers of 1:64 or greater. Hepatitis-antigen negative plasma will be pooled and lyophilized for use in the event of a new occurrence of the disease.

(P.A. Webb, K. M. Johnson, H. T. Wulff, F. A. Murphy, J. G. Bremen,
M. K. White, D. L. Heymann, S. O. Foster, J. McCormick)

(Note from editor: Reports by Belgian, British and American investigators on the African Hemorrhagic Disease outbreaks in Sudan and Zaire will appear in a March issue of Lancet. See also the report from Dr. S. R. Pattyn, Antwerp, Belgium, in this issue of the Info-Exchange)

References:

1. Pattyn, S. R., W. Jacob, G. van der Groen, P. Plot and Courteille. Isolation of a Marburg-like virus from a case of hemorrhagic fever in Zaire. Lancet. March 12, 1977.
2. Bowen, E. T. W., G. S. Platt, G. Lloyd, A. Baskerville, W. J. Harris and E. Vella. Outbreaks of Viral Hemorrhagic Fever in Southern Sudan and Northern Zaire, 1976. Preliminary studies on the etiologic agent. Lancet. March 12, 1977.
3. Johnson, K. M., P. W. Webb, J. V. Lange and F. A. Murphy. Isolation and Partial Characterization of a New Virus Causing Acute Hemorrhagic Fever in Zaire. Lancet. March 12, 1977.

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

Surveillance for Human Arboviral Infections - United States, 1976

In 1975, widespread outbreaks of St. Louis Encephalitis (SLE) resulted in more cases of SLE (1,791) from more States (30) than for any other year. Nationwide reporting by CDC largely depended on periodic telephoned surveys of the States for information. In June 1976, at the request of the Conference of State and Territorial Epidemiologists, we initiated a weekly collection of the cumulative totals of human cases and deaths from laboratory documented, arboviral infections.

A total of 692 arboviral infections were reported: 372 cases of SLE including 17 deaths, 273 cases of Colorado Tick Fever, 42 cases of California Encephalitis, 2 cases of Western Equine Encephalitis, and 1 case of dengue. The first SLE cases occurred at nearly the same time in widely separate locations (California 6/30, Mississippi 7/2, Tennessee 7/7). SLE was reported by 26 States; 3 States (Texas, Mississippi, and Alabama) reported 64% of the cases. The largest outbreaks occurred in Tuscaloosa and Mobile, Alabama, Dallas and Houston, Texas, and Coahoma County, Mississippi.

Advantages of weekly reporting were improved timeliness, specificity, and consistency with economy of effort. The major limitation remains the prolonged interval between clinical onset and laboratory diagnosis of arboviral etiology.

(Karl Kappus, John Sullivan-Bolyai, and Lawrence Schonberger)

REPORTS OF ARBOVIRAL INFECTIONS - UNITED STATES, 1976

STATE	SLE		CE	WEE	CTF
	Cases	Deaths	Cases	Cases	Cases
Alabama	67	4			
Alaska					
Arizona	2				
Arkansas	4	1	1		
California	3				10
Colorado	1			1	218
Connecticut					
Delaware					
Dist. of Col.					
Florida	1				
Georgia	2				
Hawaii					
Idaho					
Illinois	18		5		
Indiana	18	1	3		
Iowa	1				
Kansas	5				
Kentucky	5				
Louisiana	9				
Maine					
Maryland	3				
Massachusetts					
Michigan	2				
Minnesota					
Mississippi	79	6			
Missouri	19	1			
Montana					19
Nebraska					
Nevada					
New Hampshire					
New Jersey					
New Mexico					
Upstate New York	1		2		
New York City					
North Carolina					
North Dakota	2				
Ohio	9		14		
Oklahoma	4				
Oregon					
Pennsylvania					
Rhode Island					
South Carolina					
South Dakota	1				1
Tennessee	21	1			
Texas	93	3			
Utah					24
Vermont					
Virginia	1				
Washington				1	1
West Virginia					
Wisconsin	1		17		
Wyoming					
Totals	372	17	42	2	273

REPORT FROM THE VECTOR BIOLOGY AND CONTROL DIVISION (VBCD)
BUREAU OF TROPICAL DISEASES, CENTER FOR DISEASE CONTROL
ATLANTA, GEORGIA

Insecticide Susceptibility Studies

Ineffective control of mosquitoes of the Culex pipiens complex with ground ultra-low-volume (ULV) malathion applications was first suspected by the Memphis and Shelby County Health Department during the 1975 season. The St. Louis Encephalitis (SLE) epidemic during that year made it imperative that an evaluation of the effectiveness of malathion and other compounds registered for use in ULV equipment be initiated. Field studies were carried out in Memphis during August-September 1976 while laboratory studies were being completed in Atlanta with freshly colonized mosquitoes of the Culex pipiens complex from Memphis, Tenn., and Birmingham, Ala.

Organophosphorous resistance was confirmed in Culex pipiens complex mosquitoes from Memphis, Tenn. Field and laboratory studies with fenthion, malathion, naled, and resmethrin gave comparable results. Birmingham Culex pipiens quinquefasciatus were tested in the laboratory against the same four compounds and found to be susceptible to all. The organophosphorous compounds resulted in approximately 30% kill of laboratory and field specimens from Memphis at the highest dosage level. One hundred percent kill of Birmingham Culex p. quinquefasciatus was recorded at the same dosage levels in the laboratory. Both Culex spp. were susceptible to resmethrin.

(R. Taylor)

The Culex pipiens Complex in Western Tennessee

It is generally accepted among mosquito biologists that the State of Tennessee is in the "zone of intergradation" where both C. pipiens pipiens and C. p. quinquefasciatus occur as discrete taxonomic entities as well as a hybrid form or "intergrade". The significance of these entities in terms of the epidemiology and control of St. Louis Encephalitis (SLE) is not known. Since there is evidence of a vectorability differential between C. p. pipiens, the northern form, and C. p. quinquefasciatus, the southern form, as well as the possibility of some behavioral differences, e.g., attraction to light and host preference, that might bear on interpretation of epidemiologic data associated with disease transmission, the taxonomic makeup of the population in West Tennessee (Shelby County) should be determined. These studies, along with continuing surveillance activities, should strengthen our knowledge of factors resulting in epidemics of SLE and on ability to measure and control vector populations in the vicinity of Shelby County, Tenn. Results would be applicable to other areas involving this vector species complex.

During the summer of 1976, 3 shipments of preserved male specimens of the Culex pipiens complex were received from the Memphis-Shelby County Health Department. All shipments represented randomly collected males from natural daytime resting sites used by the Health Department in indexing the local population and represented early season, mid-season, and late season populations.

Slide mounts of the male genitalia were prepared for each specimen received and the specimen identified as C. p. pipiens, C. p. quinquefasciatus, or intergrade based on established characteristics. (Sundaraman 1949. Amer. J. Hyg. 50(3):307-314.)

The data are insufficient to determine significance to disease transmission or control efforts. However, there is indication that further pursuit of such studies might be fruitful.

Overall, of 119 specimens examined, 11.7% were C. p. pipiens, 41.2% C. p. quinquefasciatus, and 47.1% intergrades (hybrids) of the two. The percentages did change seasonally with a high of 42.9% C. p. pipiens in the May 16 collection, and a low of 4.6% in the July 19 collection, and 12.5% in the October 13 collection. On the other hand, C. p. quinquefasciatus seemed to be more stable with 35.7%, 35.4%, and 52.5% in the May, July, and October collections, respectively. Intergrades peaked to 60% in July when the C. p. pipiens population was lowest at 4.6%.

The Memphis-Shelby County data are shown in the following table:

Results of identification, Culex pipiens complex,
Memphis-Shelby County, Tennessee, 1976 based on male genitalia

Date of collection	No. specimens examined	<u>C. p. pipiens</u>		<u>C. p. quinquefasciatus</u>		Intergrades	
		No.	%	No.	%	No.	%
May 16	14	6	42.9	5	35.7	3	21.4
Jul 19	65	3	4.6	23	35.4	39	60.0
Oct 13	40	5	12.5	21	52.5	14	35.0
	119	14	11.7	49	41.2	56	47.1

Ten specimens collected in late summer from Birmingham, Ala., were all C. p. quinquefasciatus, revealing a less complicated population pattern than the Memphis area.

There is some indication that there are sites and localities in the Memphis area that are dominated by one of the taxonomic entities. While this limited study is insufficient to be meaningful in terms of population measurement, disease relationships and significance of insecticide

resistance, it does seem to indicate the need for more detailed study of a serial nature in precise localities which might lead to very practical associations.

(S. Breeland)

Aedes aegypti Survey

During the period from May-October, 1976, urban areas in 10 southeastern states were visited as part of a limited survey for Aedes aegypti. This is the first such survey since the US eradication effort was discontinued in 1969. Survey efforts in 1976 were limited to cities within the area of infestations reported on prior surveys.

In order to visit as many cities as possible during the 1976 breeding season, the survey method was generally limited to finding the first infestation in the city and then moving on to the next city. Tinker and Hayes (1959) reported that when Aedes aegypti were common in cities they were inspecting, specimens were usually found in the first few habitats inspected. During the present survey only suitable habitats in each city were inspected and the city was visited during a period when climatic conditions were favorable.

Using this method, Aedes aegypti were found on the first or second premises visited in 29 of the 30 cities inspected during 1976 (see table). They were not found in Memphis during the 1/2 day spent there. In all other cities inspected they were easily found and may be considered common.

The results of this survey and observations made by health officials in the areas surveyed would tend to support a conclusion that Aedes aegypti populations in the 10 southeastern states have fully recovered from the efforts made toward their eradication during the period 1964-1969. Most urban areas in the 10 southeastern states must be considered as being receptive to dengue and yellow fever viruses because of their Aedes aegypti populations and probably very low levels of immunity to the viruses in human populations.

(D. Eliason)

Results of Aedes aegypti survey - Southeastern United States - 1976

<u>Cities inspected*</u>		<u>Strain Established in Chamblee</u>
Texas	Harlingen	+
	McAllen	+
	Laredo	+
	San Antonio	
	Houston	+
	Dallas	+
Arkansas	Little Rock	
	El Dorado	+
	Texarkana	+
Louisiana	New Orleans	+
	Alexandria	+
Mississippi	Meridian	+
	Jackson	+
Tennessee	Memphis	
	Nashville	
Alabama	Birmingham	+
	Mobile	+
Georgia	Atlanta	+
	Savannah	+
North Carolina	Charlotte	+
South Carolina	Spartanburg	+
	Columbia	+
	Charleston	+
Florida	Tallahassee	+
	Jacksonville	+
	Orlando	+
	Tampa	+
	Bradenton	
	Miami	+
	Ft. Lauderdale	+

* All cities except Memphis yielded Aedes aegypti at the first or second premises inspected.

REPORT FROM THE DEPARTMENT OF ENTOMOLOGY, WALTER REED ARMY INSTITUTE
OF RESEARCH, WASHINGTON, D.C. 20012

Since the occurrence of the St. Louis Encephalitis (SLE) virus epidemic in the U.S. and Canada in 1975, investigations have been conducted to test the hypothesis that SLE virus persists in adult diapausing Culex pipiens during the winter season. These studies have involved the collection of C. pipiens from overwintering sites and the subsequent inoculation of mosquito suspensions intracerebrally into suckling mice. This report deals only with those C. pipiens collected from one site in Prince Georges County, Maryland.

Of a total of 312 C. pipiens was collected from this site during January 1977, one mouse lethal agent having an incubation period consistent with that of SLE virus has been isolated from a pool of 10 mosquitoes collected 26 January. Preliminary identification employing the smb-1 virus suspension and SLE virus hyperimmune antiserum in plaque reduction neutralization tests strongly indicated that the isolate is closely related, if not identical to prototype SLE virus. The validity of the virus isolate is supported by 1) virus reisolation from the original mosquito suspension, 2) quantity of virus in the original mosquito suspension was 2×10^4 pfu/0.2 ml as assayed in LLC-MK₂ cells 3) Infectivity of the original mosquito suspension was completely neutralized by SLE virus reference antiserum, 4) Isolation and identification was conducted in a laboratory where SLE virus had not been handled for more than six months prior to the initiation of the virus isolation studies.

(C.L. Bailey, B.F. Eldridge, D.E. Hayes, D.M. Watts, and R.F. Tammariello)

Salt-Dependent Hemagglutination with Bunyaviridae Antigens

A recent report from Japan of salt-dependent hemagglutination (SDHA) for Akabane, a Simbu group virus, prompted examination of other Bunyaviruses for SDHA. After confirming SDHA with a Simbu group antigen, we selected other Bunyavirus antigens with low or nondetectable HA titers to see if they could be satisfactory antigens when used with high NaCl molarities.

Representative sucrose-acetone extracted antigens from 10 serologic groups were used. Some of these had been sonicated. Two LaCrosse virus tissue culture antigens obtained by tween-80-ether extraction were included.

HA and HI were carried out according to Clarke and Casals using goose erythrocytes in phosphate buffers with molarities of 0.15, 0.25, and 0.4 NaCl. Antigens were titrated at final pH's 5.75, 6.0, 6.2, and 6.4 except for Congo antigens at pH 6.8, 7.0, 7.2, and 7.4. Four HA units were employed in HI tests.

Hyperimmune mouse ascitic fluids prepared to each antigen were acetone extracted and goose cell adsorbed before use in HI tests.

Table 1 shows the effect of increased NaCl molarity on 29 Bunyavirus antigens representative of 10 different serologic groups. Enhancement of titer was typically greatest at pH 5.75. Nine antigens had >4 -fold increase in HA titer in hypertonic saline. Four of these (Anopheles A, Bwamba, LaCrosse, and Oropouche) had no demonstrable HA at 0.15M, but were higher titered at higher NaCl molarities.

Table 2 demonstrates the specificity of representative enhanced agglutinins by HI. In all cases, agglutination was inhibited by homologous antiserum, and in cases of cross-reaction, the homologous antiserum inhibited to a higher titer. The two LaCrosse tissue culture antigens had titers of 1:2048 at 0.4M NaCl, but specificity could not be demonstrated by HI. The source of these non-specific salt-dependent agglutinins is undetermined.

Hypertonic saline had no demonstrable effect on the HA titer of 7 of 29 antigens. Twenty-two of the antigens had ≥ 2 -fold increase in HA. Nine of these were ≥ 4 -fold increases. Sonicated antigens accounted for most (89%) of those with ≥ 4 -fold increases, while none of those without a demonstrable HA enhancement had been sonicated.

Table 3 shows the cumulative effect of increased NaCl and sonication on the same antigen. The effect was additive resulting in increased HA titer. The LaCrosse antigen, for example, was unusable at 0.15M NaCl,

but titered 1:32 at 0.4M NaCl after sonication. The Sicilian and Tahyna antigens were functional at 0.15M NaCl without sonication, but the combination of techniques resulted in higher HA titers.

The mechanism by which high NaCl increases Bunyaviridae HA titer is not known. That some antigens in our experiments did not increase in titer at high NaCl does not mean lack of capability of enhancement since optimum conditions of virus substrate, molarity, pH, and erythrocyte type may not have been achieved in these experiments. The results do, however, indicate the broad occurrence of the salt-dependent hemagglutinin among Bunyaviridae.

Recent titration of representative Togavirus antigens using buffers of 0.15, 0.25, 0.4, 0.6, and 0.8 M NaCl ranging from pH 5.75 to 7.4 did not reveal common enhancement of HA titer as found with Bunyaviruses. A broadening of hemagglutination activity into new pH ranges was noted, and one Togavirus CF antigen lacking a useable HA at 0.15M titered 1:160 at 0.8M NaCl.

Investigation continues to determine the distribution of this phenomenon in other taxons and the nature of the salt-dependent hemagglutinins.

Barry Beaty, Robert Shope, and Delphine Clarke

TABLE 1. Effects of increased NaCl molarity on hemagglutination (HA) titers of 29 Bunyaviridae antigens

<u>Antigen</u>	<u>Sonicated</u>	Reciprocal of HA titer at NaCl Molarity		
		<u>0.15</u>	<u>0.25</u>	<u>0.4</u>
Anopheles A	yes	<4*	128	256
Apeu	no	<4	<4	<4
Bunyamwera	no	<4	8	8
Buttonwillow	yes	16	32	64
Bwamba I**	yes	<4	64	128
Bwamba II**	no	<2	<2	<2
Bwamba II	yes	<2	<2	2
California	yes	2	<2	16
Capim	yes	8	16	16
Congo I	yes	64	32	128
Congo II	yes	<2	<2	4
Guajara	yes	16	64	64
Itaqui	yes	64	64	128
Jurona	no	<2	<2	<2
Keystone	no	<4	<4	<4
LaCrosse I	no	<2	<2	2
LaCrosse I	yes	<2	4	32
Manzanilla	no	<2	<2	<2
Oropouche	yes	<4	64	128
Sicilian I**	no	<4	8	8
Sicilian II**	no	32	64	512
Sicilian II	yes	64	512	≥1024
Snowshoe hare	no	4	8	8
Tahyna I**	yes	4	8	8
Tahyna II**	no	16	32	32
Tahyna II	yes	256	128	512
Turlock	no	4	4	2
Trivittatus	no	2	<2	<2
Witwatersrand	no	16	16	32

*Viral adjusting diluents were pH 5.75, 6.0, 6.2 and 6.4 for all antigens except Congo with which diluents of pH 6.8, 7.0, 7.2 and 7.4 were used. Optimal HA pH is not listed, but enhancement was typically greatest at 5.75.

**Different antigen preparations.

TABLE 2. Specificity of enhanced hemagglutination titers as determined by hemagglutination inhibition

<u>Antigen*</u>	<u>NaCl Molarity</u>	Homologous	California (BFS-283)	<u>Antiserum</u>		
				Pongola	Lukuni	Normal
Anopheles A	0.25	320**	0	0	0	0
Anopheles A	0.4	320	0	0	0	0
Bwamba	0.25	160	0	0	0	0
Bwamba	0.4	320	0	0	0	0
Guajara	0.4	80	0	0	0	0
LaCrosse	0.4	40	0	0	N.D.	N.D.
Sicilian	0.15	80	0	0	N.D.	N.D.
Sicilian	0.4	80	0	0	N.D.	N.D.
Tahyna	0.15	80	20	0	N.D.	N.D.
Tahyna	0.4	160	40	0	N.D.	N.D.

*All antigens were sonicated.

**Reciprocal of highest serum dilution inhibiting agglutination by four units of antigen; 0, no inhibition at 1:10 serum dilution; N.D. = not done.

TABLE 3. Effects of sonication and increased NaCl molarity on hemagglutination (HA) titers of 3 Bunyaviridae antigens

<u>Antigen</u>	<u>Sonicated</u>	<u>NaCl molarity</u>	<u>HA titer (reciprocal)</u>
LaCrosse	no	.15	0
	no	.25	0
	no	.4	2
	yes	.15	0
	yes	.25	4
	yes	.4	32
Sicilian	no	.15	32
	no	.25	64
	no	.4	512
	yes	.15	64
	yes	.25	512
	yes	.4	>1024
Tahyna	no	.15	16
	no	.25	32
	no	.4	32
	yes	.15	256
	yes	.25	128
	yes	.4	512

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

Arbovirus Surveillance 1976

A total of 5,766 pools of 295,444 wild-caught mosquitoes collected statewide during the summer of 1976 and submitted to the State laboratory were inoculated into suckling mice. Fifty-three virus isolates were obtained; forty-eight of these have been identified as of February 25, 1977, the remaining 5 isolates are still under investigation (Table 1). The 9 EEE isolates were obtained from 838 pools of 33,558 mosquitoes collected in an area of central New York State where EEE virus activity has been noted repeatedly in the past and where an epizootic in horses occurred from August to mid-October 1976 (preliminary results were reported in the Arbovirus Information Exchange No. 31, September 1976).

EEE virus was also isolated from 9 of a total of 40 sick or dead horses examined; 28 of the remaining 31 horses had diagnostic serologic findings for EEE. In addition, 64 birds were studied for presence of virus and EEE virus was isolated from tissues of 4 pheasants and of 1 savannah sparrow. The widespread occurrence of EEE virus in this area was further supported by serologic conversions in 4 of 14 sentinel pheasants, 2 of which yielded EEE virus from preconversion blood samples and by findings in sera of 499 birds: HI reactions were observed in samples from 82 of these birds including the following species: Black capped chickadee, catbird, cowbird, field sparrow, ovenbird, red eyed vireo, robin, savannah sparrow, song sparrow, towhee, veery, woodthrush, yellowthroat and yellow warbler.

During the summer and fall of 1976, 551 patients were studied serologically for evidence of an infection with EEE, WEE, SLE, Powassan and California encephalitis virus. In none of these patients was an etiology of arbovirus infection confirmed.

(Sunthorn Srihongse, Margaret A. Grayson and Rudolf Deibel)

Table 1

Arboviruses Isolated from Mosquitoes in New York State, 1976

Virus	Month of Collection	County	Species	No. of Isolates
Eastern equine encephalitis	July	Oswego	<u>C. perturbans</u>	1
	August	Onondaga	<u>C. melanura</u>	6
	August	Oswego	<u>C. melanura</u>	2
California encephalitis complex	June	Albany	A. spp.	1
	June	Monroe	<u>A. stimulans</u>	1
	June	Saratoga	<u>A. communis</u>	2
	July	Albany	<u>A. canadensis</u>	2
	July	Albany	<u>A. vexans</u>	1
	July	Albany	<u>A. triseriatus</u>	1
	July	Columbia	A. spp.	1
	July	Columbia	<u>A. communis</u>	1
	July	Essex	<u>A. canadensis + vexans</u>	1
	July	Monroe	<u>A. canadensis</u>	1
	July	Oswego	<u>A. canadensis</u>	1
	July	Saratoga	<u>A. communis</u>	1
	July	Steuben	<u>A. vexans</u>	1
	July	St. Lawrence	<u>A. communis</u>	1
	Flanders	June	Schenectady	<u>A. vexans</u>
July		Cortland	<u>C. melanura</u>	1
July		Monroe	<u>C. pipiens</u>	1
July		Suffolk	<u>C. pipiens</u>	3
July		Suffolk	<u>C. perturbans</u>	1
July		Westchester	<u>Culex spp.</u>	2
August		Columbia	<u>C. pipiens</u>	1
August		Monroe	<u>C. pipiens</u>	4
August		Onondaga	<u>C. pipiens + restuans</u>	2
August		Oswego	<u>C. melanura</u>	1
August		Saratoga	<u>Culiseta spp.</u>	1
August		Suffolk	<u>C. pipiens</u>	3
August		Suffolk	<u>C. melanura</u>	1
August		Westchester	<u>Culex spp.</u>	1

UNIVERSITE DU QUEBEC, TROIS-RIVIERES, CANADA

Serological survey of some arboviruses in the area of Trois-Rivières
(Province of Quebec)

Several studies have been made in Canada on the epidemiology of arboviruses. However in the Province of Quebec only a few cases of encephalitis caused by arboviruses (EEE and Powassan) were described and only one isolation of a virus from mosquitoes (CE from Ae. communis) has been made. In order to have more information on the activity of some arboviruses (EEE, SLE, CE, Powassan, WEE) in our region, we elaborated a research program on the serological survey of these viruses in the area of Trois-Rivières with the collaboration of the National Arbovirus Reference Center (Toronto, Canada).

Sera collected from human, horses, wild birds, chipmunks, hares, pigeons, rabbits (local and sentinels) and chickens (local and sentinels) are presently under analysis. We only report here the results (confirmed by Dr. H. Artsob, Toronto) of chickens sera analysed by the hemagglutination-inhibition (HI) test using EEE, WEE, SLE antigens provided by the National Arbovirus Reference Center (Toronto). All the sera were negative for WEE and SLE and 22 (6 local and 16 sentinels) out of 25 chickens were negative for EEE. However 3 chickens sentinels showed a possible seroconversion to EEE. Chicken A was located in a semi-wooded area near St. Etienne des Grès (ten miles north of Trois-Rivières), chicken B on a farm in Yamachiche (twenty miles west of Trois-Rivières) and chicken C in the city of Trois-Rivières west.

The serological results were as follows:

	Date serum collected	Titer
	June 6, 1976	< 1 : 10
Chicken A	Aug. 2, 1976	1 : 160
	Sept. 5, 1976	1 : 640

	Date serum collected	Titer
Chicken B	June 6, 1976	< 1 : 10
	Aug. 2, 1976	1 : 1280
	Sept. 7, 1976	1 : 2560
Chicken C	June 6, 1976	< 1 : 10
	July 8, 1976	1 : 40
	Aug. 4, 1976	1 : 640
	Sept. 8, 1976	1 : 2560

The EEE serology should be confirmed by neutralisation test but high antibody titers and the patterns of response make these conversions appear to be genuine.

(S. Belloncik, C. Trudel, H. Artsob).

Studies on the Silverwater virus (SIL)

Silverwater virus was first isolated from the tick Haemaphysalis Leporis palustris in Ontario by McLean (1); later in Alberta and Wisconsin from the tick and the snowshoe hares by Hoff et al.(2). The SIL, a bunyaviruslike (Kaisodi group) not reputed pathogen for human, induces a viremia after intracerebral (IC) intraperitoneal (IP) or intravenous (IV) infection of hare, rabbit and hamster. After IC injection into suckling mice (2-4 days old) the virus causes an encephalitis and death occurs from 5 to 12 days later. In vitro after inoculation by the SIL cytopathic effects are observed in BHK 21 and Vero cell lines (2). Until today no result has been published on the replication and the characterization of this virus. In this report we summarize our observations on the morphology of the virus and its growth in suckling mice after an IC injection.

The viruses possess an envelope and almost all of them are oval in shape. Along the long axis they measure 91 nm and the nucleocapsid measures 63 nm (average of 95 measurements). Our observations are similar to that reported by Holmes (3) on the Bunyamwera super group viruses.

Two kinds of virus particles were observed: one has an electron dense membrane surrounding an electron lucent core. The other has an electron dense core covered with an electron lucent membrane. The viruses are scattered in the Golgi region of the infected cells. The viruses were detected by electron microscopy in the brain bulb and marrow cells and also in the cytoplasm of Vero cells. The infectivity assays of the virus (provided by H. Artsob) were performed on pooled brain and marrow tissue or blood at different time (fig. 1). Before 6 hours no viral replication was noted. Viremia occurred at 24 h. From 6 hours (brain), 24 h (blood) or 48 h (marrow) the titer of the virus increases to a maximum after 5 days and there after decreases (fig. 1) until the death of the mice. At that time (6th day) we are able to localize by the infectivity tests the viral infection of several organs and tissues of the animal (Table 1).

We intend to complete our information on the replication cycle of the virus in Vero cells and also on the ultrastructural and biochemical properties of the Silverwater virus.

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(S. Belloncik, R. Gagnon, P. Boucher).

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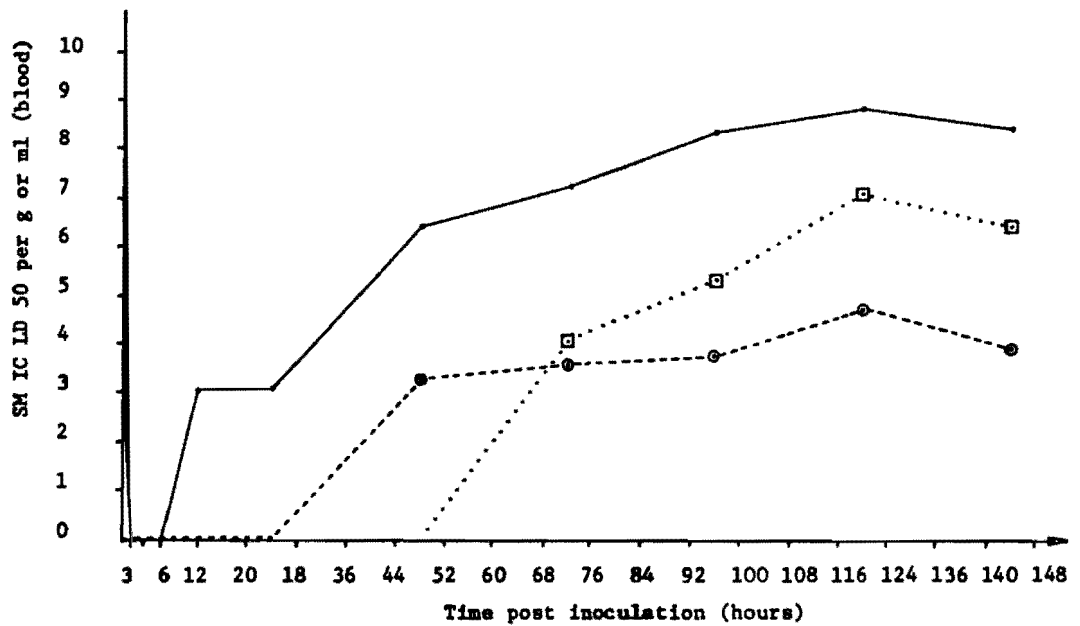


Fig. 1.: Growth curve for Silverwater virus (SIL) in brain (—), marrow (⊠··⊠) and blood (⊙---⊙) of suckling mice (intracerebral injection). Titrations were made in suckling mice by intracerebral injections (SM IC LD 50 as \log_{10}). Dose inoculated: 3.25 SM IC LD 50/g of brain.

Table 1 : Titrations of the SIL at the 6th day in different organ or tissue of the suckling mice after intracerebral inoculation.

Titre (\log_{10} SM IC LD 50/g or ml)	Brain	marrow	spleen	heart	liver	blood	bone	lung	stomach	skin
	8.5	6.48	4.6	4.6	4.3	3.9	3.8	3.8	3.3	3.3

Report from: THE NATIONAL ARBOVIRUS REFERENCE SERVICE*

Department of Medical Microbiology,
University of Toronto, Toronto, Canada.

SEROLOGICAL SURVEY FOR HUMAN ARBOVIRUS INFECTIONS IN THE PROVINCE OF QUEBEC

A study was undertaken to document the exposure of residents of the province of Quebec to selected arboviruses. Blood samples taken between 1971 and 1974 were collected from 4706 adults from five main regions of Quebec - Chicoutimi, the Eastern Townships, Montreal, Quebec City and Trois Rivieres, and from scattered areas of the rest of the province including the Gaspé peninsula, western and northern Quebec.

All sera were tested by haemagglutination inhibition (HI) against Group A (Eastern equine encephalitis, EEE, Western equine encephalitis, WEE), Group B (St. Louis Encephalitis, SLE, Powassan, POW) and California Encephalitis (snowshoe hare), CAL, antigens. Sera positive to SLE by HI were further tested against other Group B antigens including Banzi, BAN, MODOC, MOD, and Montana Myotis Leucoencephalitis, MML.

Sera were kaolin treated and all kaolin positives were confirmed by acetone extraction before being considered as genuine HI reactors. Initial dilutions for HI testing were at 1:10 except for SLE, BAN, MOD and MML antigens which were tested at starting dilutions of 1:20. Some HI positive Group B sera were further tested by complement fixation (CF) tests with initial sera dilutions of 1:4.

The results of HI reactions of the 4706 sera tested against Group A and CAL viruses are presented in Table 1. Three sera demonstrated HI antibodies to EEE (titres 1:10, 1:10 and 1:20) and one serum from a Quebec City resident had an HI titre of 1:40 to WEE.

Twenty-five sera possessed HI antibodies to CAL virus with an even distribution throughout 1971-74. The highest rate of HI antibody was found in the Chicoutimi region in which 1.1% of residents tested were found to have antibodies.

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* A Laboratory Operating in Conjunction with the Laboratory Centre for Disease Control - Department of National Health and Welfare, Canada.

The highest incidence of HI antibody in Quebec residents was found to Group B antigens (Table 2). Of the 4706 samples screened by HI, 202 or 4.3% of the sera had HI antibodies directed either to POW or SLE antigens. Twenty-two HI reactors were obtained to POW antigen alone. These serological positives were from Montreal, Quebec City and from smaller communities in the rest of the province.

The largest numbers of serological positives were obtained to SLE antigen. One hundred and eighty reactors were detected including 118 sera which cross-reacted to other Group B antigens. Twenty-three of the HI positive sera yielded CF titres to SLE ranging from 1:4 to 1:256 whereas 40 sera were CF negative.

A breakdown of SLE HI positive sera by year revealed a striking pattern with only six HI positives detected in sera drawn prior to 1974. In contrast 174 of the 1295 sera tested from 1974 showed HI titres to SLE. Reactors were found from all regions tested including Montreal (1.9%), Trois Rivieres (1.0%), Eastern Townships (2.4%), Quebec City (6.9%), Chicoutimi (3.8%) and the rest of the province (4.2%).

This survey revealed little evidence of Group A arbovirus activity in Quebec province but the data obtained indicate the widespread exposure of residents to CAL and Group B arboviruses. A high incidence of HI antibodies detected in sera taken in 1974 suggests the introduction of a Group B arbovirus, possibly SLE. Further studies including neutralization tests of SLE virus against Group B positive sera are planned.

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

TABLE I

HAEMAGGLUTINATION INHIBITION REACTIONS OF QUEBEC SERA
TO GROUP A AND CALIFORNIA ENCEPHALITIS VIRUSES

Region	Number of Sera Tested	Number of Sera Positive to:		
		EEE	WEE	CAL
Montreal	1046	0	0	5
Trois Rivieres	294	0	0	1
Eastern Townships	832	1	0	1
Quebec City	1049	1	1	7
Chicoutimi	728	1	0	8
Rest of Province	757	0	0	3

TABLE 2

SEROLOGICAL RESPONSE OF QUEBEC RESIDENTS TO GROUP B ARBOVIRUS ANTIGENS

Region	Number of Sera Tested	HI Positives to:			POW CF Results		SLE CF Results	
		POW	SLE	SLE & Gr.B	+ve	-ve	+ve	-ve
Montreal	1046	5	9	15	2	1	7	17
Trois Rivieres	294	0	3	0	-	-	-	-
Eastern Townships	832	0	8	12	-	-	4	10
Quebec City	1049	5	34	38	-	-	5	5
Chicoutimi	728	0	5	24	-	-	3	3
Rest of Province	757	12	3	29	-	-	4	5

REPORT FROM DIVISION OF LABORATORIES
ILLINOIS DEPARTMENT OF PUBLIC HEALTH, CHICAGO, ILLINOIS

In 1976, a statewide arbovirus surveillance program was established in Illinois. This was the result of the 1975 outbreak of St. Louis Encephalitis in which 578 cases were recorded in the state.

Bird and mosquito studies were initiated in southern Illinois in late-May and in Cook County, extreme northeastern Illinois, in Mid-July. SLE virus was isolated from young house sparrows in June and July (Table 1). SLE was isolated from Culex mosquitoes during the period from June through October (Table 2). A California group virus, probably LaCrosse, was isolated from Aedes triseriatus mosquitoes in early July.

Antibodies to SLE were detected in six species of birds while WEE antibodies were found in Cook County birds (Table 3). Because of its abundance and role in the transmission cycle of SLE, the house sparrow was the principal species sought. Only in September did the percentage of juvenile sparrows exceed 2 percent (Table 4).

In 1976, there were 19 confirmed SLE infections in the state. Infections due to a California group virus were detected in 8 children, ages 5 through 14. Of the 6 boys and 2 girls, 5 were from the same county.

(Gary G. Clark)

Table 1 Virus isolations* from birds in Illinois, 1976

Virus	Species	Age	Collection Date	County
SLE	House Sparrow	Nestling	June 12	St.Clair
SLE	House Sparrow	Nestling	July 2	St.Clair
SLE	House Sparrow	Nestling/Fledgling	July 12	Richland
SLE	House Sparrow	Nestling/Fledgling	July 12	Richland
SLE	House Sparrow	Nestling/Fledgling	July 12	Richland
SLE	House Sparrow	Nestling/Fledgling	July 12	Richland
SLE	House Sparrow	Nestling/Fledgling	July 27	White

* Seven isolations have come from 736 birds, primarily nestling House Sparrows, inoculated in suckling mice in pools of four. This number will increase as samples from positive pools are inoculated on an individual basis.

Table 2 Virus Isolation from Mosquitoes* in Illinois, 1976

Virus	Species	Collection Date	County	Collection Method
SLE	<u>Culex spp.</u>	June 19	Christian	Resting
SLE	<u>Culex pipiens</u>	June 19	Christian	Resting
SLE	<u>Culex pipiens</u>	June 28-29	Madison	CDC Light Trap**
SLE	<u>Culex pipiens</u>	June 29	Madison	CDC Light Trap
SLE	<u>Culex pipiens</u>	July 8-9	Christian	Light Trap/Resting
SLE	<u>Culex pipiens</u>	July 11-13	Richland	Resting
SLE	<u>Culex pipiens</u>	August 1	Will	Resting
SLE	<u>Culex spp.</u>	August 19	Hamilton	Resting
SLE	<u>Culex pipiens</u>	August 26-27	Cook	CDC Light Trap
SLE	<u>Culex spp.</u>	September 9	Cook	Resting
SLE	<u>Culex pipiens</u>	September 21	Christian	Resting
SLE	<u>Culex pipiens</u>	October 12	Cook	Resting
GEE***	<u>Aedes triseriatus</u>	July 1	Peoria	Man Landing

* 27,138 tested; additional isolates are being identified.

** Baited with dry ice as CO₂ source.

*** Probably LaCrosse Virus.

Table 3 HI Antibodies to SLE, WEE and EEE in Avian Sera, Illinois, May-October, 1976

Species	Age							
	Adult		Juvenile		Fledgling		Nestling	
	No. Tested	Positive*	No. Tested	Positive	No. Tested	Positive	No. Tested	Positive
Grackle	39	1 SLE	13	0	0		0	
House Sparrow	858	23 SLE	1474	36 SLE 1 WEE	61	1 SLE	34	0
Mourning Dove	0		0		1	1 SLE	2	0
Pigeon	93	1 SLE	107	2 SLE 3 WEE	30	0	26	0
Red-winged Blackbird	6	1 SLE	5	0	0		0	
Ring-necked Pheasant	0		159	1 SLE	0		0	
Other Species	72		41	0	7	0	6	0
Total	1068	26 SLE	1799	39 SLE 4 WEE	99	2 SLE	68	0
Percent Positive		2.4 SLE		2.2 SLE 0.2 WEE		2.0 SLE		0

* HI \geq 20

Table 4 House Sparrows with HI Antibodies* to SLE, Illinois, May-September, 1976

County	Month											
	May		June		July		August		September		Total	
	J	A	J	A	J	A	J	A	J	A	J	A
Hamilton	0/9** (0)	0/35 (0)	0/3 (0)	0/13 (0)	0/33 (0)	0/18 (0)	0/3 (0)	0/0			0/48 (0)	0/66 (0)
Saline	0/1 (0)	0/7 (0)	0/7 (0)	1/47 (2)	0/24 (0)	1/16 (17)					0/32 (0)	2/60 (3)
St. Clair	0/16 (0)	3/20 (15)	0/23 (0)	0/19 (0)	0/55 (0)	0/16 (0)	0/63 (0)	1/25 (4)	14/137 (10)	3/22 (14)	14/294 (5)	7/102 (7)
White			0/8 (0)	1/10 (10)	2/76 (3)	0/6 (0)	0/40 (0)	0/6 (0)	6/35 (17)	1/2 (50)	8/159 (5)	2/24 (8)
Richland			1/9 (11)	2/33 (6)	1/79 (1)	0/62 (0)	0/52 (0)	0/5 (0)	1/122 (1)	0/11 (0)	3/262 (1)	2/111 (2)
Christian			1/2 (50)	7/90 (8)	0/18 (0)	1/81 (1)	1/16 (6)	0/2 (0)	1/24 (4)	0/5 (0)	3/60 (5)	8/178 (4)
Cumberland			0/34 (0)	2/38 (5)	0/29 (0)	0/51 (0)					0/63 (0)	2/69 (3)
Madison			0/4 (0)	0/1 (0)					1/32 (3)	0/3 (0)	1/36 (3)	0/4 (0)
Edwards							1/16 (6)	0/4 (0)	0/20 (0)	0/0	1/36 (3)	0/4 (0)
Total Juvenile	0/26 (0)		2/90 (2)		3/314 (1)		2/190 (1)		23/370 (6)		30/990	
Adult		3/62 (5)		13/251 (5)		2/220 (1)		1/42 (2)		4/43 (9)		23/618
Cook					0/216 (0)	0/102 (0)	6/258 (2)	0/104 (0)	1/99 (1)	0/27 (0)	7/573 (1)	0/233 (0)

* HI \geq 20

** No. positive/No. tested %

Seventeen cases of California (LAC) encephalitis and 1 SLE were hospitalized and serologically diagnosed in Wisconsin during the 1976 season. The distribution of the LAC cases included 8 boys and 9 girls, 7 weeks to 14 years of age; with the first date of onset June 30th followed by 3 during July, 7 in August and 6 during September. Twelve were residents of the endemic region in southwestern Wisconsin and 5 from adjoining rural forested areas in southwestern Minnesota and northeastern Iowa. Convalescent serums from all cases had higher antibody levels in comparative neutralization tests with LAC than with 3 other California group arboviruses previously isolated from mosquitoes in Wisconsin; TVT, JC, and SSH viruses, and CF antibodies were detectable only with LAC.

Although a severe drought occurred in upper midwestern states during the summer of 1976, LAC virus continued to emerge from protected A. triseriatus oviposition sites, including basal tree-holes and old tires in dense hardwood deciduous forested hillsides as in southwestern Wisconsin.

La Crosse and other California encephalitis group arbovirus infections and disease were studied in residents of suburban La Crosse. La Crosse virus continues to be the main cause of arboviral encephalitis in Wisconsin, with over 200 cases diagnosed in children since it was originally isolated from a fatal case during 1963. Four of these serologically confirmed cases were detected in children residing in the State Road Coulee area at the east side of La Crosse. Epidemiologic studies were recently conducted to determine occurrence of past infections and clinical disease associated with La Crosse and other arboviruses.

Antibodies neutralizing La Crosse or other California group arboviruses in comparative microtiter tests in BHK₂₁ cells were found in sera from 41 (15.5%) of 256 residents including 132²¹ of the children in two grade schools and 133 other residents of all age groups in the forested suburban State Road Coulee area. Of these, 15 (5.7%) had antibodies to La Crosse (LAC), 12 (4.7%) to Trivittatus (TVT), 12 (4.7%) to Jamestown Canyon (JC) California group arboviruses; and 2 (0.8%) to Bunyamwera (BUN) group arbovirus. Antibody rates in those under 20 years of age sampled in the State Coulee area were 7/146 (4.3%) to LAC, 4 (2.4%) to TVT, 4 (2.4%) to JC and 1 (0.7%) to BUN group arbovirus. No HI antibodies to EEE, WEE, SLE, or Powassan arboviruses were found.

A high rate of associated disease was reported for children who had been infected with LAC virus; 6 of the 7 under 20 years of age with antibody evidence of past infection also had histories of encephalitis, 4 of these had been hospitalized. Similar histories of encephalitis were not reported for any of the 8 adults with antibody to LAC virus; however, one had reported a possibly associated disease characterized by prolonged fever and headache seven years ago when she was 25 years of age.

No history of encephalitis was found in those with antibodies to TVT, JC, or BUN group viruses in this limited survey, but some had reports of possibly related non-encephalitic febrile illnesses which could not be associated with their past arbovirus infections because of the lack of timely diagnostic specimens.

No new cases of La Crosse encephalitis have been detected in this State Road Coulee suburban area during the past three seasons following the closures of basal tree-holes in an adjoining woodlot and removal from the Coulee center of two old tires containing Aedes triseriatus larvae from which numerous isolates of La Crosse virus had been obtained during May of 1973. The vector, still present in nearby forested hillsides, does not usually range far from its protected habitat.

Aedes triseriatus larvae were collected during 1976 from basal tree-holes along a line extending from the known endemic area around La Crosse in the western part of the state to southeastern Wisconsin where no known histories of exposure to California encephalitis or antibodies to LAC in chipmunks and squirrels have been found.

Isolates were obtained (by FA or in mice) from 7 adults (including males and females) reared from 666 A. triseriatus from 63 basal tree-holes in the known endemic area in western Wisconsin, and no isolates were obtained from 581 processed from 54 basal tree-holes in the non-endemic southeastern part.

Transmission trials (by bite to mice) were positive with F_1 progeny reared from collections from the non-endemic area as well as the endemic area, and additional numbers are being processed to study rates of salivary, transovarial and venereal transmission. Studies are continuing on vector, host and environmental factors associated with presence or absence of LAC virus in isolated forested areas.

Results of continuing studies of venereal transmission of LAC virus in Aedes triseriatus defining distribution of viral antigen in females following insemination by infected males were presented at the fall 76 meeting of ASTMH, with Barry Beaty, who is now at YARU in a post-doctoral position. In this study LAC viral antigen was observed by FA in bursal contents of 53% (35/66) of females dissected within 24 hours post-insemination, in 20% (5/24) on days 2 and 3, and in 2.5% (1/40) on days 4 and 5.

Distribution of viral antigen was observed in tissues of other non-lower genital tract organs, in 3.4% (13/379) of females dissected on days 1 thru 14, and in 5% of females dissected 7 thru 14 days post insemination.

Transmissions of LAC virus by venereally infected females through eggs and saliva have been demonstrated in the laboratory; studies of rates of transmission in nature are underway. Venereal transmission is considered as an important supplement to transfer through feeding on viremic hosts for horizontal transmission, necessary for maintaining LAC virus in nature through transovarial transmission, when filial infection rates of progeny are less than 100%.

(Wayne Thompson)

REPORT FROM THE ARTHROPOD-BORNE
ANIMAL DISEASE RESEARCH LABORATORY,
USDA - ARS, DENVER, COLORADO

Bluetongue and Epizootic Hemorrhagic
Disease Virus Serotyping

A plaque neutralization test was recently developed for serotyping bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV); the test is also one of the most sensitive methods of quantitating antibody to BTV and EHDV. The test is done in 24-well plastic panels. The BHK₂₁ cells are added from a cell suspension so that the original cell-virus interaction occurs in suspension. A gum tragacanth overlay covers the cells during a 7-day (37C) incubation period. After 7 days the cell monolayer that has formed is stained and plaques are counted. Reference typing serums are from convalescent sheep (BTV) or calves (EHDV). These reference serums consistently reduce 80 (\pm 20) plaque-forming units of a homologous serotype virus by \geq 80%. These procedures have enabled us to serotype virtually all of the U. S. isolates of BTV and EHDV since 1972. Our early serotyping results with 10 BTV strains were checked for agreement with international serotype groupings at the World Reference Center for Bluetongue Serotyping, Veterinary Research Institute, Onderstepoort, South Africa. Their more qualitative serotyping procedure in L-929 cells gave results that were identical to those we had obtained earlier.

Four of the 17 international BTV serotypes are known to exist in the U. S. The first BTV strain isolated in California from an outbreak in sheep (1953) was serotype 10. A BTV strain isolated from sheep in Texas in 1962 was reported to be antigenically different from earlier viral

isolates and was subsequently found to be serotype 11. We recently serotyped a BTV isolate obtained from sheep in New Mexico in 1955 and found that it was also serotype 11. A BTV strain isolated from sheep in 1962 was studied in the U. S. and in South Africa in 1975 and was found to be a new international serotype; this BTV serotype has not been isolated and identified except in the U. S. The new viral serotype was designated serotype 17. A BTV strain later shown to be serotype 13 was isolated from cattle in 1967.

More BTV strains from epizootics since 1972 have been serotyped than from previous years. Serotype 11 was prevalent in 1973; isolations were made from sheep, cattle, mule deer and the Culicoides variipennis vector. In 1974 serotype 17 was the most frequent among BTV isolates received for serotyping. The serotype 17 viral strains in 1974 were obtained from domestic sheep, cattle and bighorn sheep. In 1975 BTV serotype 11 was again prevalent among the samples we received and the viral isolates serotyped were from sheep and cattle. The 1976 BTV isolates have not all been serotyped to date but serotypes 11 and 17 appear to have been predominant and widespread. The 1976 BTV isolates (serotype 17) were from antelope and sheep samples collected in California, Montana, Nebraska, Oklahoma, Texas and Wyoming. Serotype 11 isolates in 1976 were from cattle and sheep and originated in California, Idaho, Indiana, Louisiana, Nebraska and Washington. A single serotype 10 isolate was found in 1976 from sheep in California.

We have only serotyped 86 BTV strains and this number includes those collected by our staff or submitted to our laboratory from 1953 to the present. The samples we serotyped were not from a survey designed to study

the epizootiology of BTV. Thus, although these results are informational, they should not be mistakenly presumed to be a complete or accurate depiction of BTV activity in the U. S. Serologic evidence of BTV activity has come from 47 of the 50 states in the U. S.

Epizootic hemorrhagic disease virus is antigenically related to BTV. Because they contain shared antigens, EHDV and BTV are in the same serologic subgroup of the Orbivirus genus. There are 2 distinct, well-defined serotypes of EHDV. New Jersey is the prototype strain of EHDV - serotype 1; this was the first strain isolated from white-tailed deer when the disease was discovered in the early 1950s. Alberta is the prototype strain for EHDV - serotype 2; this strain was isolated from white-tailed deer in Canada in 1962. Serotype 2, EHDV, was obtained from Culicoides variipennis and cattle in Colorado (1972); from white-tailed deer in Michigan (1974), Indiana and New Jersey (1975); and from cattle in Colorado in 1974. Serotype 1 was obtained from cattle in Colorado in 1974. The EHDV serotyping results include only 15 strains collected by our staff or submitted to us for serotyping.

The only commercially available vaccine for BT is a modified-live virus product that includes only serotype 10. Since serotype 10 has been uncommon in occurrence in recent years, the value of this monovalent vaccine has been questioned. We favor the development of an inactivated polyvalent vaccine to avoid the danger of insect transmission of the vaccine virus with possible reversion to virulence as occurred with the "attenuated" egg-adapted vaccine. There is no vaccine available for EHD.

(T. L. Barber, M. M. Jochim, B. M. Bando)

Latent and Active Bluetongue Virus (BTV) Infections
in Cattle

Latent infections, activation from latency, and subsequent biological transmission by Culicoides variipennis have been demonstrated in cattle experimentally infected with BTV serotype 11.

Recent field studies at ranches north of Denver, Colorado, have revealed that BTV (serotype 11) is associated with reproductive problems in cattle; abortions, repeat breeding, anestrus, neonatal calf mortality, congenital calf anomalies, weak calves at birth, unthrifty calves, chronic diarrhea in calves, and sporadic, acute diarrhea in some of the adult cattle. BTV has been isolated from cows and calves shortly after parturition, and from some aborted fetuses and stillborn calves that appeared normal. Neutralizing, precipitating, or complement-fixing antibodies are rarely demonstrable in cattle of the herds tested during the past several years of study. Two ataxic calves (1 male and 1 female) received from one ranch, born in March and June, have been raised at the laboratory under insect security and are now 700 and 600 days of age, respectively. The female was blind for several months after birth, and is now normal in appearance but small for her age. BT viremia was demonstrated in these calves during episodes of fever and leukopenia similar to that previously described for a Hereford bull at our laboratory. In addition, activation of viremia from latency and biological recovery of BTV has occurred after feeding uninfected laboratory colonized C. variipennis females on them. Neither calf has yet developed detectable BTV antibodies.

BTV has been readily demonstrable in the semen of actively and latently infected bulls for extended periods of time. Fourteen nulliparous

heifers were naturally inseminated by a BTV-latently infected bull; all 14 heifers were impregnated and the 10 tested to date all became BT-viremic; no clinical signs of BT have been observed. One heifer aborted during the first trimester and was anestrus for almost one year. Estrus occurred 2 months after she was treated with progesterone and FSH; she is now more than 200 days pregnant. Twelve live calves were born and all had congenital anomalies or dysfunctions at birth, none of which were incompatible with life when good husbandry was employed. Anomalies observed were arthrogryposis, transient hydrocephalus, deviated lower jaws, excessive gingival tissue, and protruding tongues. Dysfunctions included locomotor disturbances, transient blindness, ataxias and inability to stand. Clinical signs of bluetongue were observed at or soon after birth in some calves; these included fevers, leukopenias, and macula and ulceration of the muzzles and buccal mucosa. BT viremias have been demonstrated at birth in all calves adequately tested to date; no calf had at birth nor developed precipitating or complement-fixing antibodies. The calves presently range from 1 through 10 months of age. Viremia assay at, and since birth, and neutralization tests have not as yet been completed. The calf of the fourteenth heifer died in utero 17 days after the expected parturition date; the calf was apparently normal and no virologic or serologic studies have yet been done.

Latent infections that are activated by C. variipennis with biological recovery and transmission to susceptible hosts, venereal transmission with fetal infection, and immunological tolerance in neonates can result from BTV infections in cattle. While none of these events are unique to BTV infections, we are unaware of similar reports for other orbivirus infections.

(A. J. Luedke, T. E. Walton, and H. E. Metcalf)

REPORT FROM THE VECTOR-BORNE DISEASE DIVISION
CENTER FOR DISEASE CONTROL, FORT COLLINS, COLORADO

New alphavirus from swallowbugs

In previous issues of the Information Exchange we described the isolation of a new alphavirus, serologically related to western equine encephalitis (WEE) virus from Oeciacus vicarius bugs (Hemiptera: Cimicidae) occupying cliff swallow nests and from nestling birds (cliff swallows and house sparrows). Characteristics of the new virus which distinguished it from standard, Culex tarsalis-borne WEE virus included: (1) lack of virulence for infant mice; (2) small plaque size in primary duck embryo cells; (3) a broad pH optimum for hemagglutination; and (4) results of standard serologic tests (see below). Further studies, to be reported in a separate publication, have also shown biochemical differences between this new WEE-related alphavirus and other members of the WEE complex [WEE (Fleming and McMillan), Sindbis, Highlands J, and Y62-33]. Briefly stated, the E1 glycoprotein of the new virus was of significantly higher molecular weight and had a significantly higher isoelectric point than the corresponding glycoprotein of the other members of the group.

Field studies conducted in eastern Colorado have documented the role of O. vicarius bugs as the local winter reservoir of the new agent. The virus survives the winter months in nest-occupying bugs; amplification occurs annually with the early summer appearance of susceptible nestling birds.

Experimental studies have shown Culex tarsalis mosquitoes to be wholly refractory to infection, both by ingestion of virus and direct intrathoracic inoculation. Indeed, the new agent fails to replicate in Aedes albopictus cells. Infection and transmission in laboratory-held O. vicarius bugs has been accomplished. The new virus thus appears to be specifically adapted and restricted to its bug vector.

The name Fort Morgan virus is proposed for the new agent.

Isolation of a second alphavirus from swallowbugs.

During cloning procedures with a strain of the WEE-related virus (CM4-146) a second virus was discovered. Briefly, the original suspension of 25 triturated bugs (CM4-146) was inoculated into fluid Vero cell cultures and two plaque types were noted on subsequent plating: small, discrete plaques typical of the Fort Morgan virus and large, hazy plaques. The latter were cloned and subjected to further study.

The virus was also sent to Dr. Peter Jahrling at USAMRIID. Dr. Jahrling noted protection in VEE-immune hamsters against lethal challenge with the large-plaque clone.

This finding stimulated us to complete the antigenic analysis, comparing Fort Morgan virus, the large plaque agent, and members of the WEE and VEE groups. These results are presented in Tables 1-6.

The serologic studies showed that Fort Morgan virus and the large plaque virus separated on the basis of plaque morphology from the same suspension of Oeciacus bugs are antigenically distinct; the former virus is placed in the WEE serologic complex, while the latter is related but distinct from strains of VEE virus representing subtypes I through IV.

The name Bijou Bridge virus is proposed for this new member of the VEE complex.

Studies now completed have documented the presence of Bijou Bridge virus in five of 11 Fort Morgan virus-positive original suspensions of pooled O. vicarius bugs and in four of 20 nestling sparrow bloods positive for Fort Morgan virus. Bijou Bridge virus is present at low concentrations in these mixed infections, at ratios of 100 to 1000:1 Fort Morgan to Bijou Bridge PFU. Therefore, direct plaquing techniques have been inadequate for separation, and Bijou Bridge virus has been demonstrated only after neutralization of Fort Morgan virus by specific antiserum.

Despite much testing of field material, we have not succeeded in obtaining individual isolations of Bijou Bridge virus, nor have we demonstrated antibody in adult birds from appropriate sites. This puzzling series of observations remain unexplained.

Other characteristics of Bijou Bridge virus include: (1) a high degree of mouse neurovirulence, similar to other VEE subtype I viruses, (2) ability to infect Culex tarsalis mosquitoes and to replicate in Aedes albopictus cells, (3) ability to infect and be transmitted by O. vicarius bugs, and (4) production of viremia and antibody in nestling and adult house sparrows, with demonstrable cross-protection between it and VEE IB and ID viruses.

(Vector-Borne Diseases Division, Bureau of Laboratories, Center for Disease Control, Fort Collins, Colorado)

Table 1

RELATIONSHIPS IN HEMAGGLUTINATION-INHIBITION TEST BETWEEN
FORT MORGAN AND WEE COMPLEX VIRUSES AND LACK OF
RELATIONSHIP WITH BLJOU BRIDGE VIRUS

ANTIGEN	A N T I S E R U M						BLJOU BRIDGE ²
	FORT MORGAN ¹	WEE FLEMING	Y62-33	SINDBIS	AURA	WHATAROA	
FORT MORGAN	<u>40</u>	320	320	320	20	20	-
WEE FLEMING	- 3	<u>320</u>					-
Y62-33	-		<u>160</u>				-
SINDBIS	-			<u>640</u>			-
AURA					<u>80</u>		-
WHATAROA ⁴							-
BLJOU BRIDGE	-	-	-	-	-	-	<u>640</u>

1. Q4-146 PLAQUE A (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)
2. Q4-146 PLAQUE C (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)
3. <10
4. NO HA ANTIGEN AVAILABLE

Table 2

RELATIONSHIP BY COMPLEMENT-FIXATION TEST BETWEEN
FORT MORGAN AND WEE COMPLEX VIRUSES AND LACK OF
RELATIONSHIP WITH BLJOU BRIDGE VIRUS

ANTIGEN	A N T I S E R U M						BLJOU BRIDGE ²
	FORT MORGAN ¹	WEE FLEMING	Y62-33	SINDBIS	AURA	WHATAROA	
FORT MORGAN	<u>256</u>	128	128	128	-	-	-
WEE FLEMING	128	<u>>1024</u>					-
Y62-33	128		<u>512</u>				-
SINDBIS	64			<u>512</u>			-
AURA	-				<u>64</u>		-
WHATAROA	-					<u>32</u>	-
BLJOU BRIDGE	-	-	-	-	-	-	<u>64</u>

1. Q4-146 PLAQUE A (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)
2. Q4-146 PLAQUE C (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)

Table 3

RELATIONSHIPS IN PRNT BETWEEN FORT MORGAN AND WEE COMPLEX VIRUSES
AND LACK OF RELATIONSHIP WITH BLJOU BRIDGE VIRUS

Virus	A N T I S E R U M						BLJOU BRIDGE ²
	FORT MORGAN ¹	WEE FLEMING	Y62-33	SINDBIS	AURA	WHATARQA	
FORT MORGAN	<u>320</u>	- ³	80	-	20	-	-
WEE FLEMING	-	<u>640</u>					-
Y62-33	20		<u>320</u>				-
SINDBIS	-			<u>160</u>			-
AURA	-				<u>160</u>		-
WHATARQA	-					<u>160</u>	-
BLJOU BRIDGE	-	-	-	-	-	-	<u>>1280</u>

1. C₄-146 PLAQUE A (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)

2. C₄-146 PLAQUE C (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)

3 <10

Table 4

RELATIONSHIPS BY HEMAGGLUTINATION-INHIBITION TEST
BETWEEN FORT MORGAN, BLJOU BRIDGE, AND VEE COMPLEX VIRUSES

ANTIGEN	A N T I S E R U M									FORT MORGAN ²
	BLJOU BRIDGE ¹	VEE ⁴								
	IA	IB	IC	ID	IE	II	III	IV		
BLJOU BRIDGE	<u>640</u>	20	- ³	20	20	10	40	-	10	-
VEE IA	20	<u>40</u>								-
IB	10		<u>20</u>							-
IC	40			<u>640</u>						-
ID	10				<u>80</u>					-
IE	10					<u>80</u>				-
II	20						<u>>320</u>			-
III	20							<u>80</u>		-
IV	80								<u>>640</u>	-
FORT MORGAN	-	-	-	-	40	-	-	-	-	<u>40</u>

1. C₄-146 PLAQUE C (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)

2. C₄-146 PLAQUE A (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)

3. <10

4. STRAINS USED: VEE IA (TrD); IB (PTF-39); IC (P-676); ID (3880);
IE (MENA II); II=EVERGLADES (FE 3-7c); III=MUCAMBO
BeAn 8007); IV=PIXUNA (BeAr 35645).

Table 5

RELATIONSHIPS IN PRNT BETWEEN BLJOU BRIDGE, FORT MORGAN,
AND VEE COMPLEX VIRUSES.

VIRUS	ANTISERUM									FORT MORGAN ²
	BLJOU BRIDGE ¹	V E E								
	IA	IB	IC	ID	IE	II	III	IV		
BLJOU BRIDGE	<u>≥1280</u>	20	20	40	80	160	10	-	20	-
VEE IA	320	<u>≥640</u>								-
IB	80		<u>320</u>							-
IC	<u>≥1280</u>			<u>>640</u>						-
ID	<u>≥1280</u>				<u>160</u>					-
IE	320					<u>160</u>				-
II	320						<u>>640</u>			-
III	160							<u>320</u>		-
IV	- ³								<u>320</u>	-
FORT MORGAN	-	-	-	-	-	-	-	-	20	<u>320</u>

1. CM4-146 PLAQUE C (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)

2. CM4-146 PLAQUE A (*Oe. vicarius*, FORT MORGAN, COLORADO, 1974)

3. <10

Table 6

LACK OF RELATIONSHIP BETWEEN FORT MORGAN, BIJOU BRIDGE, AND ALPHAVIRUSES
OTHER THAN MEMBERS OF THE WEE AND VEE COMPLEXES

	FORT MORGAN						BIJOU BRIDGE					
	ANTIGEN			ANTISERUM			ANTIGEN			ANTISERUM		
	HI	CF	N	HI	CF	N	HI	CF	N	HI	CF	N
CHIKUNGUNYA	40/160 ¹	- ² /256	/>640	-/40	-/256	-/320	-/160	-/256	-/>640	-/640	-/64	-/>1280
O'NYONG NYONG	-/40	-/32	-/160	-/	-/	-/	-/40	-/32	-/160	-/	-/	-/
SEMLIKI FOREST	-/640	-/64	-/>640	-/	-/	-/	-/640	8/64	-/>640	-/	-/	-/
MAYARO	-/40	-/32	-/160	-/	-/	-/	-/40	-/32	-/160	-/	-/	-/
UNA	-/NT ³	-/16	-/40	NT/	-/	-/	-/NT	8/16	-/40	NT/	-/	-/
MIDDELBURG	-/NT	-/16	-/320	NT/	-/	-/	-/NT	-/16	-/320	NT/	-/	-/
EEE	-/80	-/128	-/>640	-/	-/	-/	-/80	-/128	-/>640	-/	-/	-/

226

1. Ht/Ho
2. - = <10 (HI,N); <8 (CF)
3. NT = not tested

Identification and Characterization of Ft. Morgan
and Bijou Bridge, two new viruses from swallow nest bugs

Identification - Plaque purified Ft. Morgan ("plaque A") and Bijou Bridge ("plaque C") viruses were compared in HI, CF and N tests. The results are summarized in Tables 7 and 8. These viruses appear to be different from each other and new to science.

Characterization - SDC sensitivity and pH stability test results (Table 9) showed these viruses to be sensitive to SDC and pH 3 labile. As shown in Table 10, Bijou Bridge is a much more virulent virus than is Ft. Morgan and has a much wider spectrum of host susceptibility.

Growth curves were determined for both viruses in primary duck embryo and serially propagated Vero cells at 37° and 42°C. Hemagglutinin and complement-fixing antigens and infectious virus were also sampled at intervals and titrated. Titers in duck embryo cells were higher than in Vero cells (Figure 1), but patterns of replication were similar. Extracellular virus decreased from the maximum (generally 18-48 hours) and was not detectable or had decreased precipitously by the 144th hour after infection. The decrease was most acute with Ft. Morgan virus grown in duck embryo cells. This may reflect instability of the virus at 37° and 42°C, slower release of extracellular virus from Vero cells or other factors not yet investigated. Thermal stability studies are in progress.

With both viruses in both cell systems and at both temperatures, low titer (1:4 to 1:128) hemagglutinin appeared 18-24 hours after

infection and reached a maximum by 36-48 hours with Bijou Bridge virus and 72-96 hours with Ft. Morgan virus.

Complement-fixing antigens titering 1:4 to 1:8 appeared 48 hours after infection and reached a maximum by 72 hours with both viruses.

Charles H. Calisher, John S. Lazuick
and David J. Muth

New virus isolations from Colorado

During studies of Colorado tick fever virus in Colorado, 9 strains of an unidentified virus were obtained from Argas cooleyi ticks found in nests of cliff swallows (Petrochelidon pyrrhonota) in north central Colorado. By screening complement fixation tests, this virus is a member of the Kemerovo group. Further work has shown close relationships with Sixgun City and Mono Lake and more distant relationships with Great Island and Huacho viruses. Additional studies, including SDPRN tests in cell culture, are in progress.

(Arbovirus Reference Branch and Vertebrate Ecology Branch)

Table 7. Serologic reactivity of Ft. Morgan
("plaque A") virus with other alphaviruses
CM4-146 (Plaque "A")

Antigen or Antibody of Indicated Virus	Antigen			Antibody		
	HI	CF	NT	HI	CF	NT
WEE (Fleming)	320/320	128/≥1024	-/640	10/40	128/256	-/320
Y62-33	320/640	128/512	80/640	-/40	128/256	-/320
HJ	20/80	8/64	-/320	10/40	32/256	-/320
Sindbis	320/640	128/512	-/160	-/40	64/256	-/320
• Whataroa	20/?	-/32	-/160	NT	-/256	-/320
Chikungunya	40/160	-/256	-/≥640	-/40	-/256	-/320
• Aura	20/80	-/64	20/160	-/40	-/256	-/320
Mayaro	-/40	-/32	-/160	-/40	-/256	-/320
Semliki Forest	-/640	-/64	-/≥640	-/40	-/256	-/320
Una	-/?	-/16	-/40	NT	-/256	-/320
O'nyong nyong	-/40	-/32	-/160	-/40	-/256	-/320
Middleburg	-/?	-/16	-/320	NT	-/256	-/320
EEE	-/80	-/128	-/≥640	-/40	-/256	-/320
VEE IA	-/160	8/128	-/≥640	-/40	-/256	-/320
IB	-/80	-/64	-/320	-/40	-/256	-/320
IC	-/640	-/64	-/≥640	-/40	-/256	-/320
ID	40/80	16/128	-/160	-/40	-/256	-/320
IE	-/80	-/64	-/160	-/40	-/256	-/320
II	-/640	-/256	-/≥640	-/40	64/256	-/320
III	-/160	-/32	-/160	-/40	-/256	-/320
IV	-/≥640	-/256	20/160	-/40	-/256	-/320
Plaque "C"	10/640	-/64	-/640	-/40	-/256	-/320

- = <10 HI, NT; <8 CF

(Ab/Ab - Ht/Ho)

Table 8. Serologic reactivity of Bijou Bridge
("plaque C") virus with other alphaviruses
CM4-146 (Plaque "C")

Antigen or Antibody of Indicated Virus	Antigen			Antibody		
	HI	CF	NT	HI	CF	NT
WEE (Fleming)	-/320	-/≥1024	-/640	-/640	-/64	-/≥640
Y62-33	-/640	-/512	-/640	-/≥640	-/64	-/≥640
HJ	-/80	-/64	-/320	-/≥640	-/64	-/≥640
Sindbis	-/640	-/512	-/160	-/≥640	-/64	-/≥640
Whataroa	-/?	-/32	-/160	NT	-/64	-/≥640
Chikungunya	-/160	-/250	-/≥640	-/≥640	-/64	-/≥640
Aura	-/80	-/64	-/160	-/≥640	-/64	-/≥640
Mayaro	-/40	-/32	-/160	-/≥640	-/64	-/≥640
SFV	-/640	8/64	-/≥640	-/≥640	-/64	-/≥640
UNA	-/?	8/16	-/40	NT	-/64	-/≥640
ONN	-/40	-/32	-/160	-/≥640	-/64	-/≥640
MID	-/?	-/16	-/320	NT	-/64	-/≥640
EEE	-/80	-/128	-/≥640	-/≥640	-/64	-/≥640
VEE IA	40/160	32/128	20/≥640	20/≥640	-/64	320/≥640
IB	-/80	16/64	20/320	10/≥640	-/64	80/≥640
IC	20/640	64/64	40/≥640	40/≥640	-/64	≥640/≥640
ID	40/80	64/128	80/160	10/≥640	-/64	≥640/≥640
IE	10/80	32/64	160/160	10/≥640	-/64	320/≥640
II	80/640	32/256	10/≥640	20/≥640	8/64	320/≥640
III	-/160	8/32	-/160	20/≥640	-/64	160/≥640
IV	10/≥640	64/256	20/160	80/≥640	8/64	-/≥640
Plaque "A"	-/40	-/256	-/320	-/≥640	-/64	-/≥640

- = <10 HI, NT; <8 CF

(Ab/Ab - Ht/Ho)

Table 9. SDC sensitivity and pH stability of Ft. Morgan ("plaque A") and Bijou Bridge ("plaque C") viruses

SDC Sensitivity (in Vero cells)

	Plaque "A"	Plaque "C"
Untreated	5.4	6.9
Treated	<2.0	<2.0

pH Stability (in Vero cells)

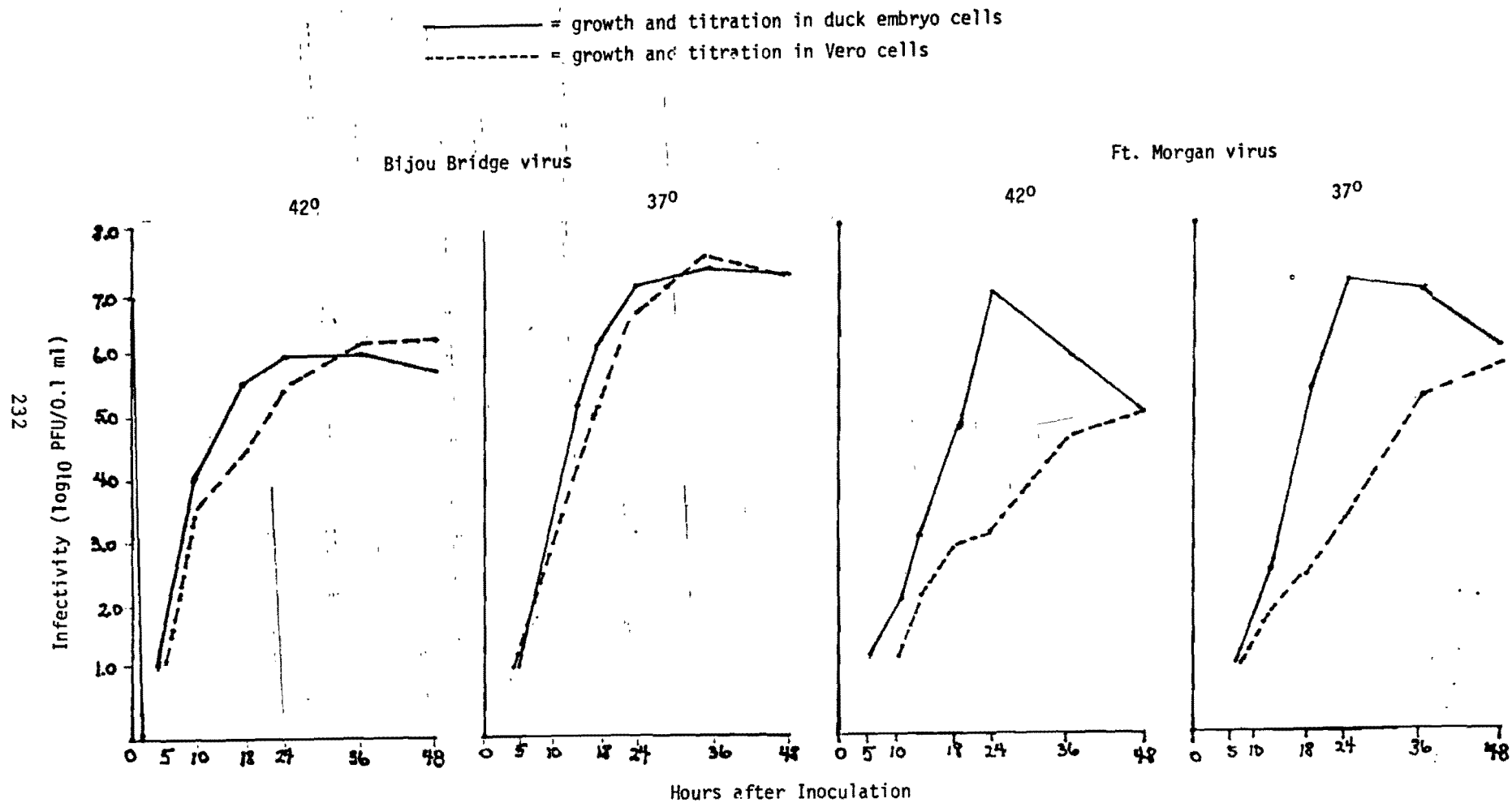
	Plaque "A"	Plaque "C"
pH 2.0-4.0	<2	<2
pH 5.0-10.0	5.0	6.7-7.1

Table 10. Titers of Ft. Morgan ("plaque A") and Bijou Bridge ("plaque C") viruses by host and route

Titer per ml of inoculum by host and route

Host (route)	Plaque "A"	Plaque "C"
SM (ic)	6.7	9.1
SM (ip)	6.8	8.3
WM (ic)	≤2.9	8.4
WM (ip)	≤2.9	8.1
WM (sc)	≤2.8	8.6
WM (im)	≤2.8	≥8.9
SH (ic)	7.1	9.7
SH (ip)	6.4	≥8.9
WH (ic)	4.9	7.8
WH (ip)	≤2.9	5.3
OM (ic)	≤2.9	8.2
OM (ip)	≤2.9	4.4
"L" cells	<2.0	7.2
DE cells	5.7	7.8
Vero cells	5.4	6.6

Figure 1. Replication of Bijou Bridge and Ft. Morgan viruses in duck embryo and Vero cells at 42° and 37°C



A new virus from Alaska, related to Sawgrass virus

Three virus strains were received from Mr. D. G. Ritter, Division of Public Health, Fairbanks, Alaska for identification attempts. These strains (0576, 0579 and 0608) were from adult female Haemaphysalis leporis-palustris ticks removed from adult hares (Lepus americanus) near New Minto, Alaska, in 1972. All three cross react to titer by CF, did not agglutinate goose erythrocytes (pH 6.0-7.0), did not kill weaned mice inoculated intraperitoneally, and did not form plaques in duck embryo or Vero cells.

In tests performed with Dr. R. E. Shope, YARU, D. J. Muth found that strain 0576 did not react with any of 30 grouping HIAF's and 0576 HIAF did not react with 203 different arbovirus antigens. A slight (3+ fixation at 1:4) CF reaction was obtained with 0576 HIAF and Sawgrass antigen. Further CF testing (Table 11) has shown the distant relationship of these two viruses. By virus dilution N tests in SM, a 20-fold reduction in titer was seen with strain 0576 and Sawgrass HIAF. Further N testing awaits development of suitable cell culture systems.

Sawgrass virus has been isolated 16 times from H. leporis-palustris ticks in the Tampa Bay area of west central Florida.

Heretofore, Sawgrass virus has been classified as Ungrouped. The weak, but significant, one-way relationship of the new virus, for which the name New Minto is suggested, with Sawgrass leads us to suggest that this is a new group--the Sawgrass group, of which New Minto is the second known member.

(Charles H. Calisher, Donald G. Ritter
and David J. Muth)

Table 11. Results of cross box CF tests with three strains of a virus from Alaska and Sawgrass virus.

Antigen	Antibody to:				
	0576	0579	0608	Sawgrass	Normal
0576	<u>512</u>	256	128	<8	<8
0579	256	<u>256</u>	256	<8	<8
0608	256	256	<u>512</u>	<8	<8
Sawgrass	16	32	16	<u>128</u>	<8
Normal	<8	<8	<8	<8	<8

Arbovirus from Ecuador

A large number of viruses have been isolated from vertebrates and invertebrates collected in Ecuador during a cooperative project between this Division and the National Institute of Hygiene, Guayaquil, Ecuador. Isolates from vertebrates were made by Dr. E. Gutierrez and his group and from invertebrates by a group directed by Dr. D. B. Francly of this Division.

Thus far, from vertebrates and mosquitoes, we have identified isolates as follows:

<u>Number</u>	<u>Source</u>	<u>Group</u>	<u>Comments</u>
1	Cormorant	A	EEE-like
1	Sentinel hamster	Capim	
5	Sentinel hamsters	A?	SDC-sensitive
3	<u>C.</u> (Mel.) sp.	A	EEE-like
>100	<u>Aedes</u> sp.	Bunyamwera	new virus
1	<u>C.</u> (Mel.) sp.	C	
1	<u>M. indubitans</u>	Bun-C	mixture
9	<u>C.</u> (Mel.) sp.	Patois	new virus
12	<u>Aed. squamipennis</u>	Gamboa	new virus
2	<u>Culex</u> sp.	Turlock	
1	<u>C. nigripalpus</u>	SLE	
1	<u>C.</u> (Mel.)EC8 (= <u>parataeniopus</u>)	A	VEE subtype

REPORT FROM THE ROCKY MOUNTAIN LABORATORY,
NATIONAL INSTITUTES OF HEALTH, HAMILTON, MONTANA

We continue to explore the occurrence, distribution, and vector relationships of tick-borne viruses, particularly of the western United States, and with special reference to those viruses that are associated with migratory and pelagic birds. Since last report, 523 pools of ticks were tested in suckling mice and Vero cells for the presence of viruses. Except for 26 pools from Tanzania, all were of ticks common to the Western Hemisphere. Colorado tick fever (CTF) virus was isolated on three occasions from 18 pools of Dermacentor andersoni [Ixodidae] collected in Malheur Co. (1 strain) and Deschutes Co. (2 strains), Oregon. Two isolations of a virus closely resembling Hughes virus were made from 36 pools of Ornithodoros (Alectorobius) capensis [Argasidae] collected on Aves Island, some 90 mi. SW of Guadalupe in the Lesser Antilles. In addition, numerous ticks of this same species were collected from abandoned brown pelican nests on islands off the coast of Texas, U.S.A. These ticks were tested as 164 pools, yielding 66 viral isolates of which all but 3 were indistinguishable from Soldado virus in complement fixation (CF), immunofluorescent (F), and plaque reduction neutralization (PRNT) tests. Soldado virus, first isolated from Trinidad, West Indies, has since been recorded from O. A. capensis group ticks in various parts of the World. However, the present report is the first record of this virus from the North American continent and the contiguous United States.

The 3 other isolates from these Texas seabird parasites are related to Upolu virus, an ungrouped virus from similar ticks taken from the Great Barrier Reef of Australia. Our isolates react markedly in CF, IF, and PRNT tests with Upolu virus antisera. However, Upolu virus reacts only slightly or not at all in these tests with potent antisera prepared from the Texas virus. The latter, which we call Aransas Bay virus, is being characterized in preparation for registration in the World Catalog of Arboviruses. It is an RNA virus, between 100-220 nm in size, possesses essential lipids, and is sensitive to exposure to heat and acid. Repeated attempts to produce a hemagglutinin from Aransas Bay virus have proven negative. The virus is lethal for suckling and weanling mice by both i.c. and i.p. routes of inoculation, and infects Vero cells with the production of CPE and plaques. Mosquito cell cultures (Aedes albopictus and Culex quinquefasciatus) do not permit growth of Aransas Bay virus but, based on preliminary tests, Dermacentor andersoni primary cultures apparently do. The finding of a virus related but not identical to Upolu requires the recognition of a new serological group, Upolu. The occurrence of this virus in proximity to urban areas of the southwestern United States has epidemiological implications.

Ornithodoros (Alectorobius) denmarki was collected from brown pelican nests on San Lorenzo Norte I. in the Gulf of California, Mexico. Two hundred and forty-five of these ticks were sent to us for identification

and virological testing. Virus isolations were made from 22 of 49 pools. All were identified as Raza virus (Hughes serogroup) by CF, IF and PRNT tests, and the infection rate of ticks was calculated as 12.7%.

A virus we isolated on three occasions in 1974 from Argas brumpti [Argasidae] has been tested exhaustively in CF test with numerous arbovirus antisera in our possession, with negative results. This virus has been sent to Dr. J. Casals (YARU) for further identification. Argas brumpti, apparently a ubiquitous parasite of vertebrates of the Ethiopian faunal region, is known to attack humans and inflict painful bites.

Two hundred and eleven blood clots from marine birds taken in the Pribiloff Islands (Alaska) during the course of studies of influenza viruses were sent to us by the collector, Dr. Bernard Easterday of the Department of Veterinary Science, University of Wisconsin. A single viral isolate was recovered from the blood of a common murre, Uria aalge. CF and IF tests of this virus indicate its relationship to Okhotskiy and Yaquina Head viruses (Kemerovo group, Cape Wrath subgroup) from the USSR and Oregon, U.S.A., respectively. Insofar as we are aware, this is the first recovery of a virus of this subgroup from a vertebrate host.

(C. E. Yunker, C. M. Clifford, J. E. Keirans, and L. A. Thomas)

REPORT FROM THE VIRAL AND RICKETTSIAL DISEASE LABORATORY
CALIFORNIA STATE DEPARTMENT OF HEALTH, BERKELEY, CALIFORNIA

The large epidemic of St. Louis encephalitis in the U.S. during 1975 resulted in concern about a possible epidemic during 1976. However, no large outbreaks occurred in the country, and surveillance in California again showed the relatively low level of arbovirus activity characteristic of the past 15 years. Attention was particularly focussed on the Imperial Valley, where late seasonal rainfall and flooding threatened to increase the mosquito population. Detailed results of our surveillance are published each year in the Proceedings and Papers of the California Mosquito Control Association, Inc. Annual Conference.

There were 455 patients who had significant febrile illness, aseptic meningitis, or encephalitis, and were tested serologically for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE), as well as other diseases. This was the lowest number in the last 5 years (peak year was 1973, with 1,037 cases tested). The majority of these cases was found to be caused by enteroviruses, mumps virus, herpes virus, leptospirosis, etc., or the cause could not be determined. Further tests of 102 cases of undetermined etiology, to see if other mosquito-borne viruses besides WEE and SLE cause human disease in California, will be made in Dr. W. C. Reeves' laboratory (the 12th year for this special study). There were 13 human brain samples tested in suckling mice, but none yielded arboviruses.

Three cases of SLE were detected during 1976. A 34 year old woman from Riverside County, probably exposed while frog-hunting June 20, became ill June 30 with severe headache, disorientation and seizures. Paired sera showed rising SLE antibody titers as follows: complement-fixation (CF) <1:8 to 1:16; indirect fluorescent antibody (IFA) 1:256 to 1:2,048; plaque-reduction neutralization (PRNT) 1:1,024 to 1:4,096; and hemagglutination-inhibition

(HAI) 1:80 to 1:160 (all HAI titers reported here were done at the School of Public Health, U.C., Berkeley). This was the first case of SLE acquired in California since 1973, when there were 5 cases. Her probable place of exposure was $1\frac{1}{2}$ miles from where SLE-positive Culex tarsalis were collected June 9.

A 48 year old woman from San Diego became ill August 6, with malaise, fever, progressive confusion, and abnormal cerebrospinal fluid and encephalogram. The SLE antibody titers were: CF \leq 1:16 to 1:16; IFA 1:256 to 1:1,024; PRNT 1:1,024 to 2:16,384; and HAI 1:40 to 1:320. She and her husband may have been exposed July 2-4 or July 23-24, while fishing in Imperial County. SLE-positive mosquitoes were found in the area during the summer. The husband was not ill, but a serum sample had SLE antibody titers of: CF 1:8; IFA 1:1,024; PRNT 1:512; and HAI 1:80, evidence of past or current infection.

A 29 year old woman from Madera became ill September 26, with stiff neck, fever, and cells in the cerebrospinal fluid. The SLE antibody titers were: CF \leq 1:4 to 1:8; IFA 1:256 to 1:512; PRNT 1:1,024 to 1:4,096; HAI - pending. Exposure was presumably near her home.

No human cases of WEE were detected in 1976. There were 35 suspect cases of encephalitis in equines from 25 counties; serum samples were submitted from 25 of them, but none had significant WEE antibody titers. There were 15 equine brain samples tested in suckling mice, none yielding arboviruses; however, 4 were positive for rabies by the FRA test.

There were 1,273 mosquito pools collected and tested in suckling mice during the year, the majority from Imperial, Riverside, and San Bernardino Counties. Arbovirus isolates included 10 SLE, 11 Turlock, and 1 Hart Park strains, all from C. tarsalis. From 19,628 vector pools (over 743,132 specimens) tested from 1969 to the present, 650 virus strains have been isolated, including: 159 WEE, 193 SLE, 199 Turlock, and 99 strains of other viruses (Hart Park,

California encephalitis group, Bunyamwera group, and a few not yet identified). Efforts to identify and characterize the latter, along with some other unknown viruses from tick species and several vertebrate species, are being made in collaboration with Drs. Johnson, Reeves, and Hardy; the Center for Disease Control; and the Yale Arbovirus Research Laboratory.

There were 12 cases of Colorado tick fever (CTF) confirmed during 1976, 3 acquired in Colorado, and the rest in endemic areas of California. Since 1954, when recordkeeping began in the state, there have been 205 confirmed cases. Research is continuing (in collaboration with Dr. Robert S. Lane, Vector Control and Solid Waste Management Section, California State Department of Health) on the natural history of CTF in previously unstudied areas of California. CTF virus was isolated and reisolated in suckling mice from the blood clot of a black-tailed jackrabbit (Lepus californicus), shot 30 March, 1976, at the University of California Hopland Field Station, Mendocino County. The rabbit's serum had CTF antibody titers of 1:32 by both the PRNT and IFA tests. This is the first reported isolation of CTF virus from this species; however, CTF neutralizing antibody was detected in L. californicus previously (Philip, C.B. and Hughes, L.E., "Disease agents found in the rabbit tick, Dermacentor parumapertus, in the Southwestern United States", 6th Intern. Congr. Microbiol., Rome, 5:541, September 6-12, 1953; and Philip, C.B., Bell, J.F., and Larson, C.L., "Evidence of infectious diseases and parasites in a peak population of black-tailed jack rabbits in Nevada", J. Wildl. Mgmt. 19(2):225-233, April, 1955). The location of our positive finding is far west of the previous known distribution of CTF virus in California, in an area where Dermacentor andersoni does not occur, indicating that there is a maintenance cycle different from the usual golden mantled ground squirrel/chipmunk/D. andersoni cycle. A serologic survey has thus far shown CTF antibody in 31 additional L. californicus (PRNT and IFA tests),

and in 21 deer (Odocoileus hemionus), 1 sheep (Ovis aries), 2 coyotes (Canis latrans), 3 ground squirrels (Spermophilus beechevi), 2 California meadow mice (Microtus californicus), 2 wood rats (Neotoma fuscipes), and 1 deer mouse (Peromyscus maniculatus) (all by PRNT tests). Further studies are in progress at this site and will be extended to other areas as well.

Laboratory studies are continuing on the phenomenon of replication of CTF virus within erythrocytes of infected hosts (including man), first reported by us (Emmons, R.W., Oshiro, L.S., Johnson, H.N., and Lennette, E.H., "Intra-erythrocytic location of Colorado tick fever virus", J. Gen. Virol. 17:185-195, 1972). Infection in early-stage hematopoietic cells has been demonstrated by electron microscopy. Fluorescent antibody staining of peripheral blood smears continues to be a rapid and reliable diagnostic test for human cases. The long-persistent (3-4 months in man) erythrocyte-associated viremia allows a definitive diagnosis to be made by FA staining and virus isolation (after separating the erythrocytes from antibody-containing plasma in a heparinized blood sample), even months after recovery of the patient from all symptoms.

Fluorescent antibody staining methods are routinely used in our laboratory also for identification of various arboviruses isolated in suckling mice or cell cultures (such as WEE, SLE, Turlock, Hart Park, CEV-group), and for the titration of antibodies (indirect method). A chapter describing our standard methods and their applications in public health and clinical virology will be published this year (Emmons, R.W. and Riggs, J.L., in Maramorosch, K. and Koprowski, H., Editors, Methods in Virology, Vol. VI, Academic Press, Inc., New York).

(R. W. Emmons, Calif. Dept. of Health; H. N. Johnson, Rockefeller Foundation, retired; and W. C. Reeves, J. L. Hardy, U. of Calif. School of Public Health)

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

Strains of California encephalitis virus (snowshoe hare subtype) were isolated from 8 of 475 pools comprising 23,747 unengorged female mosquitoes of 5 species collected at 3 of 6 locations throughout the Mackenzie Valley of the Northwest Territories, Canada, from latitudes 60 to 69°N between 10 and 24 July 1976. Minimum field infection rates included 1:2734 for Aedes communis, 1:256 to 1:3662 for A. hexodontus and 1:911 to 1:1611 for A. punctor. Northway virus was also isolated from 1:3662 A. hexodontus mosquitoes collected at Inuvik (69°N, 135°W). Wild-caught A. communis mosquitoes transmitted a zero-passage arctic isolate of CE virus (strain 74-Y-234) after 13 days or more of extrinsic incubation at 13° and 23°C following infection with 1 mouse LD₅₀ by feeding or by intrathoracic injection.

(D. M. McLean)

REPORT FROM THE VIRUS LABORATORY - LABORATORY SERVICES BRANCH
ONTARIO MINISTRY OF HEALTH
TORONTO, CANADA

ST. LOUIS ENCEPHALITIS (SLE) VIRUS IN ONTARIO

THE 1975 SLE EPIDEMIC

No data are available to exclude the possibility that cases of SLE could have occurred in Ontario before 1975. During the summer of 1975, the first recorded outbreak of SLE occurred in Canada (1,2). This outbreak appeared as an extension of a large epidemic in the U.S.A. which involved states in the Mississippi watershed and some adjoining the Great Lakes.

A total of sixty-six cases in Ontario were considered confirmed by serological tests. SLE virus was isolated from the brain in a fatal case. The epidemic involved southwestern Ontario (please see map). The majority of the cases (51 or 77%) occurred in the southwestern tip of the Province with 50 cases in the Windsor-Essex and one in the Kent-Chatham Health Units. Fifteen other cases (22.7%) were distributed as follows: 4 in Lambton-Sarnia, 6 in Niagara Regional Area, 2 in Hamilton-Wentworth and 3 in Metropolitan Toronto.

The 1975 SLE epidemic in Ontario appears to have involved areas south and west of a line drawn by the Committee on Programs for the Prevention of Mosquito-Borne Encephalitis, Ontario Ministry of Health. This line extends from the northern part of Toronto to Sarnia (please see map).

Fifty (76%) of these confirmed cases were serologically followed to determine antibody levels. Figure 1 shows the titres of residual

haemagglutination inhibiting (HAI) and complement fixing (CF) antibodies 8-9 months after infection with SLE. This information was needed to: (a) establish criteria for the interpretation of serological results in suspected cases of arboviral etiology in 1976, and (b) assist in assessing the results of a Province wide survey undertaken to investigate the spread of infection with SLE during the 1975 epidemic.

Figure 1 shows that (a) HAI antibody titres of 40 or higher were present in 10 (20%) of the cases. CF antibody titres of 16 or higher were detected in 8 (16%) of the samples. Our lowest dilution of screening for CF antibodies has been 1:8. In the majority of these 50 cases (29 or 58%), the CF antibody titre fell below 8.

SURVEILLANCE FOR SLE IN 1976

On the basis of these data, additional mosquito control measures were recommended during 1976 whenever the first serum sample from a suspected case of SLE had HAI antibody titres of ≥ 40 and/or CF antibody titre of ≥ 16 (2). This presumptive diagnosis of infection with SLE was followed for confirmation on the basis of established criteria (3). Laboratory tests were conducted also to exclude other or concurrent viral infections.

During the period between April 15 and December 31, 1976, 1094 serum samples from 737 cases with definitive or suspected involvement of the central nervous system (CNS) were referred to the Arbovirus Unit of the Laboratory Services Branch, Ontario Ministry of Health.

Many arboviruses as well as members of other groups of viruses can cause syndromes similar to those caused by SLE. These sera were tested,

therefore, through the cooperation of various sections of this laboratory for evidence of infection with: eastern equine, western equine and California encephalitis, Powassan, herpes simplex, varicella, measles, mumps, selected enteroviruses and other viruses as needed. Appropriate clinical samples were also inoculated in cell cultures as well as in new born mice to attempt virus isolation.

According to the information given in the case history sheets, the 737 cases were divided in the following groups (Table 1): a) Encephalitis, b) Possible, suspected or "rule out encephalitis", c) Meningitis or possible meningitis, d) Headache, and/or neck rigidity with or without fever, e) Other symptoms, clinical conditions or incomplete information. Large metropolitan areas and those involved in the 1975 outbreak constituted the major contributors of the samples submitted.

Four confirmed cases of infection with SLE virus were identified in 1976 in the areas of Windsor-Essex and Kent-Chatham Health Units (please see map and Tables 2 and 3). The first serum sample in three of the four confirmed cases satisfied the criteria proposed for the presumptive diagnosis and subsequent alert to further control measures as described. In these three cases, HAI antibodies appeared earlier and their titres were higher than CF antibodies. HAI antibodies were detected about four days after onset of disease.

It is interesting that a fatal case in 1975 had concurrent infections with herpes simplex and SLE viruses. In 1976, seroconversion to both SLE and vaccinia viruses occurred in Case 2. However, it should be noted that the patient had received vaccination for smallpox prior to his sickness.

The number of cases associated with or caused by other viruses in 1976 did not suggest a wide spread pattern. A number of enteroviruses has been isolated and coxsackie B5 appeared to be more common than the others.

Our experience in 1976 demonstrates the value of careful surveillance of cases with suspected involvement of the CNS. Case 1 (Table 2) was the first indication of SLE activity in 1976. Infection was recognized before sentinel chickens showed seroconversion or before virus was recovered from mosquitoes. The importance of the integrated approach to surveillance of SLE activity in critical areas is stressed.

Serum samples from the confirmed cases of 1975 and 1976 are expected now for further serological investigation. Results would then serve as guidelines in monitoring arboviral activity during 1977.

EXTENT OF INFECTION WITH SLE IN 1975

A serological survey was undertaken to: a) assess the spread of infection with SLE virus within the epidemic zone, b) test for the possibility of infections outside the epidemic zone. A total of 3507 serum samples were collected, therefore, from different parts of Ontario through various sources. The first was our routine program to test for rubella antibodies. These samples were chosen because they were available and were obtained before the 1976 mosquito activity. Other sources included the Health Units, hospitals, our Regional Laboratories, etc.

Many of the specimens tested were originally received for syphilis serology. This appeared a convenient source and allowed us to select from various age groups. The only difficulty was the scarcity of samples from children of ≤ 10 years of age.

All samples were screened for complement fixing antibodies at 1:8 dilution and for haemagglutination inhibiting antibodies at 1:20 dilution. For HAI test sera were treated by Kaolin adsorption followed by absorption with goose erythrocytes then examined against 8 units of the Parton strain of SLE antigen. Reacting sera were extracted with acetone and titrated against three Group B arbovirus antigens: SLE, Powassan and Banzi. The results of the CF tests are being confirmed and those completed for HAI antibodies are reported here.

We tested 2808 sera, 1763 (63%) were from the epidemic zone and 1045 (37%) were from the non-epidemic zone. Forty-four (1.6%) reactive sera were identified and the results were confirmed by the National Arbovirus Reference Service, Toronto. Tables 4 and 5 show the locations and percent of positive reactors. The incidence of reactors was 1.93% within the epidemic zone and to 0.96% outside it. Tables 6 and 7 show the areas involved and HAI antibody titres against the three antigens.

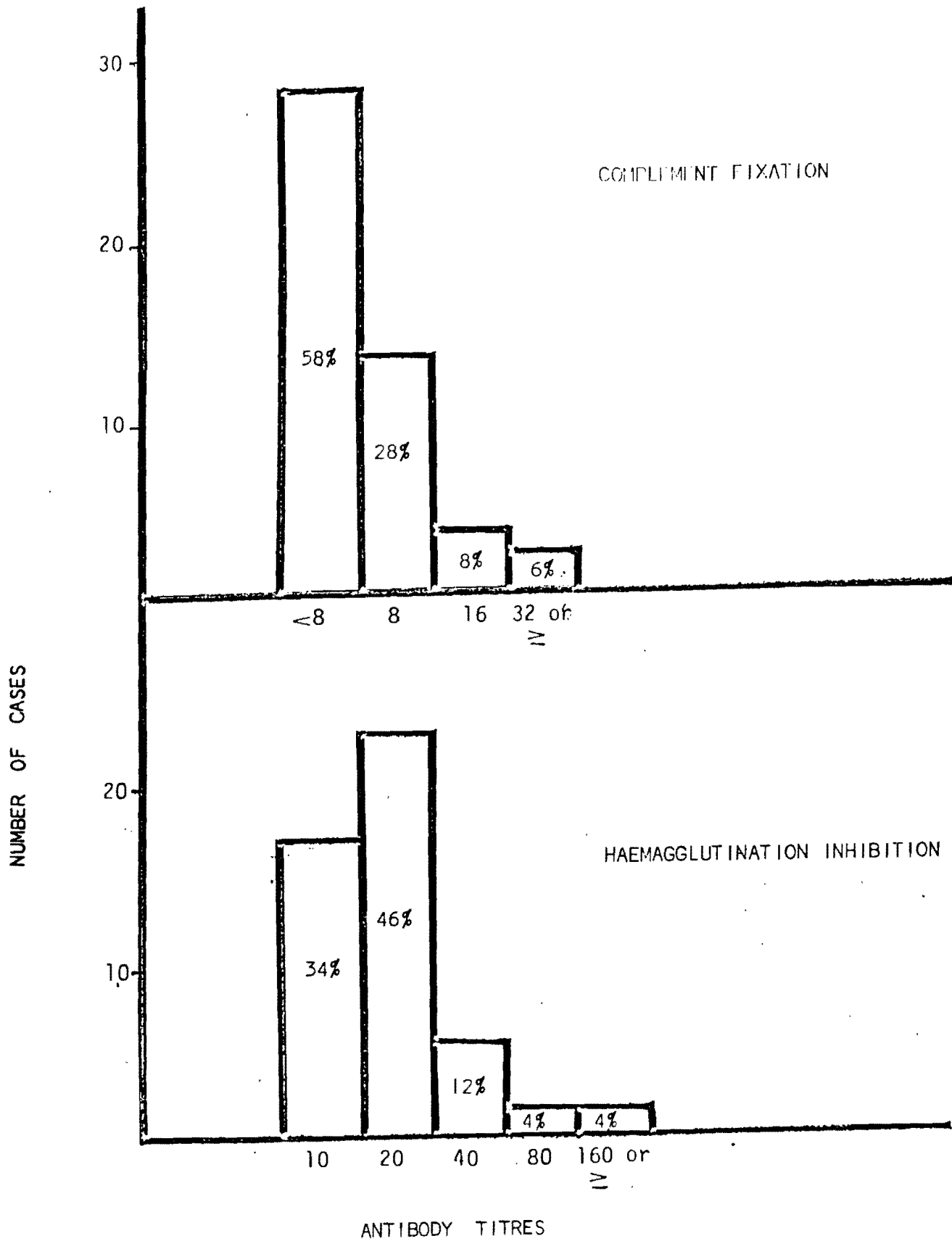
Donors of the samples which gave positive reactions indicating group B virus infection are being investigated further. The significance of these findings can be determined only after: a) the persons involved are traced and interviewed for history of travel to areas where SLE or other group B arboviruses may be present and for history of sickness during the 1975 epidemic, b) further serological tests are completed.

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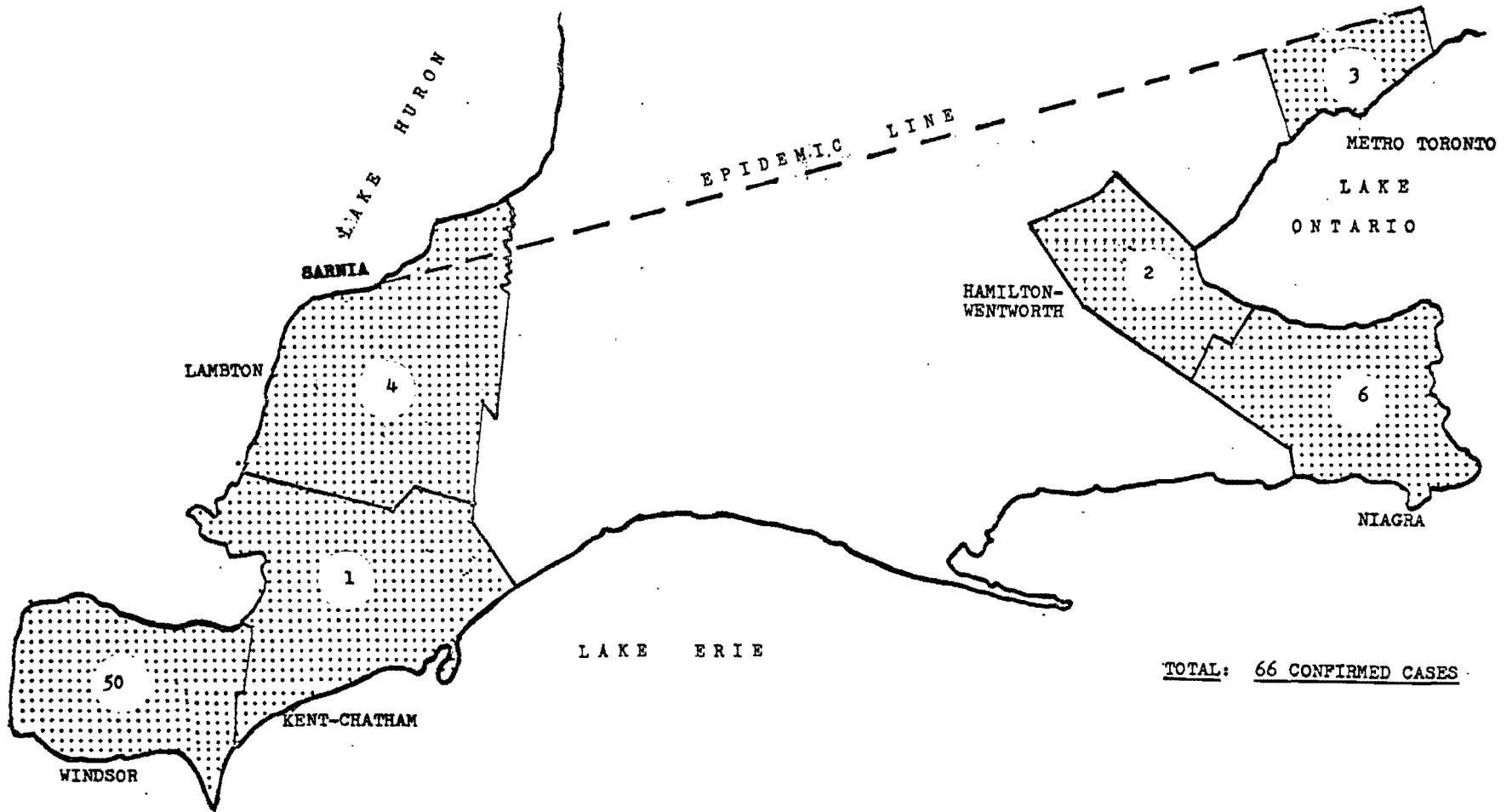
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M5W 1R5

RESIDUAL ANTIBODIES IN 50 CASES OF ST. LOUIS
ENCEPHALITIS 8 MONTHS AFTER INFECTION



ST. LOUIS ENCEPHALITIS IN SOUTHWESTERN ONTARIO IN 1975

250



T A B L E 1

INFORMATION GIVEN WITH REQUESTS FOR
LABORATORY INVESTIGATION OF 737 CASES OF
SUSPECTED NEUROTROPIC VIRAL ETIOLOGY

April 15 - December 31/1976

	Cases	Percent
Encephalitis	60	8.1
Possible, Suspected or "Rule Out Encephalitis"	154	20.9
Meningitis or Possible Meningitis	175	23.7
Headache and/or Neck Rigidity With or Without Fever	220	29.8
Other Symptoms, Clinical Conditions, or Incomplete Information	128	17.4
Total	737	100.0

TABLE 2

CONFIRMED CASES OF ST. LOUIS ENCEPHALITIS IN 1976

	Case 1	Case 2	Case 3	Case 4
Age and Sex	56 F	16 M	53 F	16 F
Date of Onset	August 8	August 8	Admitted August 18	Sept. 2
Date of First Serum Sample	August 12	August 12	August 19	Sept. 6
Second Serum Sample	August 17	August 19	August 26	Sept. 17
Location	Amherstburg Essex	Dresden Kent	Tecumseh Essex	Windsor

TABLE 3
 SEROLOGICAL REACTIONS TO ST. LOUIS ENCEPHALITIS AND POWASSAN
 VIRUS ANTIGENS² IN FOUR CASES - SUMMER 1976

Test	SLE				POWASSAN			
	Titres							
	Case 1	Case 2	Case 3	Case 4	Case 1	Case 2	Case 3	Case 4
<u>Haemagglutination</u> <u>Inhibition</u>								
First Sample	320	80	40	10	20	10	10	<10
Second Sample	640	640	160	160	20	80	40	80
<u>Complement Fixation</u>								
First Sample	4	< 8	4	< 8	< 8	< 8	< 8	< 8
Second Sample	16	32	32	32	< 8	< 8	< 8	< 8

1. Results have been confirmed by the National Arbovirus Reference Service.
2. No HAI or CF antibodies against WEE, EEE and California encephalitis viruses were detected in all samples.

TABLE 4

SURVEY FOR SEROLOGICAL REACTIONS (1) BY HAI TEST
WITH THREE GROUP B ARBOVIRUS ANTIGENS
(ST. LOUIS ENCEPHALITIS, POWASSAN AND BANZI)

Within 1975 Epidemic Zone of SLE

Health Unit/Area	Number Tested	Testing Laboratory	Number Positive (2,3)	Incidence Percent
Haldimand-Norfolk	95	CPHL	0	0
Kent-Chatham	66	WPHL	0	0
Kent-Chatham	33	CPHL	0	0
Lambton-Sarnia	519	CPHL	9	1.73
Middlesex-London	128	CPHL	4	3.12
Peel Region, Brampton, and Mississauga	81	CPHL	1	1.23
Windsor-Essex	841	WPHL/ CPHL	20	2.37
Total	1763		34	1.93

(1) Tests were performed as indicated at: Central Public Health Laboratories, Toronto (CPHL), and Windsor Public Health Laboratory, Windsor (WPHL), -Director - Miss A. Prytula.

(2) Results of WPHL were confirmed at CPHL.

(3) All positive results have been confirmed by the National Arbovirus Reference Service, Dr. H. Artsob.

TABLE 5
 SURVEY FOR SEROLOGICAL REACTIONS (1) BY HAI TEST
 WITH THREE GROUP B ARBOVIRUS ANTIGENS
 (ST. LOUIS ENCEPHALITIS, POWASSAN AND BANZI)
Outside 1975 Epidemic Zone of SLE

Health Unit/Area	Number Tested	Testing Laboratory	Number Positive	Incidence Percent
Algoma, Sault Ste. Marie	44	CPHL ⁽¹⁾	0	0
Eastern Ontario, Ottawa	676	CPHL	7 ⁽³⁾	0
Northwest, Kenora	50	CPHL	1	2
Peterborough	50	CPHL	0	0
Porcupine, Timmins	39	CPHL	0	0
Simcoe, Orillia	50	CPHL	1	2
Sudbury	50	CPHL	0	0
Thunder Bay	86	CPHL	1	0.13
Total	1045		10	0.96

(1) CPHL: Central Public Health Laboratories, Toronto.

(2) All positive results have been confirmed by the National Arbovirus Reference Service, Dr. H. Artsob.

(3) Two positive samples from Ottawa were collected prior to 1975 epidemic.

TABLE 6

POSITIVE REACTORS TO GROUP B
ARBOVIRUS ANTIGENS (SLE, POWASSAN, BANZI)
IN ONTARIO AFTER THE 1975 SLE EPIDEMIC

Within the 1975 Epidemic Zone

Health Unit/Area	CPHL* Reference No.	Age Group	Sex	HAI Titres		
				SLE	Powassan	Banzi
Lambton- Sarnia	A-141	50-59	F	80	40	20
	A-147	60-69	F	20	<10	<10
	A-187	50-59	M	160	<10	<10
	A-211	50-59	F	80	20	20
	A-280	50-59	F	160	<10	<10
	A-290	50-59	F	40	<10	<10
	A-297	50-59	M	80	<10	<10
	A-576	30-39	F	40	NA**	80
	A-614	50-59	F	20	<10	<10
Middlesex- London	A-1580	40-49	M	40	40	80
	A-1598	50-59	M	10	NA	10
	A-1605	50-59	M	10	NA	20
	A-1608	50-59	M	≥ 20	NA	80
Peel Regional- Mississauga	A-4031	60-69	M	40	80	160
Windsor-Essex	ARF7-13	40-49	M	20	40	40
	ARF7-15	30-39	F	20	80	80

TABLE 6

Health Unit/Area	CPHL* Reference No.	Age Group	Sex	IIAI Titres		
				SLE	Powassan	Banzl
Windsor-Essex	ARF7-20	20-29	F	10	< 10	20
	ARF7-21	10-19	M	20	40	80
	ARF7-24	50-59	F	<10	10	20
	ARF7-26	30-39	M	10	80	80
	ARF7-27	40-49	F	20	160	160
	ARF7-28	50-59	M	20	160	160
	ARF7-29	30-39	F	<10	10	10
	ARF7-30	50-59	F	<10	10	10
	ARF7-31	10-19	M	10	20	80
	ARF7-32	30-39	F	<10	10	10
	ARF7-33	10-19	F	<10	10	20
	ARF7-35	30-39	F	<10	10	20
	ARF7-16	50-59	F	<10	20	40
	ARF7-17	30-39	M	20	40	80
	ARF7-19	30-39	F	<10	10	20
	ARF7-23	30-39	M	<10	20	40
	ARF7-25	≥ 70	F	10	10	20
	ARF7-36	50-59	F	10	20	40

* CPHL: Central Public Health Laboratories, Toronto.

** NA: Not available because results are inconclusive or sample is exhausted.

TABLE 7

POSITIVE REACTORS TO GROUP B
ARBOVIRUS ANTIGENS (SLE, POWASSAN, BANZI)
IN ONTARIO AFTER THE 1975 SLE EPIDEMIC
Outside 1975 Epidemic Zone

Health Unit/Area	CPHL* Reference No.	Age Group	Sex	HAI Titres		
				SLE	Powassan	BanZI
Eastern Ontario- Ottawa	A-1015***	20-29	F	10	40	80
	A-1042***	20-29	F	160	80	NA**
	A-7550	10-19	M	40	NA	NA
	A-7569	20-29	M	10	40	20
	A-7661	20-29	F	40	640	80
	A-7684	20-29	F	40	320	160
	A-7747	30-39	F	20	160	80
North Western- Kenora	A-5004	10-19	M	40	40	20
Simcoe-Orillia	A-6546	60-69	M	10	<10	20
Thunder Bay	A-5529	30-39	F	20	40	10

* CPHL: Central Public Health Laboratories, Toronto.

** NA: Not available because results are inconclusive or sample is exhausted.

*** Collected in June 1975 before the epidemic.

