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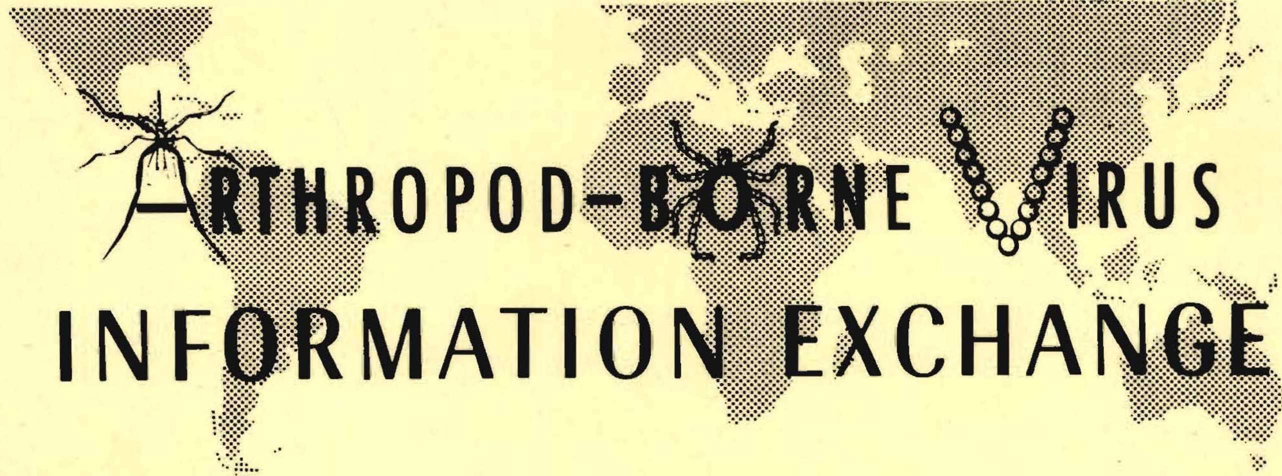

Number 38 March 1980

TABLE OF CONTENTS

Page

Announcement and Editorial Comments 1
1979 Annual Report on the Catalogue of Arthropod-Borne and Selected Vertebrate Viruses of the World 2

Reports from:

Laboratory of Enzimology, Department of Molecular Biophysics, Instituto de Biofisica, Universidade Federal do Rio de Janeiro, Brasil 58
Arbovirus Laboratory, Institut Pasteur and O.R.S.T.O.M., French Guiana 59
Gorgas Memorial Laboratory, Panama 63
Departamento de Virologia, Hospital General de Mexico, S.S.A., and Instituto de Investigaciones Biomedicas, U.N.A.M., Dengue Virus Activity in Southeastern Mexico 66
Texas Department of Health, Austin, Texas 67
Office of Laboratory Services and Entomology, Department of Health and Rehabilitative Services, Jacksonville, Florida 72
Viral Diseases Division, Center for Disease Control, Atlanta, Georgia 73
Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 75
Division of Virology and Immunology, Bureau of Laboratories, Pennsylvania Department of Health, Lionville, Pennsylvania 76
Cornell University, Department of Microbiology (Medical College, New York) and Department of Entomology (College of Agriculture, Ithaca) NY 78
State of New York Department of Health, Division of Laboratories and Research, Albany, New York 80
State of New Jersey Department of Health, John Fitch Plaza, Trenton, New Jersey 81
University of Notre Dame's Laboratory for Arbovirus Research and Surveillance (UNDLARS), Notre Dame, Indiana 83
Arbovirus Surveillance Program, Division of Laboratories, Illinois Department of Public Health, Chicago, Illinois 89

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):

Vector-Borne Diseases Division, Center for Disease Control, Fort Collins, Colorado 91
Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, California 95
National Arbovirus Reference Service, Department of Medical Microbiology, University of Toronto, Ontario, Canada 97
Division of Medical Microbiology, University of British Columbia, Vancouver, B.C., Canada 100
Pacific Research Unit, Honolulu, Hawaii 101
Arbovirus Laboratory, Institut Pasteur de Noumea, New Caledonia 103
Queensland Institute of Medical Research, Herston, Brisbane, Australia 105
Department of Microbiology, University of Western Australia, Perth, Western Australia 108
Virology and Entomology Departments, U. S. Naval Medical Research Unit No. 2, Jakarta Det., APO San Francisco, California 115
Department of Virology, U.S. Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand 128
Department of Microbiology, Kobe University School of Medicine, Kobe, Japan 137
Department of Virology and Rickettsiology, National Institute of Health, Shinagawa, Tokyo 141, Japan 138
Department of Virology, School of Tropical Medicine, Calcutta, India 139
Virus Research Centre, Medical Research Centre, Nairobi, Kenya 142
Arbovirus Research Unit, Egyptian Organization for Biological Products and Vaccines, Agouza, Cairo, Egypt 144
WHO Collaborating Centre for Arbovirus Reference and Research, Institute of Virology, Bratislava, Czechoslovakia 151
Medical Institute of Environmental Hygiene, Arbovirus Laboratory, AUF'M Hennekamp, Duesseldorf, West Germany 154
Instituut Voor Tropische Geneeskunde, Laboratory of Bacteriology and Virology, Nationalestraat , Antwerpen, Belgium 157
Arbovirus Laboratory, Unit of Viral Ecology, Institut Pasteur, Paris, France 162
Virus Laboratory, Faculty of Medicine, Brest, France 163
Special Pathogens Reference Laboratory, Public Health Laboratory Service Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, England 165
Edward Grey Institute of Field Ornithology and the Unit of Invertebrate Virology, South Parks Road, Oxford, England 167
Section of Arboviruses, Laboratory of Virology, National Institute of Health, Lisbon, Portugal 170
San Juan Laboratories, Center for Disease Control, San Juan, Puerto Rico 172

Roy W. Chamberlain, Sc.D., Editor
Bette A. Hall, Secretary

ANNOUNCEMENT AND EDITORIAL COMMENTS

1. Richard M. Taylor Award.

The Executive Council of the American Committee on Arthropod-borne Viruses will present the eighth Richard M. Taylor Award to an outstanding arbovirologist in November, 1980, at the annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia. As most of you know, this coveted award was first presented to Dr. Richard M. Taylor in 1966 in recognition of his outstanding career in vector-borne diseases and the great impact it has had on the field of arbovirology. Six other scientists have since received this medal: Jordi Casals, 1968; William McD. Hammon, 1970; William C. Reeves, 1973; Roy W. Chamberlain, 1975; Wilbur G. Downs, 1977; Pedro Galindo, 1977.

According to convention, the last three recipients have been asked to serve as a nominating committee to select an appropriate candidate for this award. I am serving as chairman. Your suggestions will be gratefully received until June 1, 1980, and will be given serious consideration. Please support your nomination with a short summary of outstanding achievements which in your judgement qualify the individual for the award.

2. Staff changes, Winches Farm Field Station.

The Arbovirus Research Unit of the London School of Hygiene and Tropical Medicine, at Winches Farm Field Station, St. Albans, Herts, England, is now under the direction of Dr. M.G.R. Varma, with Dr. E. Gould as Senior Virologist.

3. New size for the Information Exchange.

You will note that the Arthropod-borne Virus Information Exchange is now full standard letter size (8 1/2 x 11 inches) rather than its previous smaller size (8 x 10 inches). This came about through a change in U.S. government regulations concerning standard page size.

4. Deadline for reports, issue No. 39.

Mark your calendar. Reports for issue No. 39 are due September 1, 1980. Remember to use Air Mail outside the United STates.

Roy W. Chamberlain, Editor
Arthropod-borne Virus Information Exchange
Virology Division
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Atlanta, Georgia 30333

The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1979 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.

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*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 1979, 165 mailings of Catalogue material were being made. During the year, 3 new participants were added to the mailing list. At the end of the year, 168 mailings of Catalogue material were being made, including 61 within the U.S.A. and 107 to foreign addresses. Distribution by continent was: Africa 15, Asia 21, Australasia 7, Europe 36, North America 73, and South America 16.

Abstracts and Current Information: A total of 525 abstracts or references were coded by subject matter and distributed to participants during 1979. Of this total, 383 were obtained from Biological Abstracts, 137 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 5 from current journals, personal communications, or other sources. A total of 11,937 references or units of information have been issued since the start of the program.

Registration of New Viruses: Sixteen new viruses were registered during 1979. As of December 1978, the Catalogue contained 408 registered viruses. With the acceptance of sixteen new virus registrations during 1979 coupled with the recent withdrawal of the Batu Cave virus registration, the total number of registered viruses increased to 423 as of December 1979. The viruses registered during 1979 are listed below:

Virus Name	Recommended Abbreviation	Country	Source	Antigenic Group
Alenquer	ALE	Brazil	Man	PHL
Ananindeua	ANU	Brazil	Rodent	GMA
Belem	BLM	Brazil	Bird	
Benevides	BVS	Brazil	Sentinel Mouse	CAP
Benfica	BEN	Brazil	Sentinel Mouse	CAP
Timboteua	TBT	Brazil	Sentinel Mouse	GMA
Utinga	UTI	Brazil	Sloth	SIM
Barmah Forest	BF	Australia	Mosq.	TUR
Gan Gan	GG	Australia	Mosq.	MAP
Paroo River	PR	Australia	Mosq.	
Picola	PIA	Australia	Mosq.	
Termeil	TER	Australia	Mosq.	
Tilligerry	TIL	Australia	Mosq.	EUB
Yacaaba	YAC	Australia	Mosq.	
Gray Lodge	GLO	U.S.A.	Mosq.	HP
Llano Seco	LLS	U.S.A.	Mosq.	*

These recently registered viruses were isolated between 1965 and 1976. BEN and UTI were isolated in 1965, ANU in 1966, TBT in 1967, BVS and BLM in 1968, GG and YAC in 1970, TIL, GLO, and LLS in 1971, TER in 1972, PR and PIA in 1974, and ALE and BF in 1976.

* Although it has been demonstrated that Llano Seco virus is antigenically related to Umatilla virus, its antigenic relationship to other established orbivirus serogroups is uncertain.

One (BEN) of the above viruses was evaluated as an Arbovirus by the SEAS* Subcommittee. ANU, BVS, LLS, TER, and TBT were evaluated as Probable Arboviruses while all the others were evaluated as Possible Arboviruses.

Only Alenquer virus has been isolated from man, and has been reported to produce a benign febrile illness in man.

Antigenic Grouping: Recent HI and NT data indicate that Rift Valley Fever virus is conclusively an antigenic member of the Phlebotomus fever serogroup (1). The RVF HA was inhibited in HI tests by antibody preparations to three members of the PHL serogroup, and by an NIH PHL grouping immune fluid. Antibody to RVF also reacted in HI tests with HA's for several different viruses of the PHL group. Rift Valley Fever virus was neutralized by the NIH grouping fluid and by immune reagents to several members of the PHL serogroup.

Antigenic studies with established and potentially new members of the Guama group have shown that Guaratuba virus is definitely a member of this serogroup (2). The same can be said for Mirim virus and it is possible that it also is true for Minatitlan virus (2). The placement of Guaratuba, Mirim, and Minatitlan in the Guama group will await completion of these antigenic studies including the cross-testing of these two viruses with a new virus which appears to be related but not identical to both. At that point, the SIRACA† Subcommittee also will reevaluate the classification of viruses of the Guama serogroup.

Of the sixteen new viruses registered during the year, Alenquer is a new member of the PHL group, Ananindeua and Timboteua are new GMA group members, Benevides and Benfica have been placed in the CAP serogroup, Utinga has been placed in the SIM group, Barmah Forest has been linked antigenically to members of the TUR group, Gan Gan was placed in the MAP serogroup, Tilligerry in the EUB group, and Gray Lodge in the HP serogroup. Llano Seco virus was found to be related to Umatilla virus, and to react in CF with a 1:4 dilution of an NIH Palyam polyvalent grouping fluid. The placement of Llano Seco virus in any antigenic group has been left as an open question pending the determination of its relationship to other established orbiviruses.

Taxonomic Status of Registered Viruses: In almost all instances, provisional changes in the taxonomic status of a registered virus have resulted from observations obtained by electron microscopy. Therefore, Paroo River virus, a newly registered and presently ungrouped Australian virus, Tilligerry virus which is antigenically related to memebers of the Eubenangee serogroup, and Llano Seco virus which is antigenically related to Umatilla virus, demonstrate typical orbivirus morphology when examined by electron microscopy (See individual virus registration cards). Electron microscopic examination of Gray Lodge virus by ultrathin sectioning indicated that it is a typical rhabdovirus (See virus registration card). Gray Lodge virus also is antigenically related to Hart Park virus.

Several registered but ungrouped and heretofore unclassified viruses have been provided with provisional taxonomic designations by use of electron microscopy combined, in some instances, with buoyant density determinations (3,4). Thus, it has been determined that Bangui and Upolu viruses are bunyavirus-like, that Bangoran virus appears to be a rhabdovirus, and that Salanga virus is a poxvirus. Originally, Triniti virus was provisionally classified as bunyavirus-like (see covering letter for fourth quarter 1977 Catalogue mailing), but recent more precise electron microscopic observations indicate that it resembles a Togavirus (4).

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

† Subcommittee on Interrelationships Among Catalogued Arboviruses. R.E. Shope (Chairman), W.E. Brandt, C.H. Calisher, J. Casals, R.B. Tesh, and M. Wiebe.

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

Table 1. Alphabetical listing of registered viruses: Table 1 presents an alphabetical listing of the 423 viruses registered in the Catalogue as of December 1979. 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 (5). 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 54 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 12 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. Registered viruses belonging in the Bunyamwera Supergroup constitute approximately one-fourth of all registered viruses. All Supergroup viruses examined thus far possess similar, if not identical, morphologic and morphogenetic characteristics (6,7) as well as other biochemical properties, and have been designated to form the Bunyavirus genus within the family Bunyaviridae by the International Committee on Taxonomy of Viruses (ICTV) (7,8).

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

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

Tables 5 through 27 list registered viruses by serogroup with information regarding isolations from arthropod vectors and vertebrates, and geographic (by continent) distribution based on virus isolation. Data also are presented regarding production of disease in man in nature or by laboratory infection, evaluation of arbovirus status, and proved or provisional taxonomic status. Where possible, sets of viruses were grouped additionally according to their actual or suspected principal athropod 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: The data in Table 5 clearly illustrate that alphaviruses are mosquito associated, although a few have been isolated from other arthropods. A report describing the isolation of Sindbis virus from *Hyalomma marginatum* ticks collected in Sicily has been noted in the updated version of Table 5.

About one-half of the alphaviruses are associated with birds, while some of them, particularly those of the VEE complex, are associated with rodents.

Eleven alphaviruses have been isolated from man while twelve have been implicated in causing human disease either by infections acquired in nature or acquired in the laboratory. All of the latter viruses are rated as Arbovirus (11 viruses) or Probable Arbovirus (1 virus).

Tables 6, 7, and 8. Group B viruses: Of the 59 registered flaviviruses, 49% have been placed in the mosquito-associated category (Table 6), 25% are considered to be tick-borne (Table 7), and 25% are categorized as not being associated with a proven arthropod vector (Table 8).

Twenty-three of the 29 registered group B viruses which are mosquito-associated (Table 6) are rated as Probable Arbovirus or Arbovirus. With the isolation of Rocio virus from Psorophora mosquitoes, it has been removed from the "no arthropod vector demonstrated" category (Table 8) and placed in the mosquito-associated category (Table 6). 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.

With the exception of two members, none of the rest of the registered flaviviruses placed in the "no arthropod vector demonstrated" category (Table 8) are rated above Possible Arbovirus by SEAS. Seven members are rated as Probably not or Not Arbovirus.

At the end of this year, the registration for Batu Cave virus was withdrawn at the request of Dr. A. Rudnick. Cross-neutralization and CF testing have demonstrated that Batu Cave virus was indistinguishable from Phnom Penh Bat virus (9). Thus they are to be considered as strains of the same virus.

Tables 9, 10, 11, 12, 13, and 14. Bunyamwera Supergroup: There are now 12 antigenic sets of viruses plus the unassigned viruses that comprise the Supergroup. The number of individual registered viruses comprising the Bunyamwera Supergroup increased from 93 to 98 during the past year.

Table 9. Bunyamwera group: With the exception of Main Drain virus, all other members of the Bunyamwera serogroup have been isolated from Culicine or Anopheline mosquitoes. In addition, Lokern and Main Drain viruses have been isolated from Culicoides insects.

Bunyamwera, Calovo, Germiston, Ilesha, Tensaw and Wyeomyia viruses have either been isolated from man or have been shown to infect man in nature or have induced laboratory infections in man.

Thirteen of the eighteen (72%) viruses registered in the Bunyamwera serogroup have been rated as Arbovirus or Probable Arbovirus. None are rated below Possible Arbovirus.

Provisionally, SIRACA has removed Guaroa from the Bunyamwera group and placed it in the California group on the basis of its HI and NT relationships to members of the California group.

Table 10. Bwamba and Group C viruses: Both Bwamba and Pongola viruses of the Bwamba serogroup appear to be mosquito-associated, and Bwamba virus has been isolated from man. Thus far, these two viruses have been found in Africa only.

The group C viruses have been closely associated with mosquito vectors and small animals, particularly rodents. Only Gumbo Limbo virus has not been isolated from man.

Table 11. California and Capim group viruses: All the California group viruses are associated with mosquito vectors and four members have been recovered from naturally infected rodents. La Crosse, Guaroa, and Tahyna viruses have been isolated from man. In addition, California encephalitis and Inkoo viruses have been implicated in causing human infections in nature. Only Inkoo and Tahyna viruses have been isolated outside the continents of North and South America. On the basis of virus isolation, the geographic distribution of Tahyna now includes Asia as well as Africa and Europe.

The Capim group viruses are associated with mosquito vectors, and four of the members have been isolated from rodents. None of these eight viruses have been implicated in causing disease in man.

Benevides and Benfica viruses represent recently registered additions to the Capim serogroup. Both were isolated in Brazil and were obtained from both mosquitoes and sentinel mice. In addition, Benfica virus has been isolated from rodents.

Table 12. Guama, Koongol, Olifantsvlei, and Patois group viruses: Guama group viruses have been found only in the western hemisphere. Catu and Guama viruses have been isolated from man and have been implicated in causing infections of man acquired in nature.

Ananindeua and Timboteua virus constitute new members of the Guama serogroup. Both viruses were registered during the past year and were isolated in Brazil from rodents and sentinel mice. Furthermore, Ananindeua virus has been isolated from mosquitoes, birds, and marsupials.

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

The Mirim serogroup was formed in November 1978, and it is likely that the status of this serogroup will change in the near future. Both viruses were isolated in the western hemisphere, and very little is known of their basic characteristics.

The Olifantsvlei group now consists of three members, and all three were isolated in Africa from mosquitoes. Information on the properties of these viruses has not been readily available.

Viruses of the Patois group have been isolated only in North America, and they appear to be associated with mosquito vectors and rodent hosts.

Table 13. Simbu group viruses: Almost as many Simbu group viruses have been isolated from *Culicoides* insects as have been isolated from mosquitoes. None have been recovered from rodents. Most have been isolated from birds or livestock. Oropouche and Shuni viruses are the only members that have been isolated from man. Oropouche virus has caused frequent large outbreaks of disease among the human population in Brazil.

The recently registered Utinga virus represents a new addition to the Simbu serogroup although it was isolated in 1965.

Simbu group viruses have a wide distribution. Approximately 50% have been found in Africa or Africa and Asia, while others have been isolated in Asia or Asia and Australasia and North or South America.

Table 14. Tete group and unassigned (SBU) viruses: All the Tete group viruses have been recovered from birds. Only two of the members (Bahig, Matruh) have been recovered from any kind of a vector, namely ixodid ticks.

At present there are four unassigned viruses. Only Kaeng Khoi virus has not been isolated from mosquitoes. Three of the viruses have been isolated in the western hemisphere while Kaeng Khoi virus has been found in Asia.

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. It is likely that they will constitute a separate genus in the Bunyaviridae family.

The majority of the group members are associated with phlebotomine flies, while 7 of these viruses have been isolated from man or have been implicated in the production of disease in man.

Two viruses have been added to the PHL serogroup. The recently registered Alenquer virus has been isolated from man in Brazil. Recent serological evidence has demonstrated that Rift Valley fever virus is an antigenic member of the PHL serogroup (1). 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.

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.

A low-titered relationship by CF, fluorescent antibody, and indirect hemagglutination has been demonstrated between Congo and NSD viruses. SIRACA has decided that these relationships are no greater than those used to establish the Bunyamwera Supergroup and that the Congo and NSD serogroups should be kept as distinct sets.

Congo virus is antigenically indistinguishable from the agent of Crimean hemorrhagic fever (CHF). The latter virus has been implicated in hundreds of cases of disease in the U.S.S.R. Thus far Hazara has not been known to be involved in infections of man, and little is known of this antigenic relative of Congo-CHF virus.

Two of the Kaisodi group viruses were isolated from ticks in Asia while the third was isolated in North America. None of these viruses have been found to be involved in causing infections in man.

Nairobi sheep disease virus is an important cause of veterinary disease, while both Dugbe and Ganjam viruses have been isolated repeatedly from ticks taken off domestic animals. Both Dugbe and Ganjam viruses have caused a febrile illness in man. In the case of NSD, one infection in man resulted in a febrile illness, while three others resulted in subclinical serologic conversions. Pending further clarification of antigenic relationships, SIRACA considers Ganjam to be a variety of NSD.

Members of the Sakhalin group provisionally have been designated bunyavirus-like on the basis of electron microscopic observations of Avalon and Sakhalin viruses.

Thogoto virus has been isolated from man and has been involved in the production of disease in man. A presently unregistered antigenic relative of Thogoto virus has been isolated in Sicily.

All members of the Uukuniemi serogroup have been isolated from ticks. Two of the viruses were found in Asia while the other three were discovered in Europe. Hemagglutination-inhibition antibodies to Uukuniemi virus have been detected in the sera of human beings residing in Europe.

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 oribiviruses are relatively resistant to lipid solvents, are inactivated at acid pH, and possess multiple segments of a double stranded RNA genome.

Only Colorado tick fever virus of the CTF group and Kemerovo virus of the KEM group have produced disease in man.

Members of the Kemerovo group are widely distributed with at least one virus being found in each of the listed continents. Kemerovo virus has been found in both Africa and Asia while Wad Medani virus has been discovered in Africa, Asia, and North America.

Table 18. Tick-borne groups other than group B viruses: Members of these five minor antigenic groups have not been officially classified taxonomically.

Members of the DGK serogroup have not been isolated from vertebrate hosts, nor from arthropod vectors other than ticks. These viruses have been found in Africa, Asia and Australasia.

Only Hughes virus of the Hughes serogroup has been isolated from birds. It has been found in both North and South America while Soldado virus has been isolated in Africa, Europe, and South America.

Both Qalyub group viruses were found only in Africa, and both have been isolated from ticks. In addition, Bandia virus has been isolated from rodents.

Only Quaranfil virus of the QRF group has been implicated in causing disease in man in nature.

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 taxonomically as bunyavirus-like. All virus members of these minor antigenic serogroups have been primarily associated with mosquito vectors.

Members of the Anopheles A serogroup have been isolated either from Anopheline or Culicine mosquitoes, or both. All three of these viruses have been found only in South America. Tacaiuma virus has been isolated from man and a sentinel monkey, and it has been reported that it causes a febrile illness upon infecting man.

Anopheles B viruses have been isolated only from mosquitoes collected in South America.

Thus far, all viruses of the Mapputta group have been found only in Australia. The recently registered Gan Gan virus has been added to the Mapputta serogroup.

With the addition of Barmah Forest virus, isolated in Australia, the Turlock group now consists of four members. Two members of the group appear to be associated with birds.

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

Several of the viruses in these minor antigenic groups are important in causing disease in large animals. Bluetongue virus causes disease in both wild and domestic ruminants; AHS in mules, donkeys, and horses; and EHD in deer. Recently, Bluetongue virus has been isolated from Culicoides in the Northern Territory, Australia, thus extending its geographic distribution to that region. Both Bluetongue and AHS viruses have a wide geographic distribution.

Changuinola virus is the only member from these minor antigenic groups which has been isolated from man, and has been reported to produce disease in man.

Virus members of the Corriparta, Eubenangee, and Palyam serogroups appear to be primarily mosquito-associated, while members of the Wallal and Warrego serogroup appears to be associated with Culicoides insects.

Tilligerry virus which was isolated from mosquitoes collected in Australia represents a new addition to the Eubenangee serogroup.

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 Hart Park serogroup now consists of six virus members. Gray Lodge virus, from Culex mosquitoes collected in California, recently was accepted for registration, and it was demonstrated that it is related by CF and NT to Hart Park virus. All of the present members are associated with a mosquito vector and three of the viruses (Hart Park, Flanders, Mossuril) have been isolated from birds.

Thus far the antigenic relative of Kwatta virus has not been registered. Kwatta virus appears to be associated with mosquitoes. Both Sawgrass group viruses are tick-associated and have been found only in North America. Timbo and Chaco viruses of the Timbo serogroup have not been associated with any vector thus far.

Three VSV group members have been isolated from phlebotomine flies, and two others have been isolated from mosquitoes. Of the serogroups listed in this table, only members of the VSV serogroup has been implicated in causing infections of man. Chandipura, Piry, VS-Indiana, and VS-New Jersey have been isolated from man, while the former viruses plus VS-Alagoas have been found to produce disease in man during infections acquired in nature or in the laboratory. Both VS-Indiana and VS-New Jersey readily infect livestock, while Cocal has been recovered from a horse and VS-Alagoas from a mule.

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

Both Boteke groups viruses have been isolated in Africa only. Zingilamo virus was recovered from a bird and Boteke virus was isolated from mosquitoes.

Malakal and Puchong viruses of the Malakal group have been isolated from mosquitoes only.

All three Matariya group viruses have been recovered from birds collected in Africa. Nothing is known concerning their possible vector association.

Both Marburg and Ebola viruses cause human disease in nature and have been associated with laboratory-acquired infections. Nyando virus has been isolated from a single case of febrile illness in man.

Both viruses of the Tanjong Rabok serogroup have been isolated in Malaysia and neither has been associated with a vector, although Telok Forest virus was isolated from a wild monkey and Tanjong Rabok virus from a sentinel monkey.

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 nonarthropod-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). In addition to causing overt laboratory-acquired infections, Junin virus also has been reported to cause subclinical laboratory-acquired infections. A subclinical seroconversion to Tacaribe virus has been documented in a laboratory worker handling large quantities of Tacaribe virus.

Table 24. Ungrouped mosquito-associated viruses: The viruses in this table are serologically ungrouped, though they have been clustered together on the basis of their association with a mosquito vector and placed into subsets according to their taxonomic designation. Of those placed in the bunyavirus-like category, two of the African viruses are known to infect man. Both Tataguine and Zinga have been isolated from man, and both have been reported to produce disease in man during the course of infections acquired in nature.

Bocas virus was formerly included in the CAL group until it was demonstrated that it was identical to or closely related to mouse hepatitis virus.

Llano Seco and Paroo River viruses were registered during the year and both have been added to the list of viruses which are ungrouped, mosquito associated, and taxonomically designated as orbiviruses. Both were examined by electron microscopy and found to possess typical orbivirus morphology. Llano Seco virus is antigenically related to Umatilla virus but its relationship to other established orbivirus groups has not been resolved. Thus it has been placed with the ungrouped viruses pending a clarification of its antigenic relationships.

Orungo virus has caused human disease in nature, and Lebombo virus, or a closely related virus, has been isolated from human plasma, although it has not been associated with the causation of disease in man thus far.

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.

The poxvirus, Cotia, has been reported to produce disease in man during infection acquired in nature. Very little is known concerning the three rhabdoviruses listed at the bottom of the table.

Table 25. Ungrouped mosquito-associated viruses: These serologically ungrouped viruses are associated with a mosquito vector but are taxonomically unclassified. Three Australian viruses registered during the year have been added to the list in Table 25. Picola, Termeil, and Yacaaba viruses were isolated from Culicine mosquitoes, but little else is known of these viruses.

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. Approximately two-thirds of the listed viruses are taxonomically unclassified. A laboratory-acquired infection with Bhanja virus has been documented. None of the other viruses have been implicated in causing human disease.

Dhori virus was isolated from ticks collected in Portugal. Its geographic distribution has now been expanded to include Europe as well as Africa and Asia.

Tettnang virus was shown to cross-react in CF tests with mouse hepatitis virus (MHV). It remains to be determined whether it is identical or closely related to MHV or that it became contaminated with MHV subsequent to its isolation.

Issyk-Kul and Keterah viruses have been shown to be closely related or identical by CF. The decision to designate them as either the same virus or as antigenic relatives must await results of cross-neutralization testing. Pending that decision, these viruses are being listed in the ungrouped category.

Table 27. Ungrouped viruses, no arthropod vector known: None of the listed viruses have been isolated from an arthropod vector, and they are not rated higher than Possible Arbovirus. Several of the viruses are rated Probably not Arbovirus or Not Arbovirus. More than 50% have been isolated from rodents or birds.

Bangui and Le Dantec viruses have been isolated from man, and Bangui virus has been reported to cause a febrile illness with rash in man.

Approximately one-third of the viruses listed in Table 27 have been taxonomically classified.

Belem virus was isolated in 1968 from a bird collected in Brazil. It was registered this year and has been added to the list of viruses which are unclassified taxonomically. A CF antigen of Belem virus cross-reacted to low titer with an NIH group C immune grouping fluid. That was considered to be insufficient serological evidence to warrant its inclusion in the Bunyamwera Supergroup.

Table 28 gives continental distribution of viruses in different antigenic groups on the basis of virus isolation. Most of, the registered viruses are very limited in their distribution. Approximately 85% have been isolated on a single continent only, while 18 or 4.2% have been found on 3 or more continents. The largest number of viruses have been isolated in Africa.

Table 29 shows the number of viruses, according to antigenic group, which have been isolated from various classes of arthropods. Fifty percent have been recovered from mosquitoes, about 22% from ticks, and 14% from all other classes. Eighty-eight (21%) registered viruses have never been recovered from any arthropod vector. The largest number of viruses which have been isolated from any arthropod have been recovered from a single class only (307 of 335, 91.6%).

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. Most of the viruses isolated from vertebrates have been recovered from a single class only (173 of 251, 69%).

Table 31 lists the viruses in each antigenic group which cause disease in man. Approximately 24% of all registered viruses have been associated with human disease, either in nature, or by laboratory-acquired infections, or both. Members of group A, group B, and Bunyamwera Supergroup, which constitute 43% of all registered viruses, account for 66% of the instances of association of registered viruses with disease in man.

An analysis of the SEAS ratings for all registered viruses is presented in Table 32, and it shows that 223 (53%) registrations are rated as Possible Arboviruses. Clearly, additional data are required if we are to have a more precise rating of the arthropod-borne status of these viruses. Sufficient data are available for about 47% of all registered viruses so that 41% are rated Probable Arbovirus or Arbovirus, while 6% are rated Probably not Arbovirus or Not Arbovirus.

REFERENCES

1. Shope, R.E. and Peters, C.J. Serologic relationships between Rift Valley fever virus and viruses of the phlebotomus fever serogroup. 1979. Information Exchange, No. 37, pp. 175-177.

2. Calisher, C.H. Personal communication. 1979.

3. El Mekki, A., van der Groen, G., and Pattyn, S.R. Personal communication. 1980.

4. El Mekki, A., Nieuwenhuysen, P., van der Groen, G., and Pattyn, S.R. Personal communication. 1980.

5. American Committee on Arthropod-borne Viruses. Arbovirus names. 1969. Am. J. Trop. Med. Hyg. 18: 731-734.

6. Murphy, F.A. et al. Bunyaviridae: Morphologic and morphogenetic similarities of Bunyamwera serologic supergroup viruses and several other arthropod-borne viruses. 1973. Intervirol. 1: 297-316.

7. Porterfield, J.S. et al. Bunyaviruses and Bunyaviridae. 1973/74. Intervirol. 2: 270-272.

8. Fenner, F. Classification and nomenclature of viruses. Second Report of the International Committee on Taxonomy of Viruses. 1976. Intervirol. 7: 1-116.

9. Wesley, I.V. and Calisher, C.H. Personal communication. 1979.

Table 1

ALPHABETICAL LISTING OF 424 VIRUSES REGISTERED AS OF 31 DEC. 1979
WITH RECOMMENDED ABBREVIATIONS AND ANTIGENIC GROUPING

NAME	ABBR.	ANTI-GENIC GROUP
ABSETTAROV	ABS	B
ABU HAMMAD	AH	DGK
ACADO	ACD	COR
ACARA	ACA	CAP
AFRICAN HORSESICKNESS	AHS	AHS
AFRICAN SWINE FEVER	ASF	
AGUACATE	AGU	PHL
AINO	AINO	SIM
AKABANE	AKA	SIM
ALENQUER	ALE	PHL
ALFUY	ALF	B
ALMPIWAR	ALM	
AMAPARI	AMA	TCR
ANANINDEUA	ANU	GMA
ANHANGA	ANH	PHL
ANHEMBI	AMB	BUN
ANOPHELES A	ANA	ANA
ANOPHELES B	ANB	ANB
APEU	APEU	C
APOI	APOI	B
ARAGUARI	ARA	
ARIDE	ARI	
ARKONAM	ARK	
ARUAC	ARU	
ARUMOWOT	AMT	PHL
AURA	AURA	A
AVALON	AVA	SAK
BAGAZA	BAG	B
BAHIG	BAH	TETE
BAKAU	BAK	BAK
BAKU	BAKU	KEM
BANDIA	BDA	QYB
BANGORAN	BGN	
BANGUI	BGI	
BANZI	BAN	B
BARMAH FOREST	BF	TUR
BARUR	BAR	
BATAI	BAT	BUN
BATAMA	BMA	TETE
BATKEN	BKN	
BATU CAVE *	BC	B
BAULINE	BAU	KEM

* Batu Cave virus registration withdrawn after publication of this list. Batu Cave virus is identical to Phnom-Penh Bat virus.

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
BEBARU	BEB	A	BUSSUQUARA	BSQ	B
BELEM	BLM		BUTTONWILLOW	BUT	SIM
BELMONT	BEL		BWAMBA	BWA	BWA
BENEVIDES	BVS	CAP	CABASSOU	CAB	A
BENFICA	BEN	CAP	CACAO	CAC	PHL
BERTIOGA	BER	GMA	CACHE VALLEY	CV	BUN
BHANJA	BHA		CAIMITO	CAI	PHL
BIMBO	BBO		CALIFORNIA ENC.	CE	CAL
BIMITI	BIM	GMA	CALOVO	CVO	BUN
BIRAO	BIR	BUN	CANDIRU	CDU	PHL
BLUETONGUE	BLU	BLU	CAPE WRATH	CW	KEM
BOBAYA	BOB		CAPIM	CAP	CAP
BOBIA	BIA	OLI	CARAPARU	CAR	C
BOCAS	BOC		CAREY ISLAND	CI	B
BORACEIA	BOR	ANB	CATU	CATU	GMA
BOTAMBI	BOT	OLI	CHACO	CHO	TIM
BOTEKE	BTK	BTK	CHAGRES	CHG	PHL
BOUBOUI	BOU	B	CHANDIPURA	CHP	VSV
BOVINE EPHEMERAL FEVER	BEF		CHANGUINOLA	CGL	CGL
BUENAVENTURA	BUE	PHL	CHARLEVILLE	CHV	
BUJARU	BUJ	PHL	CHENUDA	CNU	KEM
BUNYAMWERA	BUN	BUN	CHIKUNGUNYA	CHIK	A
BURG EL ARAB	BEA	MTY	CHILIBRE	CHI	PHL
BUSHBUSH	BSB	CAP	CHIM	CHIM	

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
CHOBAR GORGE	CG.		EYACH	EYA	CTF
CLO MOR	CM	SAK	FLANDERS	FLA	HP
COCAL	COC	VSV	FORT MORGAN	FM	A
COLORADO TICK FEVER	CTF	CTF	FRIJOLES	FRI	PHL
CONGO	CON	CON	GAMBOA	GAM	SBU
CORRIPARTA	COR	COR	GAN GAN	GG	MAP
COTIA	COT		GANJAM	GAN	NSD
COWBONE RIDGE	CR	B	GARBA	GAR	MTY
D'AGUILAR	DAG	PAL	GERMISTON	GER	BUN
DAKAR BAT	DB	B	GETAH	GET	A
DENGUE-1	DEN-1	B	GOMOKA	GOM	
DENGUE-2	DEN-2	B	GORDIL	GOR	PHL
DENGUE-3	DEN-3	B	GOSSAS	GOS	
DENGUE-4	DEN-4	B	GRAND ARBAUD	GA	UUK
DERA GHAZI KHAN	DGK	DGK	GRAY LODGE	GLO	HP
DHORI	DHO		GREAT ISLAND	GI	KEM
DUGBE	DUG	NSD	GUAJARA	GJA	CAP
EAST. EQUINE ENC.	EEE	A	GUAMA	GMA	GMA
EBOLA	EBO	MBG	GUARATUBA	GTB	SBU
EDGE HILL	EH	B	GUAROA	GRO	CAL
ENTEBBE BAT	ENT	B	GUMBO LIMBO	GL	C
EP. HEM. DIS.	EHD	EHD	HANZALOVA	HAN	B
EUBENANGEE	EUB	EUB	HART PARK	HP	HP
EVERGLADES	EVE	A	HAZARA	HAZ	CON

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
HUACHO	HUA	KEM	JOHNSTON ATOLL	JA	QRF
HUGHES	HUG	HUG	JOINJAKAKA	JOI	
HYPR	HYPR	B	JUAN DIAZ	JD	CAP
IBARAKI	IBA		JUGRA	JUG	B
ICOARACI	ICO	PHL	JUNIN	JUN	TCR
IERI	IERI		JURONA	JUR	SBU
ILESHA	ILE	BUN	JUTIAPA	JUT	B
ILHEUS	ILH	B	KADAM	KAD	B
INGWAVUMA	ING	SIM	KAENG KHOI	KK	SBU
INHANGAPI	INH		KAIKALUR	KAI	SIM
ININI	INI	SIM	KAIRI	KRI	BUN
INKOO	INK	CAL	KAISODI	KSO	KSO
IPPY	IPPY		KAMESE	KAM	HP
IRITUIA	IRI	CGL	KAMMAVANPETTAI	KMP	
ISFAHAN	ISF	VSV	KANNAMANGALAM	KAN	
ISRAEL TURKEY MEN.	IT	B	KAO SHUAN	KS	DGK
ISSYK-KUL	IK		KARIMABAD	KAR	PHL
ITAITUBA	ITA	PHL	KARSHI	KSI	B
ITAPORANGA	ITP	PHL	KASBA	KAS	PAL
ITAQUI	ITQ	C	KEMEROVO	KEM	KEM
JAMESTOWN CANYON	JC	CAL	KERN CANYON	KC	
JAPANAUT	JAP		KETAPANG	KET	BAK
JAPANESE ENC.	JE	B	KETERAH	KTR	
JERRY SLOUGH	JS	CAL	KEURALIBA	KEU	

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
KEYSTONE	KEY	CAL	LE DANTEC	LD	
KHASAN	KHA		LIPOVNIK	LIP	KEM
KLAMATH	KLA		LLANO SECO	LLS	* *
KOKOBERA	KOK	B	LOKERN	LOK	BUN
KOLONGO	KOL		LONE STAR	LS	
KOONGOL	KOO	KOO	LOUPING ILL	LI	B
KOUTANGO	KOU	B	LUKUNI	LUK	ANA
KOWANYAMA	KOW		MACHUPO	MAC	TCR
KUMLINGE	KUM	B	MADRID	MAD	C
KUNJIN	KUN	B	MAGUARI	MAG	BUN
KUNUNURRA	KNA		MAHOGANY HAMMOCK	MH	GMA
KWATTA	KWA	KWA	MAIN DRAIN	MD	BUN
KYASANUR FOR. DIS.	KFD	B	MALAKAL	MAL	MAL
KYZYLAGACH	KYZ	A	MANAWA	MWA	UUK
LA CROSSE	LAC	CAL	MANZANILLA	MAN	SIM
LAGOS BAT	LB	*	MAPPUTTA	MAP	MAP
LA JOYA	LJ		MAPRIK	MPK	MAP
LANDJIA	LJA		MARBURG	MBG	MBG
LANGAT	LGT	B	MARCO	MCO	
LANJAN	LJN	KSO	MARITUBA	MTB	C
LASSA	LAS	TCR	MATARIYA	MTY	MTY
LATINO	LAT	TCR	MATRUH	MTR	TETE
LEBOMBO	LEB		MATUCARE	MAT	

* Rabies related

** Llano Seco virus is related to Umatilla virus. Its relationship to other orbivirus serogroups has not been determined.

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
MAYARO	MAY	A	NEGISHI	NEG	B
MELAO	MEL	CAL	NEPUYO	NEP	C
MERMET	MER	SIM	NEW MINTO	NM	SAW
MIDDELBURG	MID	A	NGAINGAN	NGA	
MINATITLAN	MNT	MIR	NIQUE	NIQ	PHL
MINNAL	MIN		NKOLBISSON	NKO	
MIRIM	MIR	MIR	NODAMURA	NOD	
MITCHELL RIVER	MR	WAR	NOLA	NOLA	SIM
MODOC	MOD	B	NORTHWAY	NOR	BUN
MOJU	MOJU	GMA	NTAYA	NTA	B
MONO LAKE	ML	KEM	NUGGET	NUG	KEM
MONT. MYOTIS LEUK.	MML	B	NYAMANINI	NYM	
MORICHE	MOR	CAP	NYANDO	NDO	NDO
MOSQUEIRO	MQO	HP	OKHOTSKIY	OKH	KEM
MOSSURIL	MOS	HP	OKOLA	OKO	
MOUNT ELGON BAT	MEB		OLIFANTSVLEI	OLI	OLI
M'POKO	MPO	TUR	OMSK HEM. FEVER	OMSK	B
MUCAMBO	MUC	A	O'NYONG NYONG	ONN	A
MURRAY VALLEY ENC.	MVE	B	ORIBOCA	ORI	C
MURUTUCU	MUR	C	OROPOUCHE	ORO	SIM
NAIROBI SHEEP DIS.	NSD	NSD	ORUNGO	ORU	
NARIVA	NAR		OSSA	OSSA	C
NAVARRO	NAV		OUANGO	OUA	
NDUMU	NDU	A	OUBANGUI	OUB	

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
PACORA	PCA		RAZDAN	RAZ	
PACUI	PAC	PHL	RESTAN	RES	C
PAHAYOKEE	PAH	PAT	RIFT VALLEY FEVER	RVF	
PALYAM	PAL	PAL	RIO BRAVO	RB	B
PARAMUSHIR	PMR		RIO GRANDE	RG	PHL
PARANA	PAR	TCR	ROCHAMBEAU	RBU	
PAROO RIVER	PR		ROCIO	ROC	B
PATA	PATA	EUB	ROSS RIVER	RR	A
PATHUM THANI	PTH	DGK	ROYAL FARM	RF	B
PATOIS	PAT	PAT	RUSS. SPR. SUM. ENC.	RSSE	B
PHNOM-PENH BAT	PPB	B	SABO	SABO	SIM
PICHINDE	PIC	TCR	SABOYA	SAB	B
PICOLA	PIA		SAGIYAMA	SAG	A
PIRY	PIRY	VSV	SAINT-FLORIS	SAF	
PIXUNA	PIX	A	SAKHALIN	SAK	SAK
PONGOLA	PGA	BWA	SAKPA	SPA	
PONTEVES	PTV	UUK	SALANGA	SGA	
POWASSAN	POW	B	SALEHABAD	SAL	PHL
PRETORIA	PRE	DGK	SAN ANGELO	SA	CAL
PUCHONG	PUC	MAL	SANDFLY F. (NAPLES)	SFN	PHL
PUNTA SALINAS	PS	HUG	SANDFLY F. (SICILIAN)	SFS	PHL
PUNTA TORO	PT	PHL	SANDJIMBA	SJA	
QALYUB	QYB	QYB	SANGO	SAN	SIM
QUARANFIL	QRF	QRF	SANTA ROSA	SAR	BUN

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
SATHUPERI	SAT	SIM	STRATFORD	STR	B
SAUMAREZ REEF	SRE	B	SUNDAY CANYON	SCA	
SAWGRASS	SAW	SAW	TACAIUMA	TCM	ANA
SEBOKELE	SEB		TACARIBE	TCR	TCR
SELETAR	SEL	KEM	TAGGERT	TAG	SAK
SEMBALAM	SEM		TAHYNA	TAH	CAL
SEMLIKI FOREST	SF	A	TAMDY	TDY	
SEPIK	SEP	B	TAMIAMI	TAM	TCR
SERRA DO NAVIO	SDN	CAL	TANGA	TAN	
SHAMONDA	SHA	SIM	TANJONG RABOK	TR	TR
SHARK RIVER	SR	PAT	TATAGUINE	TAT	
SHUNI	SHU	SIM	TELOK FOREST	TF	TR
SILVERWATER	SIL	KSO	TEMBE	TME	
SIMBU	SIM	SIM	TEMBUSU	TMU	B
SIMIAN HEM. FEV.	SHF		TENSAW	TEN	BUN
SINDBIS	SIN	A	TERMEIL	TER	
SIXGUN CITY	SC	KEM	TETE	TETE	TETE
SLOVAKIA	SLO		TETTNANG	TET	
SNOWSHOE HARE	SSH	CAL	THIMIRI	THI	SIM
SOKOLUK	SOK	B	THOGOTO	THO	THO
SOLDADO	SOL	HUG	THOTTAPALAYAM	TPM	
SOROROCA	SOR	BUN	TILLIGERRY	TIL	EUB
SPONDWENI	SPO	B	TIMBO	TIM	TIM
ST. LOUIS ENC.	SLE	B	TIMBOTEUA	TBT	GMA

NAME	ABBR.	ANTI-GENIC GROUP	NAME	ABBR.	ANTI-GENIC GROUP
TLACOTALPAN	TLA	BUN	VS-NEW JERSEY	VSNJ	VSV
TONATE	TON	A	WAD MEDANI	WM	KEM
TOURE	TOU		WALLAL	WAL	WAL
TRIBEC	TRB	KEM	WANOWRIE	WAN	
TRINITI	TNT		WARREGO	WAR	WAR
TRIVITTATUS	TVT	CAL	WESSELSBRON	WSL	B
TRUBANAMAN	TRU	MAP	WEST. EQUINE ENC.	WEE	A
TSURUSE	TSU	TETE	WEST NILE	WN	B
TURLOCK	TUR	TUR	WHATAROA	WHA	A
TYULENIY	TYU	B	WITWATERSRAND	WIT	
UGANDA S	UGS	B	WONGAL	WON	KOO
UMATILLA	UMA		WONGORR	WGR	
UMBRE	UMB	TUR	WYEOMYIA	WYO	BUN
UNA	UNA	A	YACAABA	YAC	
UPOLU	UPO		YAQUINA HEAD	YH	KEM
URUCURI	URU	PHL	YATA	YATA	
USUTU	USU	B	YELLOW FEVER	YF	B
UTINGA	UTI	SIM	YOGUE	YOG	
UUKUNIEMI	UUK	UUK	ZALIV TERPENIYA	ZT	UUK
VELLORE	VEL	PAL	ZEGLA	ZEG	PAT
VEN. EQUINE ENC.	VEE	A	ZIKA	ZIKA	B
VENKATAPURAM	VKT		ZINGA	ZGA	
VS-ALAGOAS	VSA	VSV	ZINGILAMO	ZGO	BTK
VS-INDIANA	VSI	VSV	ZIRQA	ZIR	HUG

Table 2. Antigenic Groups of 423 Viruses Registered in Catalogue

Antigenic Group	Abbreviation		No. Registered Viruses in Group		%
A	A		24		5.7
African horsesickness	AHS		1		0.2
Anopheles A	ANA		3		0.7
Anopheles B	ANB		2		0.5
B	B		59		14.0
Bakau	BAK		2		0.5
Bluetongue	BLU		1		0.2
Boteke	BTK		2		0.5
Bunyamwera Supergroup			98		23.2
Bunyamwera	BUN	18			
Bwamba	BWA	2			
C	C	11			
California	CAL	13			
Capim	CAP	8			
Guama	GMA	8			
Koongol	KOO	2			
Mirim	MIR	2			
Olifantsvlei	OLI	3			
Patois	PAT	4			
Simbu	SIM	18			
Tete	TETE	5			
Unassigned	SBU	4			
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		1.2
Epizootic hemorrhagic disease	EHD		1		0.2
Eubenangee	EUB		3		0.7
Hart Park	HP		6	1.4	1.4
Hughes	HUG		4		0.9
Kaisodi	KSO		3		0.7
Kemerovo	KEM		16		3.8
Kwatta	KWA		1		0.2
Malakal	MAL		2		0.5
Mapputta	MAP		4		0.9
Marburg	MBG		2		0.5
Matariya	MTY		3		0.7
Nairobi sheep disease	NSD		3		0.7
Nyando	NDO		1		0.2
Palyam	PAL		4		0.9
Phlebotomus fever	PHL		27		6.4
Qalyub	QYB		2		0.5
Quaranfil	QRF		2		0.5
Sakhalin	SAK		4		0.9
Sawgrass	SAW		2		0.5
Tacaribe	TCR		9		2.1
Tanjong Robok	TR		2		0.5
Thogoto	THO		1		0.2
Timbo	TIM		2		0.5
Turlock	TUR		4		0.9
Uukuniemi	UUK		5		1.2
Vesicular stomatitis	VSV		7		1.7
Wallal	WAL		1		0.2
Warrego	WAR		2		0.5
Ungrouped viruses			95		22.5
Total			423		

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

Decade	Continent	Country	Virus
1900-09	Africa	S. Africa	BLU
1910-19	Africa	Kenya	ASF,NSD
1920-29	Africa	Nigeria	YF
	Europe	Scotland	LI
	N. 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
		S. Africa	BAN,GER,ING,LEB,MID,MOS,NDU,NYM, PGA,SIM,SPO,TETE,USU,WIT,WSL
		Uganda	CHIK,CON,ENT,NDO,ONN,ORU
	Asia	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	MVE
		Philippines	DEN-3*,DEN-4*
	Europe	Czechoslovakia	HYPR,TAH
		Finland	KUM
		U.S.S.R	ABS
	N. America	Canada	POW
		Panama	BOC,LJ,PCA
		U.S.A.	CV,EHD,HP,MML,MOD,RB,SA,SSH,TUR,VSNJ
	S. America	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,BIA,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.	KYZ,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
		U.S.A.	BUT,CR,EVE,FLA,GL,HUG,JC,JS,KC,KEY,KLA, LAC,LOK,LS,MER,MD,MH,ML,PAH,SAW,SC,SHF, SR,TAM,TEN,UMA
	S. America	Bolivia	MAC**
		Brazil	ACA,AMA,AMB,ANH,ANU,ARA,BEN,BER,BLM,BOR, BUJ,BVS,CDU,CHO,COT,GTB,ICO,INH,IRI,ITP, JUR,MCO,PAC,PIRY,PIX,SDN,SOR,TBT,TIM,TME URU,UTI,VSA
		Colombia	BUE,PIC
		French Guiana	CAB
		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)

Decade	Continent	Country	Virus
1970-79	Africa	Cent.Afr.Rep.	BBO,BGI,BMA,BOB,GAR,GOM,GOR,IPPY,KOL,LJA, NOLA,OUA,OUB,SAF,SEB,SGA,SJA,SPA,ZGO
		Egypt	AH,KS,PTH
		Seychelles	ARI***
		S. Africa	PRE***
		Zaire	EBO
	Asia	India	CG,KAI
		Iran	ISF*
		Malaysia	CI,TF
		U.S.S.R.	BKN,CHIM,IK,KHA,KSI,PMR,RAZ,SOK,TDY
	Australasia	Australia	BF,GG,KNA,NGA,NUG,PIA,PR,SRE,TAG,TER,TIL, WAL,WGR,YAC
	Europe	Czechoslovakia	SLO
		Germany	EYA,TET
		Scotland	CM,CW
		U.S.S.R.	BAKU
	N. America	Canada	AVA,BAU*,GI*
		Mexico	SAR*
		Panama	CAC,CAI,NIQ
		U.S.A.	FM,GLO,LLS,NM,NOR,RG,SCA,YH
	S. America	Brazil	ITA,MQO,ROC
		French Guiana	INI,RBU,TON

* Isolated in U.S.A. laboratory
** Isolated in Panama laboratory
*** Isolated in Egypt laboratory

Table 4. Initial Isolation of 423 Registered Viruses by Continent, Country, and Chronological Period.

Continent	Country or Area	Before 1930	1930 -39	1940 -49	1950 -59	1960 -69	1970 -79	Totals
AFRICA	Cameroon					2		2
	Cent.Afr.Rep.					11	19	30
	Egypt				5	7	3	15
	Kenya	2	1			1		4
	Nigeria	1			2	6		9
	Senegal					10		10
	Seychelles						1	1
	S. Africa	1	1		15	1	1	19
	Sudan					1		1
	Uganda		2	5	6	4		17
	Zaire						1	1
	Totals	4	4	5	28	43	25	109
ASIA	Cambodia					1		1
	India				12	9	2	23
	Iran					2	1	3
	Israel				1			1
	Japan		1	1	6	1		9
	Malaysia				7	5	2	14
	W. Pakistan					3		3
	Persian Gulf					1		1
	Singapore					1		1
	Thailand					1		1
	U.S.S.R. (East)		1	1		5	9	16
	Totals	0	2	2	26	29	14	73
AUSTRAL-ASIA and PACIFIC ISLANDS	Australia				1	32	7	40
	Hawaii			1				1
	Johnston Island					1		1
	New Guinea			1				1
	New Zealand					1		1
	Philippines				2			2
	Totals	0	0	2	3	34	7	46
EUROPE	Czechoslovakia			1	2	4	1	8
	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	6	23
NORTH AMERICA	Canada				1	1	3	5
	Guatemala					1		1
	Mexico					2	1	3
	Panama				3	15	3	21
	U.S.A.	1	3	3	10	26	8	51
	Totals	1	3	3	14	45	15	81
SOUTH AMERICA	Argentina				1			1
	Bolivia					1		1
	Brazil			1	18	33	4	56
	Colombia			3	2	2		7
	French Guiana					1	3	4
	Peru					2		2
	Surinam					1		1
	Trinidad				13	5		18
	Venezuela		1					1
	Totals	0	1	4	34	45	7	91
	Grand Totals	6	10	19	109	205	74	423

Table 5. Group A Arboviruses

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq.: Culicine	Mosq.: Anopheline	Ticks: Ixodid	Ticks: Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: Africa	Asia	Australasia	Europe	North America	South America	HUMAN DISEASE: Natural Infection	Lab Infection	SEAS RATING*	TAXONOMIC STATUS
Aura	+																				+			22	Alphavirus
Bebaru	+																+							22	"
Cabassou	+										+	+	+		+						+			21	"
Chikungunya	+							+	+		+	+			+	+	+					+	+	20	"
Eastern equine enc.	+	+				+	+	+	+	+	+		+	+			+	+	+	+	+	+	+	20	"
Everglades	+	+								+			+							+		+		20	"
Fort Morgan							+				+									+				20	"
Getah	+	+												+			+	+						20	"
Kyzylagach	+																+							22	"
Mayaro	+						+	+						+							+	+	+	20	"
Middelburg	+															+								20	"
Mucambo	+							+		+	+		+		+						+	+	+	20	"
Ndumu	+													+		+								21	"
O'nyong-nyong		+						+							+	+						+		20	"
Pixuna	+	+								+											+			22	"
Ross River	+							+			+		+					+				+	+	20	"
Sagiyama	+																+							21	"
Semliki Forest	+	+						+			+				+	+	+						+	20	"
Sindbis	+	+	+				+	+			+				+	+	+	+	+			+		20	"
Tonate	+	+						+			+				+						+	+		21	"
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: ARTHROPODS: Mosq.: Culicine	Mosq.: Anopheline	Ticks: Ixodid	Ticks: Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: Africa	Asia	Australasia	Europe	North America	South America	HUMAN DISEASE: Natural Infection	Lab Infection	SEAS RATING*	TAXONOMIC STATUS
Alfuy	+										+							+						20	Flavivirus
Bagaza	+															+								22	"
Banzi	+							+							+	+						+		20	"
Bouboui	+	+							+							+								22	"
Bussuquara	+							+		+					+					+	+	+		20	"
Dengue-1	+							+								+	+	+				+	+	20	"
Dengue-2	+							+	+							+	+	+			+	+	+	20	"
Dengue-3	+							+									+	+			+	+	+	20	"
Dengue-4	+							+									+	+				+		20	"
Edge Hill	+	+																+						20	"
Ilheus	+							+			+				+					+	+	+	+	20	"
Japanese encephalitis	+	+						+			+	+		+			+					+	+	20	"
Jugra	+											+					+							22	"
Kokobera	+																	+						21	"
Kunjin	+							+			+							+					+	20	"
Murray Valley enc.	+							+										+				+		20	"
Ntaya	+															+								21	"
Rocio	+							+			+										+	+	+	22	"
Sepik	+																	+				+		21	"
St. Louis encephalitis	+	+					+	+			+	+		+						+	+	+	+	20	"
Spondweni	+							+								+						+	+	20	"
Stratford	+																	+						22	"
Tembusu	+	+													+		+	+						21	"
Uganda S	+										+				+	+								20	"
Usutu	+									+	+					+								22	"
Wesselsbron	+	+						+		+				+		+	+					+	+	20	"
West Nile	+	+	+	+				+		+	+	+		+	+	+	+		+			+	+	20	"
Yellow Fever	+							+	+				+			+					+	+	+	20	"
Zika	+							+							+	+	+					+	+	20	"

* See footnote Table 5

Table 7. Group B Arboviruses, Tick-Borne

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

* See footnote Table 5

Table 8. Group B Viruses, No Arthropod Vector Demonstrated

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq.: Culicine	ISOLATED FROM: ARTHROPODS: Mosq.: Anopheline	ISOLATED FROM: ARTHROPODS: Ticks: Ixodid	ISOLATED FROM: ARTHROPODS: Ticks: Argasid	ISOLATED FROM: ARTHROPODS: Phlebotomine	ISOLATED FROM: ARTHROPODS: Culicoides	ISOLATED FROM: ARTHROPODS: Other	ISOLATED FROM: VERTEBRATES: Man	ISOLATED FROM: VERTEBRATES: Other Primates	ISOLATED FROM: VERTEBRATES: Rodents	ISOLATED FROM: VERTEBRATES: Birds	ISOLATED FROM: VERTEBRATES: Bats	ISOLATED FROM: VERTEBRATES: Marsupials	ISOLATED FROM: VERTEBRATES: Other	ISOLATED FROM: VERTEBRATES: Sentinels	ISOLATED IN: Africa	ISOLATED IN: Asia	ISOLATED IN: Australasia	ISOLATED IN: Europe	ISOLATED IN: North America	ISOLATED IN: South America	HUMAN DISEASE: Natural Infection	HUMAN DISEASE: Lab Infection	SEAS RATING*	TAXONOMIC STATUS
Apoi										+							+						+	22	Flavivirus
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																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Anhembi	+									+											+			22	Bunyavirus
Batai	+	+															+		+					21	"
Birao		+														+								22	"
Bunyamwera	+							+								+						+	+	20	"
Cache Valley	+	+																		+				20	"
Calovo**		+																	+			+		21	"
Germiston	+							+		+					+	+							+	20	"
Ilesha		+						+								+						+		21	"
Kairi	+								+	+					+						+			20	"
Lokern	+					+								+						+				20	"
Maguari	+	+												+	+						+			20	"
Main Drain						+								+						+				20	"
Northway	+														+					+				21	"
Santa Rosa	+																			+				22	"
Sororoca	+																				+			22	"
Tensaw	+	+												+	+					+		+		20	"
Tlacotalpan	+	+																		+				22	"
Wyeomyia	+	+						+												+	+	+		21	"

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

Table 10. Bunyamwera Supergroup: Bwamba Group and Group C Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
BWAMBA GROUP																									
Bwamba		+						+								+						+		21	Bunyavirus
Pongola	+	+														+								20	"
GROUP C																									
Apeu	+							+					+		+						+	+	+	20	Bunyavirus
Caraparu	+							+		+					+					+	+	+		20	"
Gumbo Limbo	+									+										+				21	"
Itaqui	+							+		+			+		+						+	+		20	"
Madrid	+							+		+					+					+		+		20	"
Marituba	+							+					+		+						+	+		20	"
Murutucu	+							+		+			+	+	+						+	+		20	"
Nepuyo	+							+		+		+			+					+	+			20	"
Oriboca	+							+		+			+		+						+	+	+	20	"
Ossa	+							+		+					+					+		+		20	"
Restan	+							+													+	+		20	"

* See footnote Table 5

TABLE 11. Bunyamwera Supergroup: California and Capim Group Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
<u>CALIFORNIA GROUP</u>																									
California enc.	+																			+		+		20	Bunyavirus
Guaroa		+						+												+	+	+		20	"
Inkoo	+																		+			+		22	"
Jamestown Canyon	+						+								+					+				20	"
Jerry Slough	+																			+				20	"
Keystone	+	+								+					+					+				20	"
La Crosse	+							+		+					+					+		+		20	"
Melao	+																				+			21	"
San Angelo		+																		+				22	"
Serra do Navio	+																				+			22	"
Snowshoe Hare	+									+				+	+					+				20	"
Tahyna	+	+						+							+	+	+		+			+		20	"
Trivittatus	+									+					+					+				20	"
<u>CAPIM GROUP</u>																									
Acara	+									+					+					+	+			21	Bunyavirus
Benevides	+														+						+			21	"
Benfica	+									+					+						+			20	"
Bushbush	+														+						+			20	"
Capim	+									+			+		+						+			20	"
Guajara	+									+					+					+	+			20	"
Juan Diaz															+					+				22	"
Moriche	+																				+			22	"

* See footnote Table 5

TABLE 12. Bunyamwera Supergroup: Guama, Koongol, Mirim, Olifantsvlei, and Patois Group Viruses

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq.: Culicine	Anopheline	Ticks: Ixodid	Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: Africa	Asia	Australasia	Europe	North America	South America	HUMAN DISEASE: Natural Infection	Lab Infection	SEAS RATING*	TAXONOMIC STATUS
GUAMA GROUP																									
Ananindeua	+									+	+		+		+						+			21	Bunyavirus
Bertioga															+						+			22	"
Bimiti	+									+					+						+			20	"
Catu	+	+						+		+		+	+		+						+	+		20	"
Guama	+				+			+		+		+	+		+					+	+	+		20	"
Mahogany Hammock	+									+										+				22	"
Moju	+									+			+		+						+			20	"
Timboteua										+					+						+			21	"
KOONGOL GROUP																									
Koongol	+	?																+						21	Bunyavirus
Wongal	+																	+						21	"
MIRIM GROUP																									
Minatitlan															+					+				22	Bunyavirus
Mirim	+														+						+			20	"
OLIFANTSVLEI GROUP																									
Botambi	+															+								22	Bunyavirus
Bobia	+															+								22	"
Olifantsvlei	+															+								22	"
PATOIS GROUP																									
Pahayokee	+																			+				22	Bunyavirus
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																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
Aino	+					+											+	+						22	Bunyavirus
Akabane	+					+								+			+	+						21	"
Buttonwillow						+								+						+				20	"
Ingwavuma	+										+			+		+	+							20	"
Inini											+										+			22	"
Kaikalur	+																+							22	"
Manzanilla									+												+			22	"
Mermet											+									+				22	"
Nola	+															+								20	"
Oropouche	+							+						+							+	+	+	21	"
Sabo						+								+		+								22	"
Sango	+					+								+		+								22	"
Sathuperi	+					+								+		+	+							22	"
Shamonda						+								+		+								22	"
Shuni	+					+		+						+		+						+		22	"
Simbu	+															+								21	"
Thimiri											+					+	+							22	"
Utinga														+							+			22	"

* See footnote Table 5

Table 14. Bunyamwera Supergroup: Tete Group and Unassigned Viruses

VIRUS	Isolated From: Arthropods: Mosq. Culicine	Anopheline	Ticks Ixodid	Argasid	Phlebotomine	Culicoides	Other	Vertebrates: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Isolated In: Africa	Asia	Australasia	Europe	North America	South America	Human Disease: Natural Infection	Lab infection	SEAS RATING*	TAXONOMIC STATUS
TETE GROUP																									
Bahig			+								+					+			+					21	Bunyavirus
Batama											+					+								22	"
Matruh			+								+					+			+					22	"
Tete											+					+								22	"
Tsuruse											+						+							22	"
UNASSIGNED - "SBU"																									
Gamboa	+																			+				22	Bunyavirus
Guaratuba	+										+				+						+			21	"
Jurona	+																				+			22	"
Kaeng Khoi							+					+			+		+							22	"

* See footnote Table 5

TABLE 15. Phlebotomus Fever Group Viruses

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

* See footnote Table 5

Table 16. Tick-Borne Groups Other Than Group B Viruses

	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
VIRUS	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		TAXONOMIC STATUS
CHF-CONGO GROUP																									
Congo			+			+		+						+		+	+		+			+	+	20	Bunyavirus-like
Hazara			+														+							22	"
KAISODI GROUP																									
Kaisodi			+								+						+							22	Bunyavirus-like
Lanjan			+						+								+							22	"
Silverwater			+											+						+				21	"
NAIROBI SHEEP DISEASE																									
Dugbe	+		+			+		+		+				+		+						+		22	Bunyavirus-like
Ganjam	+		+					+									+					+	+	22	"
Nairobi sheep disease			+					+						+		+						+	+	20	"
SAKHALIN GROUP																									
Avalon			+								+									+				21	Bunyavirus-like
Clo Mor			+																+					22	"
Sakhalin			+														+			+				22	"
Taggert			+															+						22	"
THOGOTO GROUP																									
Thogoto			+					+						+		+			+			+		22	Bunyavirus-like
UUKUNIEMI GROUP																									
Grand Arbaud				+															+					20	Bunyavirus-like
Manawa			+	+													+							22	"
Ponteves				+															+					22	"
Uukuniemi			+							+	+								+					21	"
Zaliv Terpeniya			+														+							22	"

* 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																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
COLORADO TICK FEVER																									
Colorado tick fever			+	+				+		+				+						+		+	+	20	Orbivirus
Eyach			+																+					22	"
KEMEROVO																									
Baku				+															+					22	Orbivirus
Bauline			+																	+				22	"
Cape Wrath			+																+					22	"
Chenuda				+												+								22	"
Great Island			+																	+				22	"
Huacho				+																	+			22	"
Kemerovo			+					+			+					+	+					+	+	21	"
Lipovnik			+																+					22	"
Mono Lake				+																+				22	"
Nugget			+															+						22	"
Okhotskiy			+														+							22	"
Seletar			+														+							22	"
Sixgun City				+																+				22	"
Tribec			+							+					+				+					21	"
Wad Medani			+													+	+			+				22	"
Yaquina Head			+																	+				22	"

* See footnote Table 5

Table 18. Tick-Borne Groups Other Than Group B Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
DERA GHAZI KHAN GROUP																									
Abu Hammad				+												+	+							22	Unclassified
Dera Ghazi Khan			+														+							22	"
Kao Shuan				+													+	+						22	"
Pathum Thani				+													+							22	"
Pretoria				+												+								22	"
HUGHES GROUP																									
Hughes				+							+									+	+			21	Unclassified
Punta Salinas				+																	+			22	"
Soldado				+												+			+		+			20	"
Zirqa				+													+							22	"
QALYUB GROUP																									
Bandia				+						+						+								22	Unclassified
Qalyub				+												+								22	"
QUARANFIL GROUP																									
Johnston Atoll				+														+						20	Unclassified
Quaranfil				+				+			+					+						+		20	"

* See footnote Table 5

Table 19. Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq.: Culicine	Mosq.: Anopheline	Ticks: Ixodid	Ticks: Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: Africa	Asia	Australasia	Europe	North America	South America	HUMAN DISEASE: Natural Infection	Lab Infection	SEAS RATING*	TAXONOMIC STATUS
ANOPHELES A GROUP																									
Anopheles A		+																			+			21	Bunyavirus-like
Lukuni	+	+																			+			22	"
Tacaiuma	+	+						+	+						+						+	+		21	"
ANOPHELES B GROUP																									
Anopheles B		+																			+			22	Bunyavirus-like
Boraceia	+	+																			+			22	"
BAKAU GROUP																									
Bakau	+			+					+								+							22	Bunyavirus-like
Ketapang	+																+							21	"
MAPPUTTA GROUP																									
Gan Gan	+																	+						22	Bunyavirus-like
Mapputta		+																+						22	"
Maprik	+	+																+						21	"
Trubanaman		+																+						22	"
TURLOCK GROUP																									
Barmah Forest	+																	+						22	Bunyavirus-like
M'Poko (=Yaba-1)	+															+								22	"
Turlock	+										+			+	+					+	+			20	"
Umbre	+										+						+							21	"

* See footnote Table 5

Table 20. Minor Antigenic Groups of Viruses

VIRUS	Isolated From – Arthropods: Mosq. – Culicine	Mosq. – Anopheline	Ticks – Ixodid	Ticks – Argasid	Phlebotomine	Culicoides	Other	Isolated From – Vertebrates: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Isolated In: Africa	Asia	Australasia	Europe	North America	South America	Human Disease: Natural Infection	Lab Infection	SEAS RATING*	TAXONOMIC STATUS
AFRICAN HORSESICKNESS																									
African horsesickness						+								+		+	+		+					20	Orbivirus
BLUETONGUE GROUP																									
Bluetongue						+								+		+	+	+	+	+				20	Orbivirus
CHANGUINOLA GROUP																									
Changuinola					+			+												+		+		21	Orbivirus
Irituia										+											+			22	"
CORRIPARTA GROUP																									
Acado	+															+								22	Orbivirus
Corriparta	+										+							+						21	"
EHD GROUP																									
Epizootic hem. dis.														+		+				+				21	Orbivirus
EUBENANGEE GROUP																									
Eubenangee	+?	+?																+						22	Orbivirus
Pata	+															+								22	"
Tilligerry		+																+						22	"
PALYAM GROUP																									
D'Aguilar						+								+				+						22	Orbivirus
Kasba	+																+							22	"
Palyam	+																+							22	"
Vellore	+																+							22	"
WALLAL GROUP																									
Wallal						+												+						22	Orbivirus
WARREGO GROUP																									
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																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotumine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
HART PARK GROUP																									
Flanders	+										+									+				22	Rhabdovirus
Grey Lodge	+																			+				22	"
Hart Park	+										+									+				21	"
Kamese	+															+								22	"
Mosqueiro	+																				+			22	"
Mossuril	+										+					+								22	"
KWATTA GROUP																									
Kwatta	+																				+			22	Rhabdovirus
SAWGRASS GROUP																									
New Minto			+																	+				22	Rhabdovirus
Sawgrass			+																	+				22	"
TIMBO GROUP																									
Chaco														+							+			22	Rhabdovirus
Timbo														+							+			22	"
VESICULAR STOMATITIS GR.																									
Chandipura					+			+						+		+	+					+		20	Rhabdovirus
Cocal	+						+			+				+	+						+			20	"
Isfahan					+												+							22	"
Piry								+					+								+		+	22	"
VS-Alagoas														+							+	+		22	"
VS-Indiana	+				+			+						+						+	+	+	+	20	"
VS-New Jersey							+	+						+						+	+	+	+	22	"

* See footnote Table 5

Table 22. Minor Antigenic Groups of Viruses

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

* See footnote Table 5

Table 23. Tacaribe (LCM) Group Viruses

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

* See footnote Table 5

Table 24. Ungrouped Mosquito-Associated Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
Belmont	+																	+						22	Bunyavirus-like
Kowanyama		+																+						22	"
Tataguine	+	+						+								+						+		21	"
Witwatersrand	+									+					+	+								20	"
Zinga	+							+								+						+		22	"
Bocas	+											+								+	+			22	Coronavirus
Japanaut	+											+						+						21	Orbivirus
Lebombo	+						+	+		+						+								21	"
Llano Seco**	+																			+				21	"
Orungo	+	+														+						+		21	"
Paroo River	+																	+						22	"
Umatilla	+										+									+				20	"
Nodamura	+																+							23	Picornavirus
Cotia	+				+			+							+						+	+		24	Poxvirus
Bangoran	+										+					+								22	Rhabdovirus
Joinjakaka	+																	+						22	"
Kununurra	+																	+						22	"
Yata	+															+								22	"
Triniti	+																				+			21	Togavirus

* See footnote Table 5.

** Although it has been demonstrated that Llano Seco virus is antigenically related to Umatilla virus, its antigenic relationship to other established orbivirus serogroups is uncertain.

Table 25. Ungrouped Mosquito-Associated Viruses

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

* See footnote Table 5

Table 26. Ungrouped Tick-, Culicoides-, or Phlebotomus-Associated Viruses

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

* See footnote Table 5
** Cuba

Table 27. Ungrouped Viruses: No Arthropod Vector Known

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
Bangui								+								+						+		22	Bunyavirus-like
Bobaya											+					+								22	"
Ibaraki														+			+							22	Orbivirus
Nariva										+											+			23	Paramyxovirus
Salanga										+						+								22	Poxvirus

* See footnote Table 5

Table 27. Ungrouped Viruses: No Arthropod Vector Known (cont'd)

	ISOLATED FROM: ARTHROPODS: Mosq.		Ticks					VERTEBRATES								ISOLATED IN						HUMAN DISEASE			
VIRUS	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	SEAS RATING*	TAXONOMIC STATUS
Kern Canyon												+								+				23	Rhabdovirus
Keuraliba										+						+								22	"
Klamath										+										+				22	"
Lagos bat												+				+								24	"
Marco														+							+			22	"
Mount Elgon bat												+				+								23	"
Navarro											+										+			22	"
Almpiwar														+				+						21	Unclassified
Araquari													+								+			22	"
Belem											+										+			22	"
Bimbo											+					+								22	"
Gossas												+				+								23	"
Ippy										+						+								22	"
Kammavanpettai											+						+							22	"
Kannamangalm											+						+							22	"
Kolongo											+					+								22	"
Landjia											+					+								22	"
Le Dantec								+								+								22	"
Ouango											+					+								22	"
Sakpa										+						+								22	"
Sandjimba											+					+								22	"
Sebokele										+						+								22	"
Sembalam											+						+							22	"
Simian hem. fever									+								?			+				24	"
Thottapalayam														+			+							22	"
Toure										+						+								22	"
Yogue												+				+								22	"

* See footnote Table 5

Table 28. Continental Distribution of Grouped and Ungrouped Viruses.

Supergroup	Antigenic Group	Total in Group	Africa	Asia	Aus-tral-asia	Eur-ope	North Amer-ica	South Amer-ica	No. of Conti-nents involved 1	2	3	4	5	6
	A	24	6	8	5	2	6	10	16	6	0	1	1	0
	AHS	1	1	1	0	1	0	0	0	0	1	0	0	0
	ANA	3	0	0	0	0	0	3	3	0	0	0	0	0
	ANB	2	0	0	0	0	0	2	2	0	0	0	0	0
	B	59	18	23	13	7	10	7	45	10	3	1	0	0
	BAK	2	0	2	0	0	0	0	2	0	0	0	0	0
	BLU	1	1	1	1	1	1	0	0	0	0	0	1	0
	BTK	2	2	0	0	0	0	0	2	0	0	0	0	0
Bunyamwera Supergroup	BUN	18	4	1	0	2	8	5	16	2	0	0	0	0
Bunyamwera Supergroup	BWA	2	2	0	0	0	0	0	2	0	0	0	0	0
Bunyamwera Supergroup	C	11	0	0	0	0	5	8	9	2	0	0	0	0
Bunyamwera Supergroup	CAL	13	1	1	0	2	9	3	11	1	1	0	0	0
Bunyamwera Supergroup	CAP	8	0	0	0	0	3	7	6	2	0	0	0	0
Bunyamwera Supergroup	GMA	8	0	0	0	0	2	7	7	1	0	0	0	0
Bunyamwera Supergroup	KOO	2	0	0	2	0	0	0	2	0	0	0	0	0
Bunyamwera Supergroup	MIR	2	0	0	0	0	1	1	2	0	0	0	0	0
Bunyamwera Supergroup	OLI	3	3	0	0	0	0	0	3	0	0	0	0	0
Bunyamwera Supergroup	PAT	4	0	0	0	0	4	0	4	0	0	0	0	0
Bunyamwera Supergroup	SIM	18	9	6	2	0	2	4	13	5	0	0	0	0
Bunyamwera Supergroup	TETE	5	4	1	0	2	0	0	3	2	0	0	0	0
Bunyamwera Supergroup	SBU	4	0	1	0	0	1	2	4	0	0	0	0	0
	CGL	2	0	0	0	0	1	1	2	0	0	0	0	0
	CTF	2	0	0	0	1	1	0	2	0	0	0	0	0
	CHF-CON	2	1	2	0	1	0	0	1	0	1	0	0	0
	COR	2	1	0	1	0	0	0	2	0	0	0	0	0
	DGK	5	2	4	1	0	0	0	3	2	0	0	0	0
	EHD	1	1	0	0	0	1	0	0	1	0	0	0	0
	EUB	3	1	0	2	0	0	0	3	0	0	0	0	0
	HP	6	2	0	0	0	3	1	6	0	0	0	0	0
	HUG	4	1	1	0	1	1	3	2	1	1	0	0	0
	KSO	3	0	2	0	0	1	0	3	0	0	0	0	0
	KEM	16	3	4	1	4	6	1	14	1	1	0	0	0
	KWA	1	0	0	0	0	0	1	1	0	0	0	0	0
	MAL	2	1	1	0	0	0	0	2	0	0	0	0	0
	MAP	4	0	0	4	0	0	0	4	0	0	0	0	0
	MBG	2	2	0	0	0	0	0	2	0	0	0	0	0
	MTY	3	3	0	0	0	0	0	3	0	0	0	0	0
	NSD	3	2	1	0	0	0	0	3	0	0	0	0	0
	NDO	1	1	0	0	0	0	0	1	0	0	0	0	0
	PAL	4	0	3	1	0	0	0	4	0	0	0	0	0
	PHL	27	6	4	0	2	10	11	23	2	2	0	0	0
	QYB	2	2	0	0	0	0	0	2	0	0	0	0	0
	QRF	2	1	0	1	0	0	0	2	0	0	0	0	0
	SAK	4	0	1	1	1	2	0	3	1	0	0	0	0
	SAW	2	0	0	0	0	2	0	2	0	0	0	0	0
	TCR	9	1	0	0	0	1	7	9	0	0	0	0	0
	THO	1	1	0	0	1	0	0	0	1	0	0	0	0
	TIM	2	0	0	0	0	0	2	2	0	0	0	0	0
	TR	2	0	2	0	0	0	0	2	0	0	0	0	0
	TUR	4	1	1	1	0	1	1	3	1	0	0	0	0
	UUK	5	0	2	0	3	0	0	5	0	0	0	0	0
	VSV	7	1	2	0	0	2	5	4	3	0	0	0	0
	WAL	1	0	0	1	0	0	0	1	0	0	0	0	0
	WAR	2	0	0	2	0	0	0	2	0	0	0	0	0
	Ungrouped	95	37	23	15	6	10	15	89	1	5	0	0	0
	Totals	423	122	98	54	37	94	107	359	45	15	2	2	0

Table 29. Number of Viruses Isolated From Wild Caught Arthropods

Antigenic Group		Total in Group	Isolated From: Mosq.	Ticks	Phlebotomine Flies	Culicoides	Other	No. of Classes Involved: 1	2	3
A		24	23	1	0	1	5	20	2	2
AHS		1	0	0	0	1	0	1	0	0
ANA		3	3	0	0	0	0	3	0	0
ANB		2	2	0	0	0	0	2	0	0
B		59	29	16	0	0	2	41	3	0
BAK		2	2	1	0	0	0	1	1	0
BLU		1	0	0	0	1	0	1	0	0
BTK		2	1	0	0	0	0	1	0	0
Bunyamwera Supergroup	BUN	18	17	0	0	2	0	17	1	0
	BWA	2	2	0	0	0	0	2	0	0
	C	11	11	0	0	0	0	11	0	0
	CAL	13	13	0	0	0	1	12	1	0
	CAP	8	7	0	0	0	0	7	0	0
	GMA	8	6	0	1	0	0	5	1	0
	KOO	2	2	0	0	0	0	2	0	0
	MIR	2	1	0	0	0	0	1	0	0
	OLI	3	3	0	0	0	0	3	0	0
	PAT	4	3	0	0	0	0	3	0	0
	SIM	18	10	0	0	8	0	8	5	0
	TETE	5	0	2	0	0	0	2	0	0
	SBU	4	3	0	0	0	1	4	0	0
CGL		2	0	0	1	0	0	1	0	0
CTF		2	0	2	0	0	0	2	0	0
CHF-CON		2	0	2	0	1	0	1	1	0
COR		2	2	0	0	0	0	2	0	0
DGK		5	0	5	0	0	0	5	0	0
EHD		1	0	0	0	0	0	0	0	0
EUB		3	3	0	0	0	0	3	0	0
HP		6	6	0	0	0	0	6	0	0
HUG		4	0	4	0	0	0	4	0	0
KSO		3	0	3	0	0	0	3	0	0
KEM		16	0	16	0	0	0	16	0	0
KWA		1	1	0	0	0	0	1	0	0
MAL		2	2	0	0	0	0	2	0	0
MAP		4	4	0	0	0	0	4	0	0
MBG		2	0	0	0	0	0	0	0	0
MTY		3	0	0	0	0	0	0	0	0
NSD		3	2	3	0	1	0	1	1	1
NDO		1	1	0	0	0	0	1	0	0
PAL		4	3	0	0	1	0	4	0	0
PHL		27	5	0	16	0	0	16	1	1
QYB		2	0	2	0	0	0	2	0	0
QRF		2	0	2	0	0	0	2	0	0
SAK		4	0	4	0	0	0	4	0	0
SAW		2	0	2	0	0	0	2	0	0
TCR		9	1	1	0	0	3	3	1	0
THO		1	0	1	0	0	0	1	0	0
TIM		2	0	0	0	0	0	0	0	0
TR		2	0	0	0	0	0	0	0	0
TUR		4	4	0	0	0	0	4	0	0
UUK		5	0	5	0	0	0	5	0	0
VSV		7	2	0	3	0	2	3	2	0
WAL		1	0	0	0	1	0	1	0	0
WAR		2	0	0	0	2	0	2	0	0
Ungrouped		95	39	22	3	2	1	59	4	0
Totals		423	213	94	24	21	15	307	24	4

Table 30. Number of Viruses Isolated From Naturally Infected Vertebrates

Antigenic Group	Total in Group	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Livestock	All Others	Number of Classes Involved 1	2	3	4	5	6
A	24	11	2	6	10	3	6	6	3	6	5	2	3	1	1
AHS	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
ANA	3	1	1	0	0	0	0	0	0	0	1	0	0	0	0
ANB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	59	28	4	17	15	13	1	5	6	26	8	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
Bunyamwera Supergroup															
BUN	18	4	1	3	0	0	0	1	3	8	2	0	0	0	0
BWA	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0
C	11	10	0	8	0	1	5	0	1	2	5	3	1	0	0
CAL	13	3	0	4	0	0	0	0	1	4	2	0	0	0	0
CAP	8	0	0	4	0	0	1	0	0	3	1	0	0	0	0
GMA	8	2	0	7	1	2	4	0	0	3	1	1	2	0	0
KOO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MIR	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLI	3	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	18	2	1	0	4	0	0	6	4	11	3	0	0	0	0
TETE	5	0	0	0	5	0	0	0	0	5	0	0	0	0	0
SBU	4	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	0	1	0	0	0
CHF-CON	2	1	0	0	0	0	0	1	1	0	1	0	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	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HP	6	0	0	0	3	0	0	0	0	3	0	0	0	0	0
HUG	4	0	0	0	1	0	0	0	0	1	0	0	0	0	0
KSO	3	0	1	0	1	0	0	0	1	3	0	0	0	0	0
KEM	16	1	0	1	1	0	0	1	0	0	2	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	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MBG	2	2	0	0	0	0	0	0	0	2	0	0	0	0	0
MTY	3	0	0	0	3	0	0	0	0	3	0	0	0	0	0
NSD	3	3	0	1	0	0	0	2	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	1	0	1	0	0	0	0	0
PHL	27	7	0	8	2	0	2	1	2	14	4	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	1	0	0	0	0	0
SAW	2	0	0	0	0	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
TR	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0
TUR	4	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	1	5	0	0	0	0
WAL	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WAR	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped	95	8	1	12	15	9	1	4	4	49	1	1	0	0	0
Totals	423	96	13	87	69	30	21	35	33	173	48	15	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		24	11	8	12	50.0
Afr. horsesickness		1	0	0	0	
Anopheles A		3	1	0	1	33.3
Anopheles B		2	0	0	0	
Group B		59	28	25	31	52.5
Bakau		2	0	0	0	
Bluetongue		1	0	0	0	
Boteke		2	0	0	0	
Bunyamwera Supergroup	Bunyamwera	18	5	2	6	33.3
	Bwamba	2	1	0	1	50.0
	C	11	9	2	9	81.8
	California	13	5	0	5	38.5
	Capim	8	0	0	0	
	Guama	8	2	0	2	25.0
	Koongol	2	0	0	0	
	Mirim	2	0	0	0	
	Olifantsvlei	3	0	0	0	
	Patois	4	0	0	0	
	Simbu	18	2	1	2	11.1
	Tete	5	0	0	0	
	SBU	4	0	0	0	
Changuinola		2	1	0	1	50.0
Colorado tick fever		2	1	1	1	50.0
CHF-Congo		2	1	1	1	50.0
Corriparta		2	0	0	0	
Dera Ghazi Khan		5	0	0	0	
Epizoot. hem. dis.		1	0	0	0	
Eubenangee		3	0	0	0	
Hart Park		6	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		4	0	0	0	
Marburg		2	2	2	2	100.0
Matariya		3	0	0	0	
Nairobi sheep dis.		3	3	2	3	100.0
Nyando		1	1	0	1	100.0
Palyam		4	0	0	0	
Phlebotomus fever		27	6	1	6	22.2
Qalyub		2	0	0	0	
Quaranfil		2	1	0	1	50.0
Sakhalin		4	0	0	0	
Sawgrass		2	0	0	0	
Tacaribe		9	3	5	5	55.6
Tanjong Rabok		2	0	0	0	
Thogoto		1	1	0	1	100.0
Timbo		2	0	0	0	
Turlock		4	0	0	0	
Uukuniemi		5	0	0	0	
Vesicular stom.		7	4	3	5	71.4
Wallal		1	0	0	0	
Warrego		2	0	0	0	
Ungrouped		95	5	1	6	6.3
Totals		423	94	55	103	24.3

Table 32. Evaluation of Arthropod-Borne Status of 423 Registered Viruses (SEAS)

Supergroup	Antigenic Group	Total in Group	Arbovirus	Probably Arbovirus	Possible Arbovirus	Probably not Arbovirus	Not Arbovirus	Arbo or Probably Arbo		Not or Probably Not Arbo	
								No.	%	No.	%
	A	24	15	5	4	0	0	20	83.0	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	59	29	8	15	2	5	37	62.7	7	11.8
	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	
Bunyamwera Supergroup	BUN	18	8	5	5	0	0	13	72.2	0	
Bunyamwera Supergroup	BWA	2	1	1	0	0	0	2	100.0	0	
Bunyamwera Supergroup	C	11	10	1	0	0	0	11	100.0	0	
Bunyamwera Supergroup	CAL	13	9	1	3	0	0	10	76.9	0	
Bunyamwera Supergroup	CAP	8	4	2	2	0	0	6	75.0	0	
Bunyamwera Supergroup	GMA	8	4	2	2	0	0	6	75.0	0	
Bunyamwera Supergroup	KOO	2	0	2	0	0	0	2	100.0	0	
Bunyamwera Supergroup	MIR	2	1	0	1	0	0	1	50.0	0	
Bunyamwera Supergroup	OLI	3	0	0	3	0	0	0		0	
Bunyamwera Supergroup	PAT	4	1	1	2	0	0	2	50.0	0	
Bunyamwera Supergroup	SIM	18	3	3	12	0	0	6	33.3	0	
Bunyamwera Supergroup	TETE	5	0	1	4	0	0	1	20.0	0	
Bunyamwera Supergroup	SBU	4	0	1	3	0	0	1	25.0	0	
	CGL	2	0	1	1	0	0	1	50.0	0	
	CTF	2	1	0	1	0	0	1	50.0	0	
	CHF-CON	2	1	0	1	0	0	1	50.0	0	
	COR	2	0	1	1	0	0	1	50.0	0	
	DGK	5	0	0	5	0	0	0		0	
	EHD	1	0	1	0	0	0	1	100.0	0	
	EUB	3	0	0	3	0	0	0		0	
	HP	6	0	1	5	0	0	1	16.7	0	
	HUG	4	1	1	2	0	0	2	50.0	0	
	KSO	3	0	1	2	0	0	1	33.3	0	
	KEM	16	0	2	14	0	0	2	12.5	0	
	KWA	1	0	0	1	0	0	0		0	
	MAL	2	0	0	2	0	0	0		0	
	MAP	4	0	1	3	0	0	1	33.3	0	
	MBG	2	0	0	0	2	0	0		2	100.0
	MTY	3	0	0	3	0	0	0		0	
	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	27	4	8	15	0	0	12	44.4	0	
	QYB	2	0	0	2	0	0	0		0	
	QRF	2	2	0	0	0	0	2	100.0	0	
	SAK	4	0	1	3	0	0	1	25.0	0	
	SAW	2	0	0	2	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	
	TR	2	0	0	2	0	0	0		0	
	TUR	4	1	1	2	0	0	2	50.0	0	
	UUK	5	1	1	3	0	0	2	40.0	0	
	VSV	7	3	0	4	0	0	3	42.9	0	
	WAL	1	0	0	1	0	0	0		0	
	WAR	2	0	0	2	0	0	0		0	
	Un-grouped	95	3	11	72	5	3	14	14.7	8	8.4
	Totals	423	105	68	223	9	17	173	40.8	26	6.1

REPORT FROM THE LABORATORY OF ENZIMOLOGY, DEPARTMENT OF MOLECULAR BIOPHYSICS. INSTITUTO DE BIOFISICA, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - R.J., BRASIL

Aspects of Marituba Virus Replication

Marituba (MTB) Virus, was first isolated from sentinel monkeys captured in Oriboca Forest during the research project developed by Instituto Evandro Chagas, Brasil, and the Rockfeller Foundation in 1954-62 to study arboviruses diseases in Amazon region (Causey et al Am. J. Trop. Med. Hyg 10:227-249, 1961). MTB strain (Be AN15) obtained as a 10% suspension of infected suckling mouse brain, was adapted for tissue culture by growing it in BHK-21 cells. The virus was cloned by three sequential isolations from single plaques and virus stocks were grown from individual plaques to prevent the accumulation of defective interfering particles. A very reproducible plaque assay was established using BHK or L-A9 cells. Two plaque size variants was found: a small plaque (S) 0,5-1,0 mm and a large plaque (L) 1,5-2,0 mm. Single step growth cycle of the virus was carried out in $L\text{-}A_9$ cells and the results show that after a initial lag of approximatly 10-12 hours there is an exponential increase in virus production, reaching a maximum yield (5×10^6 PFU/ml) after 24-30 hours post-infection. Cythophatic effect became evident 30-36 hours after infection and appeared as a generalized deterioration of the cell monolayer. In the course of infection of L-A9 cells by MTB virus, during the lag period of the virus replication (before start the releasing of virus particles into the culture medium), the cellular protein and RNA synthesis is substantially inhibited. ^{3}H-Uridine and ^{3}H-leucine, incorporation into acid insoluble material during 30 minutes pulses, declined exponentially in infected cells by comparison to uninfected cells and this decrease was a function of the multiplicity of infection. The RNA and protein synthesis decreases from about 6 hours (at a moi of 1) to less than 3 hours for a moi of 10. Studies are being developed in our laboratory in order to investigate the mechanism by which MTB virus infection exerts this dramatic change in the macromolecular synthesis in mouse ($L\text{-}A_9$) fibroblasts cells.

(M.A. REBELLO, N. VOLKMER, I.C. FRUGULHETTI and M.C. SOARES)

REPORT FROM ARBOVIRUS LABORATORY
INSTITUT PASTEUR AND O.R.S.T.O.M.
B. P. 304
97300 CAYENNE - FRENCH GUIANA

I - ARBOVIRUS ISOLATED IN FRENCH GUIANA.

Human blood specimens were collected from febrile patients with a dengue-like illness.

Mosquitoes and wild vertebrates were caught in the field stations with a view to elucidate the epidemiology of arboviruses in French Guiana.

Following the 1977-1978 outbreak of dengue virus fever, only one strain of dengue I was isolated from a patient.

On the 22 virus strains isolated from mosquitoes collected on human bait, 9 were isolated from *Culex (M) portesi* and 8 from *Wyeomyia occulta*.

Two strains were isolated from wild vertebrates : I Guama virus from *Didelphis marsupialis* collected in Cacao village, and I Murutucu virus from sentinel mice at Gallion field station.
Table I summarize virus isolations in 1979.

2 - SEROLOGICAL STUDIES.

2.1. HUMAN. Human serum samples were collected from febrile patients in French Guiana, Martinique and Guadeloupe. A number of secondary type responses for flavivirus was observed in paired sera.

Blood samples from 48 asiatic people were investigated for alphavirus and flavivirus antibodies using HI and CF tests.

All sera were negative for alphavirus antibodies. 23 (48 %) have antibodies for at least one of the flavivirus antigen used (Yellow fever, SLE, dengue 1, 2, 3, Ilheus). HI titers were between 1/10 and 1/320 with a mean titer of 1/80. CF mean titer was 1/32.

2.2. WILD VERTEBRATES. Wild vertebrate serum samples were collected mostly from animals caught during a marsupial recapture program in Cabassou Forest in 1978-1979. All animals caught were marked and released after bleeding. Retrapped animals were bled once a month. During exactly one year, 267 marsupials were trapped and 465 blood samples (table 2) were taken and examined for HI antibodies for Pixuna, Tonate, Cabassou, Yellow fever, Saint-Louis encephalitis, Ilheus, Murutucu, Oriboca, Maguari and Bimiti antigens.

Five species of marsupials were studied : *Didelphis marsupialis*, *Philander opossum*, *Caluromys philander*, *Marmosa cinerea* and *Marmosa murina*.

Results concerning animals caught only once are summarized in table 3. As we can see, antibody titers are rather low and antibodies against flavivirus are the most frequent.

A curious evolution of HI antibodies was seen in some animals, especially in *Philander opossum* and *Caluromys philander* , antibody titers dropping from 1/160 - 1/320 to less than 1/10 within a month.

M. LHUILLIER, G. GIRAULT, Y. ROBIN. INSTITUT PASTEUR
M. DEGALLIER, F.X. PAJOT, ORSTOM.

	HUMAN		Mosquitoes	Vertebrates	Total
	Cells	Suckling mice			
ALPHAVIRUS					
Tonate (410d)		1	2		3
Ca Ar 16102			1		1
FLAVIVIRUS					
Dengue 1	1				1
BUNYAVIRUS					
1 - Group C					
Murutucu		2		1	3
2 - Bunyamwera group					
Wyeomyia			8		8
3 - Guama group			5	1	6
BUNYAVIRUS-LIKE					
Phlebotomus group					
Itaporanga			1		1
UNIDENTIFIED			5		5
TOTAL	1	3	22	2	28

Table 1 - Virus isolation during 1979

SPECIES	Number	Animals caught once		Animals caught more than once		Number of réactions by species	Seroconversion by month												Irregular evolution of antibodies	No Evolution of antibodies
		Sera +	Sera -	Sera +	Sera -		J	F	M	A	M	J	J	A	S	O	N	D		
Caluromys philander	107	27	32	39	9	200	1		1		1	1	3						8	24
Philander opossum	94	29	29	32	4	169				2	4	2							7	21
Didelphis marsupialis	54	25	13	10	6	83						1							3	6
Marmosa murina	9	7	2	0	0	9														
Marmosa cinerea	3	0	2	1	0	4	1												0	
Total	267	88	78	82	19	465	2		1	2	5	4	3						18	51

Table 2. Serological studies of marsupials (Cabassou Forest)

	Caluromys philander						Philander opossum						Didelphis marsupialis						TOTAL by Antigen
	10	20	40	80	160	320	/ 10	20	40	80	160	320	/ 10	20	40	80	160	320	/
Pixuma	5	2	2				3		2				1	4	3				22
Tonate 410d	2	1	2				2	5	4				1	1	2		1		21
Cabassou 508	7	1	1	1			2	1	1				2	3	2		1		22
Yellow fever	7	2	1	2	1		7	5	1	2	1		5	4	4	1	3	1	47
Saint-Louis Encephalitis	2	7	2	2	1		3	3	3	2			1	8	6		1	1	42
Ilheus	6	5	2				7	5	1	1	1		4	2	3	3		2	42
Murutucu	1	2					1	2						3	1				10
Oriboca	1	1	1				1	3						1	1				9
Maguari		1		1					1							1			4
Bimiti	3	1		1			1		1					1			1		9
TOTAL	34	23	11	7	2		27	24	14	5	2		14	27	22	5	7	4	

Table 3 Titer of antibody for three principals species of marsupialis. Animals caught once.

REPORT FROM THE GORGAS MEMORIAL

LABORATORY, PANAMA, R. P.*

Surveillance for Yellow Fever Activity

Continuing the Yellow Fever (YF) surveillance initiated in April 1979 in the Darien Province, Eastern Panama, in September we collected monkey sera in the Cerro Sasardi area, about 100 km northwest of the Cerro Tacarcuna massif. Twenty two howler monkeys were tested for presence of YF neutralizing (N) antibodies. Only 2 adult monkeys (8 yrs or older) had titers as high as 1:128 and 1:256.

In January 1980 a team went into the forest above Puerto Obaldia which is on the Caribbean coast of Panama close to the Panama-Colombia border, but about 40km. northwest of Tacarcuna. Only 12 howlers were obtained and of these only one (probably 6 yrs. or older) had N titer of 1:128; another (7 yrs. or older) showed a titer of 1:32.

These antibody patterns thus failed to indicate new areas of recent YF activity inspite of the strong serologic evidence last April-July that YF had gone through the Cerro Nique and Cerro Tacarcuna areas in south-east Panama (very close to Colombia) within the last 2 years.

(G. Justines, P. Peralta and A. Adames)

Phlebotomus Fever Group Viruses in Panama

In 1979 a cooperative program was begun between the Walter Reed Army Institute of Research (WRAIR) and the Gorgas Memorial Laboratory (GML). The initial topic of study for this program is to determine the incidence and prevalence of Phlebotomus fever group viruses in Panama, with special emphasis being placed on the impact of these agents on military populations. The study design for this project calls for close monitoring of 4 military units permanently assigned to Panama which frequently engage in field exercises. These units have a combined total strength of approximately 2,500 individuals. Serum samples are being collected from people as they rotate in and out of these units and these people are monitored throughout their tour for episodes of acute febrile illness which may be due to arboviruses.

Preliminary results are available for 2 of the 4 units under study. Of people assigned to a unit on the Pacific side of Panama, 847

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were tested by plaque reduction neutralization test for antibody to Chagres (CHG) virus, with 2 (0.24%) positive. Of 888 tested for antibody to Punta Toro (PT) virus, 6 (0.67%) were positive. Of people assigned to a unit on the Atlantic side of Panama, 2 (0.37%) of 542 had N antibody to CHG virus and 5 (0.74%) of 673 had N antibody to PT virus. From 56 acute samples processed for virus isolation, 3 arboviruses have been isolated; one strain each of PT, CHG and Venezuelan Equine Encephalitis (VEE) virus. All 3 patients were acutely ill and 1 patient (VEE) required hospitalization. No fatalities were recorded and all patients have subsequently recovered completely.

All 3 of these viruses have been recovered from acutely ill people in Central Panama in the past, and these results confirm that active transmission continues. The low antibody prevalence rates to CHG and PT viruses indicate, however, that transmission to military personnel under current training regimes is infrequent.

(J. LeDuc)

Infection of Guinea Pigs With Plaque Variants of Three Venezuelan Equine Encephalitis Strains.

The P-676 and MF-8 epizootic Venezuelan Equine Encephalitis (VEE) strains contained minute plaques (MP) in addition to the small plaques (SP) which are predominant in the virus pool.

Inoculation of SP or MP of the P-676 strain into horses (SP into six and MP into four horses), produced high fever, high viremia with no clinical signs and no deaths in horses. While the SP of MF-8 produced high fever, high viremia, severe signs of encephalitis and three of four horses infected died, the MP of MF-8 induced viremia in two of four horses infected, but no fever, clinical signs or deaths were observed.

Since it has been proposed that guinea pig lethality may correlate with equine virulence of VEE strains, we inoculated subcutaneously guinea pigs (C-13 strain) weighting 200-500 grams with the SP and MP of P-676 and MF-8 strains and also the SP and large plaque (LP) of the Magangue strain, an enzootic strain, which produces viremia but no deaths in equines.

The guinea pigs inoculated with the MP of MF-8 developed antibodies with no signs of illness; the SP of MF-8 produced fatal infection as did both plaque sizes of P-676. The SP of the enzootic Magangue strain produced asymptomatic infection, as did LP in two of four guinea pigs inoculated (Table 1). Thus, VEE virulence in guinea pigs and horses does not seem to correlate well; however, guinea pigs could be good animal models for detecting differences within viral subpopulations present in epizootic and enzootic VEE strains.

(B. Dutary and G. Justines)

Table 1

Plaque Variants of Three Strains of VEE

Inoculated in Guinea Pig (*Cavia porcellus*)

	P-676		MF-8		Magangue	
	Small plaque	Minute plaque	Small plaque	Minute plaque	Small plaque	Large plaque
Guinea pigs inoculated (dead/total)	2/2	4/4	2/2	0/4	0/4	2/4
Infection dose (pfu)	2000	12000	2000	11000	200	200
Peak viremia* (dex/ml blood)	5.8	3.5	6.4	Nt+	Nt	5.5
Range survival time (days)	5-6	6-11	4-5	-	-	6-7
Antibody titer††	-	-	-	$\geq$64	$\geq$16	$\geq$4

* Titer in guinea pigs which died
\+ Not tested
†† Reciprocal of serum titer neutralizing 90% of the virus dose

REPORT FROM THE DEPARTAMENTO DE VIROLOGIA, HOSPITAL GENERAL DE MEXICO, S.S.A.
AND
INSTITUTO DE INVESTIGACIONES BIOMEDICAS, U.N.A.M.

DENGUE VIRUS ACTIVITY IN SOUTHEASTERN MEXICO*

AGE GROUP	DENGUE TYPE 1	%	DENGUE TYPE 2	%	DENGUE TYPE 3	%
5 - 14	0/10**	-	0/10	-	2/10	20.0
15 - 44	6/84	7.1	27/84	32.1	20/84	23.8
45 - 54	0/15	-	12/15	80.0	15/15	100.0

* 109 human sera collected from Yucatán and Campeche, México, on June 1978.

** Number of positives/number of sera studied by the hemagglutination-inhibition (HI) test. HI titers ranged from 1:10 to ≥ 1:160 against 4 HA units of each antigen.

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REPORT FROM THE TEXAS DEPARTMENT OF HEALTH
AUSTIN, TEXAS 78756

ARVOVIRUS SURVEILLANCE, JULY 1, 1979-Dec. 31, 1979

MOSQUITO ISOLATES

For the period indicated above, 1290 litters of mice were inoculated for arbovirus isolation. This represents 2933 pools totaling 42,966 mosquitoes. Listed below are the positives:

Locality	Collection Date	Pooled Species	Number of Isolations	Virus
Galveston Co.	May 16	A. sollicitans A. taeniorhyncus C. quinquefasciatus	1	California (Keystone strain)
Cameron Co.	June 5	C. (melanoconion) species C. quinquefasciatus C. quinquefasciatus A. scapularis	1	Hart Park
Hidalgo Co.	June 18	C. quinquefasciatus C. quinquefasciatus C. quinquefasciatus	1	Hart Park
Dallas	June 15-19	C. salinarius C. salinarius C. restuans	1	Hart Park
Jefferson Co.	June 20	A. quadrimaculatus A. quadrimaculatus C. salinarius	1	Tensaw
Corpus Christi	June 20	C. (melanoconion) species C. quinquefasciatus	1	Hart Park
Dallas	June 19-21	A. vexans A. quadrimaculatus C. tarsalis	1	Hart Park
Dallas	June 19-20	A. quadrimaculatus C. tarsalis C. salinarius	1	Hart Park
Dallas	June 22-25	C. tarsalis C. quinquefasciatus C. salinarius	1	Hart Park

Locality	Collection Date	Pooled Species	Number of Isolations	Virus
Willacy Co.	June 26	C. quinquefasciatus	1	Hart Park
Dallas	June 26-29	C. quinquefasciatus A. crucians A. quadrimaculatus C. restuans	1	Hart Park
Hudspeth Co.	July 3	C. tarsalis	1	WEE
Dallas	July 2-3	A. quadrimaculatus C. restuans C. quinquefasciatus	1	Hart Park
Dallas	July 2-5	C. salinarius C. quinquefasciatus C. restuans	1	Hart Park
Dallas	July 2-5	C. salinarius C. restuans C. quinquefasciatus	1	Tensaw
Liberty Co.	July 10	A. quadrimaculatus A. crucians A. quadrimaculatus	1	Tensaw
Liberty Co.	July 10	C. (melanoconion) species P. columbiae C. quinquefasciatus	1	Hart Park
Dallas	July 10	A. quadrimaculatus C. (melanoconion) species C. salinarius	1	Hart Park
Port O'Connor	July 10	P. columbiae C. quinquefasciatus	1	Hart Park
Dallas	July 10-11	A. quadrimaculatus A. species C. restuans	1	Hart Park
Dallas	July 10-11	C. (melanoconion) species C. salinarius C. quinquefasciatus	1	Hart Park
Castro Co.	July 12	C. tarsalis A. vexans	1	Hart Park
Randall Co.	July 12	C. salinarius A. vexans C. tarsalis	1	Hart Park
Cameron Co.	July 17	C. quinquefasciatus P. columbiae A. sollicitans	1	Hart Park

Locality	Collection Date	Pooled Species	Number of Isolations	Virus
Jefferson Co.	July 18	A. quadrimaculatus C. quinquefasciatus A. crucians A. quadrimaculatus	1	Tensaw
Jefferson Co.	July 18	C. quinquefasciatus C. salinarius C. quinquefasciatus	1	Hart Park
Jefferson Co.	July 18	A. crucians C. (melanoconion) species A. quadrimaculatus	1	Tensaw
Dallas	July 18	C. quinquefasciatus C. (melanoconion) species C. quinquefasciatus	1	Hart Park
Dallas	July 23	C. restuans C. quinquefasciatus A. vexans	1	Hart Park
Ft. Worth	July 23	C. quinquefasciatus A. quadrimaculatus C. quinquefasciatus	1	Hart Park
Dallas	July 24-26	C. quinquefasciatus C. quinquefasciatus C. restuans	1	Hart Park
Hidalgo Co.	Aug. 6-7	C. quinquefasciatus C. quinquefasciatus C. quinquefasciatus	1	Hart Park
Matagorda Co.	Aug. 8	A. crucians A. taeniorhyncus P. columbiae	1	Tensaw
Dallas	Aug. 7-8	C. quinquefasciatus C. (melanoconion) species C. quinquefasciatus	1	Hart Park
Ft. Worth	Aug. 13	A. aegypti C. (melanoconion) species C. quinquefasciatus	1	Hart Park
Dallas	Aug. 10-13	C. (melanoconion) species C. quinquefasciatus A. quadrimaculatus	1	Hart Park
Brazos	Aug. 11	A. aegypti C. quinquefasciatus	1	Hart Park
El Paso Co.	Aug. 21	C. quinquefasciatus A. vexans	1	SLE

Locality	Collection Date	Pooled Species	Number of Isolations	Virus
Dallas	Sept. 4-5	C. (melanoconion) species C. quinquefasciatus C. quinquefasciatus	1	Hart Park
Dallas	Sept. 7-10	C. (melanoconion) species C. quinquefasciatus C. quinquefasciatus C (melanoconion) species	1	Hart Park
Ector Co.	Sept. 11	A. vexans C. tarsalis C. tarsalis	1	WEE
Nolan Co.	Sept. 18	P. cyanescens C. (melanoconion) species C. quinquefasciatus C. tarsalis	1	WEE
Dallas	Sept 18-20	C. (melanoconion) species C. tarsalis C. territans C. quinquefasciatus	1	WEE
Dallas	Sept. 28-Oct. 1	C. (melanoconion) species C. salinarius C. restuans	1	Hart Park
Dallas	Oct. 8-10	C. restuans C. quinquefasciatus A. aegypti	1	Hart Park

BIRD BLOODS FOR ISOLATION

Wild bird bloods were submitted for arbovirus isolation from San Antonio, Lubbock, and Dallas. Two WEE isolates were obtained from the Lubbock area. The first isolate was from a fledgling collected on July 17, 1979, while the second was collected August 31, 1979.

BIRD BLOODS FOR SEROLOGY

The following regions submitted chicken or wild bird bloods for arbovirus surveillance: Dallas City, Dallas County, Lubbock, and Cameron County. We received a total of 1660 sera on which Hemagglutination-Inhibition tests were performed. Following are the positives:

Locality	Collection Date	Species	# Positives	Antibodies Detected
Lubbock	July 31	Chicken	17	WEE $\gtrsim$ 1:40
	Sept. 6	Chicken	50	WEE ≥ 1:20
	Oct. 15	Chicken	24	WEE ≥ 1:20
	Nov. 5	Chicken	6 1	Wee ≥ 1:20 SLE ≥ 1:20
Cameron Co.	Sept. 12	Chicken	2	SLE $\lesssim$ 1:20
	Oct. 10	Chicken	2	WEE ≥ 1:20

(Charles C. Sweet)

REPORT FROM THE OFFICE OF LABORATORY SERVICES AND ENTOMOLOGY
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES
JACKSONVILLE, FLORIDA

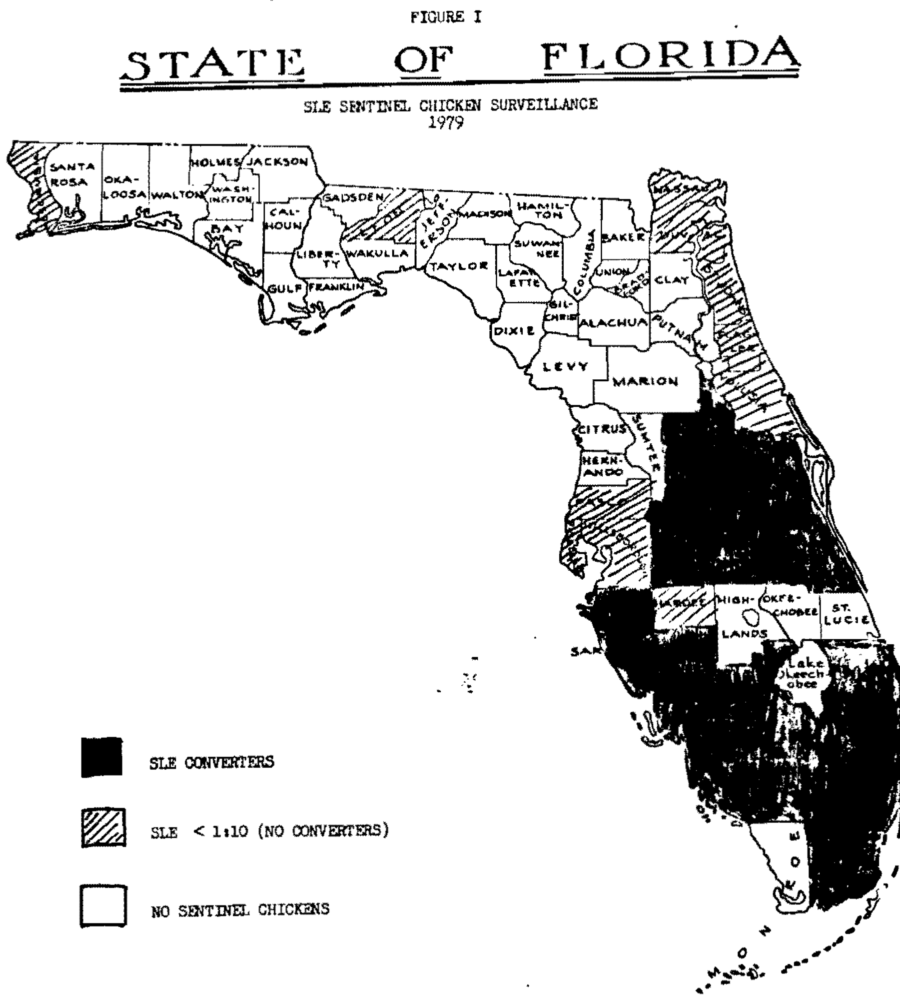
Extensive SLE surveillance in Florida, August through December, 1979 was productive in yielding a large number of sero-conversions in the strategically placed sentinel chicken flocks. EEE continued to circulate at a low level in the sentinel chickens, small mammals and horses.

A total of 1,729 sera from patients with CNS symptoms were tested by HI against EEE, SLE, VEE, Dengue 2 and CAL antigens. Five patients had laboratory confirmed SLE infections. There was one fatal case of EEE in a 15 year old male and five confirmed Dengue infections presumably acquired in the Caribbean area. Forty four patients from endemic areas had constant titers to Group B antigen.

A total of 4,023 sera from sentinel chickens yielded 290 with SLE HI antibodies. (See Figure I). Four SLE isolates were made from 978 mosquito pools of <u>Culex nigripalpus</u>. 1,186 sera from raccoons, o'possums, and other mammals yielded 64 with SLE HI antibodies.

(N.J. Schneider, F.M. Wellings, E.E. Buff and J.A. Mulrennan, Jr.)

FIGURE I



REPORT FROM THE VIRAL DISEASES DIVISION, CENTER FOR DISEASE CONTROL

ATLANTA, GEORGIA

California Encephalitis in the Southeastern United States: Identification of a Focus Around Cherokee, North Carolina

A cluster of 4 cases of California encephalitis (CE) were reported from western North Carolina in 1977, the first group of documented CE cases from North Carolina or surrounding states since 1965. Three of the patients were children from the Cherokee Indian Reservation, and the fourth child from an adjoining county who camped in the nearby Smoky Mountains National Park during his period of infection. We reviewed clinical records at the Cherokee hospital and a regional reference hospital but found no evidence of unusual clinical activity in 1977. In May 1978, we collected vector mosquito larvae from around the residences of the diagnosed and clinically suspect CE patients in the Cherokee Reservation and from campgrounds in the Smoky Mountains National Park where the other diagnosed patient had stayed. Breeding containers were numerous and vector larvae abundant near the residences on the reservation but relatively few larvae were found in the park. LaCrosse strain CE virus was isolated from 3 pools of *Aedes triseriatus* mosquitoes reared from larvae collected in the Cherokee Reservation. Neutralizing antibodies to CEV were compared in second grade children from the Cherokee school in 1978 (3/67-4.5%), second grade children in 1969 (1/50-2%). and a small group of teenagers from the reservation in 1978 (2/18-11%). With increased surveillance, CE infection was diagnosed in 2 children from the area in 1978, and in 3 children in 1979. The results suggest that LaCrosse strain CEV is endemic in the Cherokee Reservation and surrounding Jackson County.

(K. Kappus, R. Baron, J. Davenport[1], C. Calisher[2], B. Francey[2], and R. Williams[3])
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2. Vector-Borne Diseases Division, CDC, Fort Collins, CO
3. Vector Biology and Control Division, Bureau of Tropical Diseases, CDC

St. Louis Encephalitis (SLE), Mississippi, 1979

In September 1979 an increase in the number of cases of encephalitis was noted in the Washington County area of northwestern Mississippi, where St. Louis encephalitis (SLE) is endemic. Through health department surveillance and review of hospital records, a total of 32 cases of either encephalitis (22 cases) or aseptic meningitis (10 cases) were found. SLE antibodies were confirmed in 13 (9 encephalitis and 4 aseptic meningitis) of these cases and 2 cases (1 encephalitis and 1 aseptic meningitis) have thus far been ruled out. An additional 2 cases without complete clinical information have also been confirmed serologically. Dates of onset for confirmed or presumptive cases ranged from July 31 - September 28. Nine were in males and 6 were in females. The age range was 11 - 90 years (mean = 49.3 years). There were 2 deaths.

The additional 17 suspected SLE cases are awaiting laboratory confirmation. Also being tested are 193 "control" blood specimens collected from patients seen at the Delta Medical Center (the major referral hospital in Washington County) from September to October 1979.

Within the past 7 years, Washington County had had one large outbreak of SLE in 1975 and one smaller outbreak in 1974. A review of emergency room visits at the Delta Medical Center from June-September, 1973-79 revealed a significantly higher percentage of patients presenting with fever and/or headache--the two most common symptoms of SLE--in 1979 than in 1973, 1976, 1977, or 1978, the years in which no SLE epidemic had been reported ($p < .05$).

We concluded that there had indeed been a focal outbreak of SLE in the Washington Co⁻nty area similar in magnitude to the epidemic of 1974, but less severe than the epidemic of 1975. With early ongoing active surveillance, the prompt implementation of mosquito control measures would have a better chance of reducing the incidence of disease.

(P. Katona, C. Calisher[1], L. Schonberger)

1. Vector-Borne Diseases Division, CDC, Fort Collins, CO

Imported Dengue, United States, 1977-1979

The Center for Disease Control maintains a system of surveillance for laboratory-confirmed dengue cases within the continental United States. Through this system, 151 confirmed cases in U.S. residents returning from dengue-active areas have been reported for the years 1977-1979: 56 in 1977, 88 in 1978, and 7 in 1979 (through November 7, Table 1).

In 1977, 24 (43%) were imported from Jamaica, 3 (5%) from Puerto Rico, 9 (16%) from other Caribbean areas, and 1 (22%) from India; the area of travel was unknown for 19 (34%). In 1978, most cases were imported from Tahiti (29; 33%) and Puerto Rico (24; 27%). Additionally, 10 (11%) were from Caribbean islands, 7 (8%) from Central America, and 2 (2%) from Colombia; a travel history was not available for the remaining 16 (18%) cases. In 1979, of the 7 cases reported, 2 were from the Caribbean islands (excluding Puerto Rico), 1 was from Tahiti, and 1 was from the Philippines (1); travel information was unavailable for the remaining 3 cases.

Of the 151 cases imported into the continental United States between 1977 and 1979, (19%) occurred in persons returning to one of the southeastern states that has abundant vector populations. An additional 54 (36%) were in persons returning to states estimated to have the vector, but in lesser abundance. The remaining 68 (45%) were in states without *Aedes aegypti*. Based on both temporal and geographic estimates of *Aedes aegypti* distribution, 34% (28/83) of the cases in high-risk receptive states were during months when vector populations are abundant. Nonetheless, dengue transmission within the continental United States was not documented during this period.

(M. Moore, G. Taylor[1], and D. Eliason[1])

1. Vector Biology and Control Division, Bureau of Tropical Diseases, CDC

REPORT FROM THE INSTITUTE FOR CANCER RESEARCH
THE FOX CHASE CANCER CENTER
PHILADELPHIA, PENNSYLVANIA 19111

Studies of the bedbug Cimex hemipterus (Fabr.) as a possible vector of hepatitis B virus

In earlier experiments (Information Exchange No. 35, September 1978), we showed that hepatitis B surface antigen (HBsAg) is excreted in the feces of Cimex hemipterus fed on HBsAg-positive blood. We noticed that females excreted more HBsAg than did males and thought that this might be due to the females taking more blood than the males (Ogston and London, in press).

Using ^{125}I as a radioactive tracer, we have now estimated the amount of blood taken by individual bedbugs from an artificial feeder. The average blood meal of female C. hemipterus was between 2.3 and 5.3 microliters in three different strains, whereas males took between 1.5 and 3.2 microliters on average. The females took between 1.4 and 2.0 times as much blood to engorge as did males of the same strain fed at the same time.

This difference between males and females was already evident in fifth instar nymphs. The nymphs were fed on radioactively labeled blood and the amount each ingested was measured. Then the insects were kept in individual test tubes and reared to adulthood. The average blood meal of 34 nymphs which turned out to be females was 2.5 microliters while among 32 nymphs which emerged as males the average blood meal was 2.0 microliters ($t_{64} = 2.66$, $p \sim 0.005$, one-tailed). Four nymphs failed to develop to adulthood.

In another experiment making use of a radioactive tracer, C. hemipterus were shown to be capable of transferring fluid from one artificial feeder to another in an experimental setup simulating interrupted feeding. The insects were allowed to probe briefly first in an artificial feeder containing a highly radioactive solution, then immediately afterwards in a second feeder containing non-radioactive liquid. In two experiments, each of which involved more than 100 insects, each insect was found to transfer on average radioactivity equivalent to about 150 cubic microns of the first feeder's contents. Whether this amount of mechanical transfer would be sufficient to bring about significant transmission of hepatitis B virus depends, among other things, on the infectious titer of virus present in the subcutaneous tissue fluids sampled by the bedbug when probing.

C. Walter Ogston
Anna D. Yanovski

REPORT FROM THE DIVISION OF VIROLOGY AND IMMUNOLOGY
BUREAU OF LABORATORIES
PENNSYLVANIA DEPARTMENT OF HEALTH
LIONVILLE, PENNSYLVANIA

Arbovirus Surveillance in Pennsylvania, 1979

The Pennsylvania Department of Health and the Department of Environmental Resources conducted an arbovirus surveillance program during the summer and fall of 1979, similar to that conducted in 1978.

On June 27, cockerels were distributed to their sentinel locations and bled. During the course of the program, 247 chickens stationed at 51 different sites in 27 of the state's 67 counties (Figure 1) were bled weekly by Department of Environmental Resources personnel, and the blood or serum was submitted to our laboratory for testing. Upon receipt, the serum samples were treated with acetone to remove non-specific inhibitors of hemagglutination and with goose erythrocytes to remove non-specific agglutinins. The sera were then tested for hemagglutination-inhibition (HI) antibodies directed against St. Louis Encephalitis Virus (SLE), Eastern Equine Encephalitis Virus (EEE), Western Equine Encephalitis Virus (WEE), and California Encephalitis Virus (CEV). When the program was terminated on October 11, a total of 2,364 serum samples had been received and a total of 9,892 HI tests had been performed.

Three chickens seroconverted to WEE. Chicken No. 70, stationed in Monroe County in Region II North, converted during the week of September 24, when an HI titer of 1:20 was obtained; no further samples were available. Chicken No. 99, stationed in Northampton County in Region II South, converted during the week of October 8, when an HI titer of 1:20-1:40 was obtained; no further samples were available. Chicken No. 100, stationed at the same location as Chicken No. 99, converted during the week of October 1, when an HI titer of 1:40 was obtained. A sample obtained during the week of October 8 titered 1:20-1:40. No further samples were available.

One chicken, No. 253, stationed in Crawford County in Region VI, converted to SLE. Serum obtained during the week of August 6 titered 1:80. Subsequent samples had decreasing titers until the chicken reverted to negative during the week of August 27. The rapid rise and fall of HI antibodies cannot be interpreted.

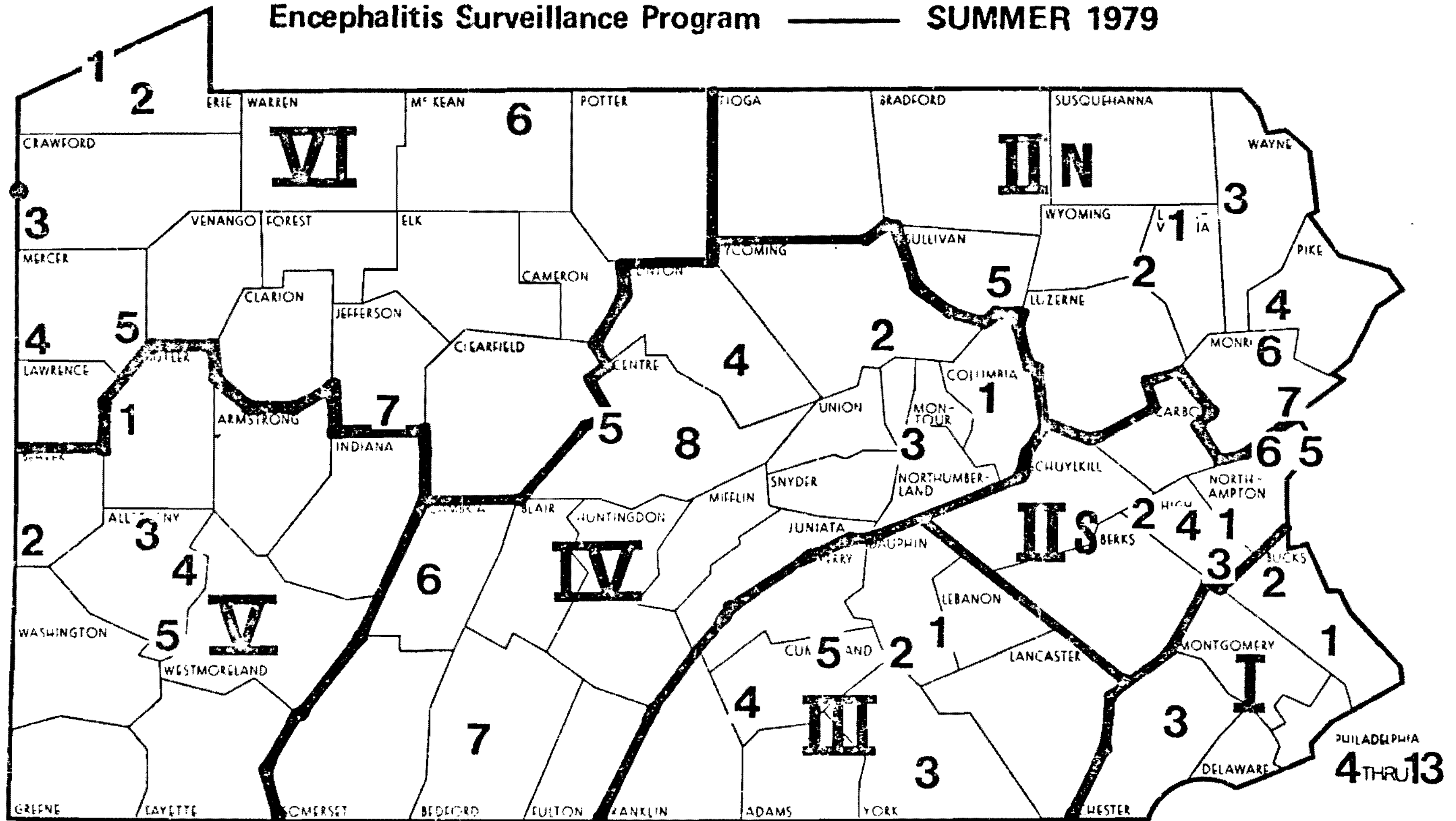
Another aspect of the surveillance program involved the testing of all serum samples submitted from patients with neurologic symptoms for antibodies to SLE, EEE, WEE, and CEV. One patient, a 46-year old woman hospitalized in Bryn Mawr, Montgomery County, was diagnosed serologically and by viral isolation procedures as infected by EEE. The date of onset of the patient's illness was September 10. From August 20 to September 8, she had resided in the Somers Point area of New Jersey, returning to her home in Pennsylvania on September 9. The diagnosis, on the basis of an elevated titer on a single serum sample collected on Septmeber 13, was made on September 14. Subsequent serum samples showed a significant rise in titer and EEE virus was isolated from spinal fluid. The patient is still alive on this date (March 1, 1980), but has never regained consciousness.

(Bruce Kleger and Vern Pidcoe)

FIGURE 1

SENTINEL POULTRY FLOCK LOCATIONS BY REGION*

Encephalitis Surveillance Program —— SUMMER 1979



COOPERATIVE WORKING AGREEMENT

PA DER — Vector Control

PA DH — Div. Comm. Disease Control; Bureau of Laboratories

● SLE

***COOPERATING COUNTY HEALTH DEPTS.**

Allegheny
Philadelphia
Bucks
Chester
Erie

REPORT FROM CORNELL UNIVERSITY, DEPARTMENT OF MICROBIOLOGY (MEDICAL COLLEGE, NEW YORK) AND DEPARTMENT OF ENTOMOLOGY (COLLEGE OF AGRICULTURE, ITHACA)

Mosquito vector studies during 1977-78 at an enzootic marsh habitat on the Pacific coast of Guatemala where VE virus is active yearly during the rainy season have shown that Cu. (Mel.) opisthopus is the predominant species of Cu. (Mel.) mosquitoes. Mansonia titillans and Aedes taeniorhynchus are also present with seasonal variations, and during July-September 1978, Culex nigripalpus appeared in large numbers. Parity rate determinations at dusk and dawn in 1978 suggested that partitioning of biting activity of parous Cu. (Mel.) opisthopus did not occur during a particular crepuscular time segment. Precipitin tests with antibodies to serum proteins of known vertebrate species are beginning to indicate that over 50% of bloods found in Cu. (Mel.) opisthopus are mammalian whereas the majority of bloods in Cu. nigripalpus are avian.

Experiments to determine the intestinal infection and transmission thresholds of Cu. (Mel.) opisthopus for an enzootic strain of VE virus were begun in 1978, and so far indicate that large percentages of these mosquitoes from the marsh habitat in Guatemala can become infected by small amounts of VE virus (measured as plaque forming units in primary chicken embryonic cell cultures). Available data suggest that 50% transmission thresholds may be low (i.e., between <5 and 20 pfu). Observations of Cu. (Mel.) opisthopus caught in nature and kept in cages indicate that they survive well and are relatively long-lived. Some gravid Cu. (Mel.) opisthopus were observed to bite and engorge blood a second time 12-19 days after the first engorgement.

Evaluations of Mansonia titillans and Culex nigripalpus for evidence of infection by VE virus and ability to transmit are in progress. To date, no evidence of VE infection or ability to transmit VE virus has been found with these species captured at the marsh habitat in Guatemala. However, larger numbers need to be examined. Aedes taeniorhynchus also needs to be tested for vector potential since it is a predominant species throughout much of the year although in 1978, it was in low numbers during the rainy season (possibly related to the abundance of fresh rather than brackish water).

Sentinel hamsters again showed in 1978 that VE virus was actively being transmitted to them by biting arthropods during the July-September rainy season at the marsh study site in Guatemala. Fifty sentinel guinea pigs were exposed at this habitat during July-September 1978 in an attempt to detect guinea pig-lethal and thus possibly equine-virulent strains of VE virus. Most guinea pigs became infected by VE virus since they developed antibodies. Four guinea pigs died, and one yielded a VE strain which has characteristics of enzootic virus. Some guinea pigs lost weight during exposure in the small sentinel cages utilized during 1978, and our conclusion is that the guinea pig that died and yielded VE virus, died of other causes at a time when it happened to be viremic.

A strain of St. Louis encephalitis virus was isolated from Culex nigripalpus collected on 29 August 1978 at the marsh study site on the Pacific coast of Guatemala. This is the first isolation of SLE virus at this habitat during studies conducted there since 1968.

Four epizootic VE virus isolates from Middle America eluted from hydroxylapatite between 0.23 M and 0.31 M phosphate. Plaque size differences between epizootic and enzootic (subtype I-E) VE viruses in Vero cells were confirmed, but found to be dependent upon the lot of agar used in the medium. Most of the virus in a cloned Middle American enzootic VE

virus strain did not bind to hydroxylapatite at 0.05 M phosphate and eluted immediately following the void volume, although about 1% of the virus bound and eluted broadly between 0.15 and 0.40 M phosphate. Late-eluting enzootic VE virus could be easily distinguished from epizootic VE virus by plaque size in Vero cells. Artificial mixture experiments showed that the combined techniques of hydroxylapatite chromatography and Vero plaque selection could detect 1 epizootic in 10,000 enzootic VE viruses.

Seventy-nine VE virus isolates from enzootic habitats before and after the 1969 Middle American and 1966 Tampico, Mexico outbreaks were examined individually or as isolate pools by hydroxylapatite chromatography. Virus eluting between 0.20 and 0.33 M phosphate of single isolates or isolate pools (representing 54 isolates) have been examined to date for small, "epizootic-like" plaques in Vero cells. Thirty-one of thirty-five isolates or isolate pools (representing 47 of 54 isolates) contained various quantities of small plaques which were selected for further characterization. To date, amplified candidate epizootic VE virus plaques from four isolates have been shown to be stable and homogeneous with respect to plaque size in Vero cells.

(W.F. Scherer, M.E. Wiebe, R.W. Dickerman (NY) and E.W. Cupp (Ithaca)

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

Arbovirus Surveillance, 1979

During 1979 580 patients with signs of central nervous system infection were studied. Serologic evidence of current California encephalitis (CE) virus infection was obtained in a 29-year-old male resident of Westchester County where a fatal CE case was diagnosed in 1978. In fourteen additional patients serologic findings were interpreted as presumptive evidence of recent infection with CE virus. Ten of these patients resided in the eastern part of New York State. Infections with other arboviruses were not observed.

From 2,236 pools of more than 100,000 wild-caught mosquitoes collected statewide, 13 isolates of California encephalitis complex (CAL) viruses were obtained. Other arboviruses were not isolated.

In the Capital District where CE is endemic, 16 sentinel rabbits and 17 hamsters exposed from May to November, 1979 in areas previously associated with human cases failed to show evidence of CAL virus infection. However, antibodies to a local strain of CAL virus were detected in 37 of 94 sheep and 4 of 33 goats maintained in a suburb of Albany during 1979. Eleven of these sheep exhibited seroconversion indicating that transmission of CAL virus occurred in the area during 1979. An extensive search for transovarial transmission of CAL virus in the tree-hole breeding mosquito, *Aedes triseriatus*, yielded negative results.

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

REPORT FROM THE STATE OF NEW JERSEY
DEPARTMENT OF HEALTH, JOHN FITCH PLAZA
TRENTON, NEW JERSEY 08625

The following tabulations comprise the New Jersey arbovirus activity report for the 3rd and 4th quarters (July-December), 1979.

THIRD QUARTER

Table 1. Isolations from Arthropods*

Group	Virus and No.of Strains EE	WE	Isolated from	Collected in
A		1	C. melanura	Atsion
A	9	13	C. melanura	Bass River
A	7	14	C. melanura	Dennisville
A		1	Coq. perturbans	Dennisville
A	2		C. melanura	Burlington Co.
A	10	9	C. melanura	New Gretna
A	3	15	C. melanura	Woodbine
A		1	Culex salinarius	Woodbine
Totals	31	54		

* These include collections from the New Jersey Agricultural Experiment Station, Rutgers University

Table 2. Isolation and/or Serology of Animals for EE

Location	Animal Species	Date Specimen Collected	Isolation Data		Serology Data		
			Organs	Blood	HI	CF	Neut.
Mays Landing Atlantic Co.	Equine	9/7/79	No brain submitted	neg.	Bl 640	8	<0.2
Medford Burlington Co.	Equine	9/8/79	No brain submitted	Bl-EE	Bl 640	16	2.0

Table 3. Isolation and/or Serology of Humans

Location	Date Specimen Collected	Isolation Data		Serology Data		
		Specimen	Blood	HI	CF	Neut.
Somers Point Atlantic Co.	9/11/79	CSF - EE	Neg.	B1 10 B2 160 B3 160	<8 8 16	3.3 ND 3.8

FOURTH QUARTER

Table 4. Isolations from Arthropods*

Group	EE	WE	Isolated from	Collected in	Month
A	2		C. melanura	Bass River	Oct.
A	1	1	C. melanura	Woodbine	Sept.
A	1		C. melanura	Dennisville	Oct.
Totals	4	1			

* These include collections from the New Jersey Agricultural Experiment Station, Rutgers University.

Table 5. Isolation and/or Serology of Animals for EE

Location	Animal Species	Date Specimen Collected	Isolation Data		Serology Data	
			Organs	Blood	HI	Neut
Jackson Ocean Co.	Equine	9/22/78	No Brain submitted	Neg	5120	2.6
Marlton Burlington Co.	Equine	9/14/79	No Brain submitted	Neg.	640	3.0
Forked River Ocean Co.	Pheasant Flock	10/22/79	Brians EE	ND	ND	ND

(Wayne Pizzuti)

REPORT FROM THE UNIVERSITY OF NOTRE DAME'S LABORATORY FOR ARBOVIRUS RESEARCH AND SURVEILLANCE (UNDLARS), NOTRE DAME, INDIANA 46556

A large-scale serological survey of the State's human population for antibodies to Eastern equine encephalomyelitis, Western equine encephalomyelitis, St. Louis encephalitis, and California group viruses was initiated in the late fall of 1978. Serum samples were obtained from over 10,000 individuals and were collected by 97 different blood banks, clinical laboratories, hospital laboratories, and volunteer programs. Samples were obtained from individuals from all 92 counties of the State of Indiana; the total sample size was approximately 0.2% of the State's population. Informed consent was obtained with samples and included specific information on age, sex, county of residence, duration of residence, military history, etc.

Samples were tested using a screening microneutralization test we developed. All samples were tested at a final 1:2 dilution in duplicate; the serum control was also duplicated. In this manner 20 samples could be tested in duplicate per 96-well plate, leaving two full columns for cell controls. Over 40,000 such screening microneutralization tests were completed in less than one year.

Samples positive at 1:2 were further tested in a standard serum dilution microneutralization test. Reference EEE, WEE, SLE virus strains were obtained through the courtesy of Dr. Charles Calisher; the LAC prototype virus strain was obtained through the courtesy of Dr. Thomas Yuill. A vero cell line was used for testing EEE, WEE, and CE group antibodies and a BHK_{21} line for SLEV (again provided by Dr. Calisher as reference strains).

Of 10,208 samples tested for antibodies to SLEV, 367 were positive for antibodies at a 1:2 dilution; SDNT titers ranged from 1:2 to 1:256 when the 367 positives were titered to endpoint. Using this statewide prevalence rate of 3.6%, an estimate was made that approximately 190,000 persons in the State possibly might thus have detectable antibodies to SLEV. Indiana has consistently ranked high in incidence of SLEV, ranking third nationally in number of cases in 1975 (323), for example. A computer-drawn diagram was used to visualize the SLEV antibody prevalence rates (Fig. 1); this provided a means of associating the data with a geographic region. One must be aware that while the diagram does not represent the precise geographic proportions of the state, it is close to the geographic proportions and gives a rather reliable regional picture of antibody prevalence rates.

We fit the frequency of titer values observed for the SLEV data to the negative binomial and Poisson frequency distributions. There was a highly significant difference between the Poisson expected and our observed values; when the negative binomial expected and our observed values were compared the fit was quite good, but just significantly different at the 0.1 level (Fig. 2). The significance of this will be discussed following the La Crosse virus antibody prevalence data below.

Of 10,194 individual samples tested for antibodies to the California group, 239 were positive for La Crosse virus, and an additional 90

positive for either Jamestown Canyon virus (64) or trivittatus virus (26). Significant cross reaction prohibited us from positively identifying the causal agent for an additional 41 CE group positive samples. Thus the total CE group prevalence rate statewide was also 3.6%, again representing a possibly 190,000 persons with detectable CE group antibodies. Specifically the LACV antibody prevalence rate was 2.3%, and that of JC + TVT 0.9%. The geographic distribution of the LACV antibodies is shown in Figure 3.

If we sample a population where a disease frequently occurs at some level, and we measure that disease incidence by antibody titer, we would expect to find some high titered samples (indicative of very recent infections), some low titered samples (indicative of much older infections), with the majority of samples falling in between. If the disease is endemic in the human population, or enzootic in an animal population, infections should be neutrally dispersed through time and the frequency distribution of individuals with positive titers should be Poisson.

When we fit the frequency of LACV observed titers data to the Poisson distribution, we found no significant difference (Fig. 2). This "good fit" indicates that La Crosse encephalitis occurs as an endemic disease. Since the SLEV observed titer frequency data does not fit the Poisson, but more closely approximates a fit to the negative binomial distribution, the suggestion is that SLEV antibodies are the result primarily of epidemics, with low level endemicity accounting for a small proportion of the total antibodies present in the population. The significance of these results for each of the two diseases is obvious. Both disease systems are resident to the State and exhibit a continuous seasonal endemicity. La Crosse is far more prevalent yearly than SLEV in the human population, while SLEV is most obvious only when it occurs in explosive epidemics as in 1975.

Paul R. Grimstad (University of Notre Dame), Charles L. Barrett, M.D. (Indiana State Board of Health) and Michael J. Sinsko (ISBH)

<u>Preliminary report on new projects</u>:

Behavioral studies currently in progress comparing the blood-feeding pattern of *Aedes triseriatus* in LACV enzootic and non-enzootic areas prompted serologic surveys of woodlots for use as potential study sites. Evaluation of the status of LACV in these woodlots is being accomplished by trapping and bleeding Sciurid rodents and SDNT testing sera against CE group viruses. Trapping in a single 10ha woodlot, which will be used as a non-enzootic site, yielded 37 chipmunks, 10 red squirrels, and 3 fox squirrels, all negative for antibody to LACV, JCV, and TVTV. A search for other non-enzootic and enzootic woodlots, both rural and urban, is in progress.

Other serosurveys under way in northern Indiana include testing a variety of serum samples provided by a local fur trapper. Of 11 samples from red fox, 3 were JCV antibody positive and 1 TVTV antibody positive. Also, serum samples provided by the Indiana Department of

Natural Resources Wildlife Disease Section drawn from deer in an area with a high human antibody prevalence rate for JCV have been tested. In an initial survey, 32% of sera drawn from 31 deer were JCV antibody positive.

In conjunction with the study of *Ae. triseriatus* bloodfeeding patterns, we are attempting to determine if engorged mosquitoes were infected with LACV prior to the blood meal, or if the bloodmeal was taken from a viremic host, and the species of that host. Sensitive virus assays utilizing minute quantities of reagent are thus necessary to allow multiple tests to be run on an individual mosquito. For this purpose, techniques for LACV detection involving observation of CPE in vero cells grown in 96-well plates are being developed. Virus identification techniques under study include injection of the suspected virus suspension into *Toxorhynchites* spp. mosquitoes (we have 5 species in colony at present) followed by screening head squashes using direct immunofluorescence. Verification of virus identification will be by CF or SDNT/PRNT using whole body *Toxorhynchites* preparations.

Roger S. Nasci and Paul R. Grimstad

Figure 1. SLEV antibody prevalence rates per county (summary of 367 antibody positive samples).

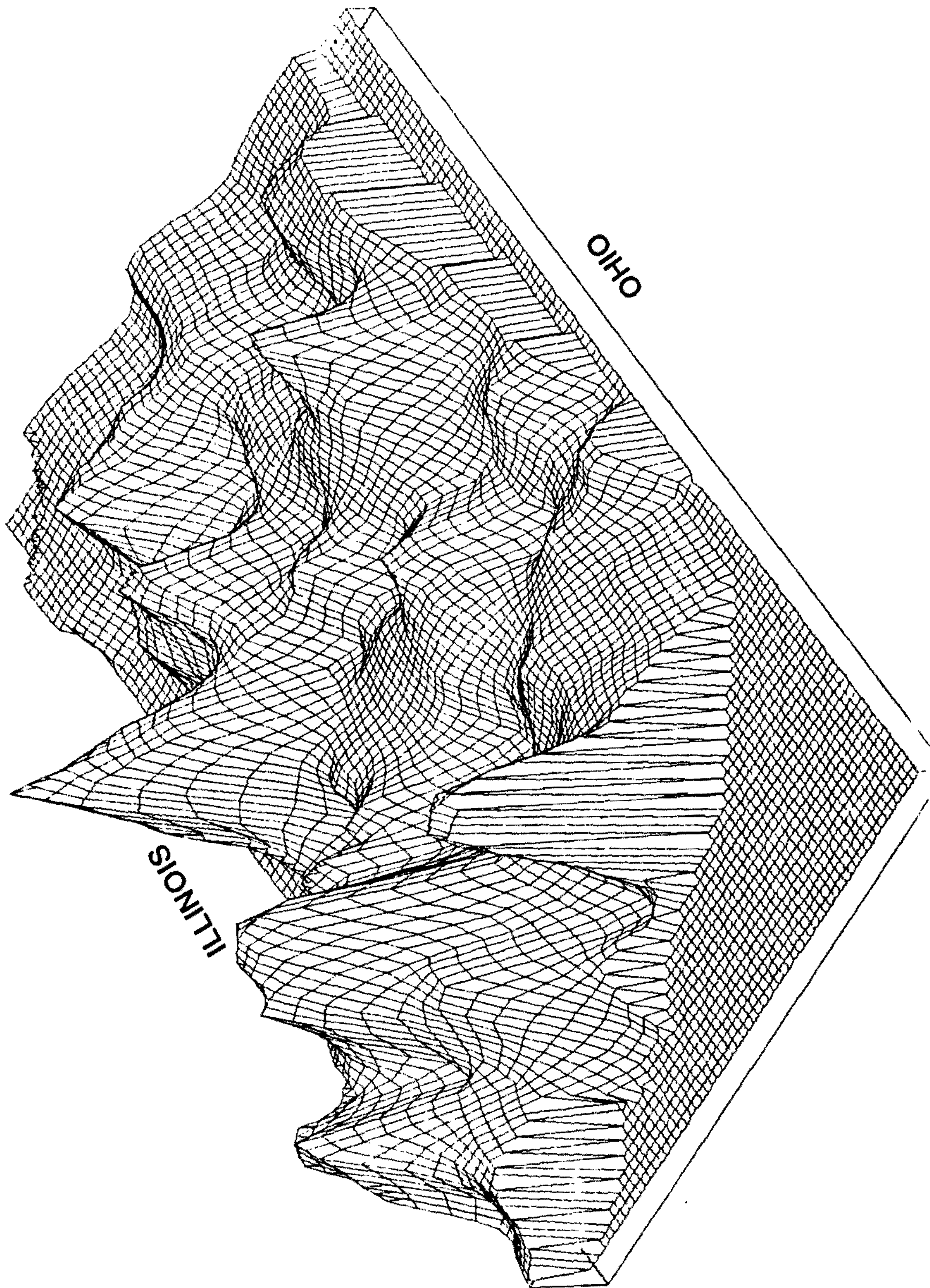
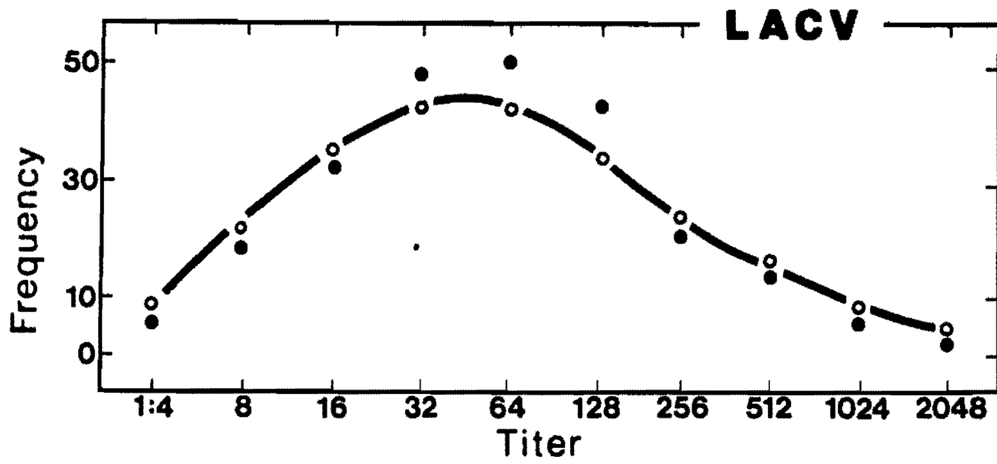


Figure 2. Frequency of observed SDNT titers for human samples positive for antibodies to LACV or SLEV. (open symbols connected by solid line is expected value, solid symbol is observed value. LACV fit is to the Poisson distribution, SLEV to the negative binomial distribution).



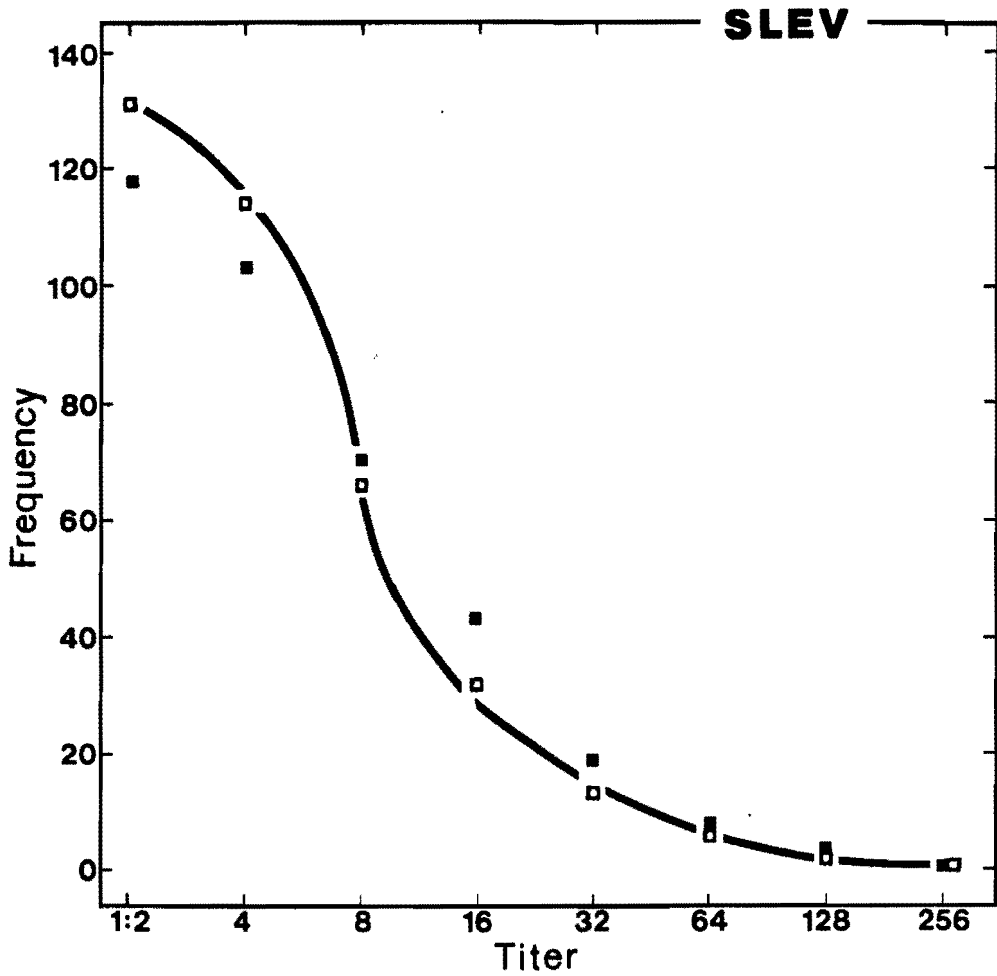
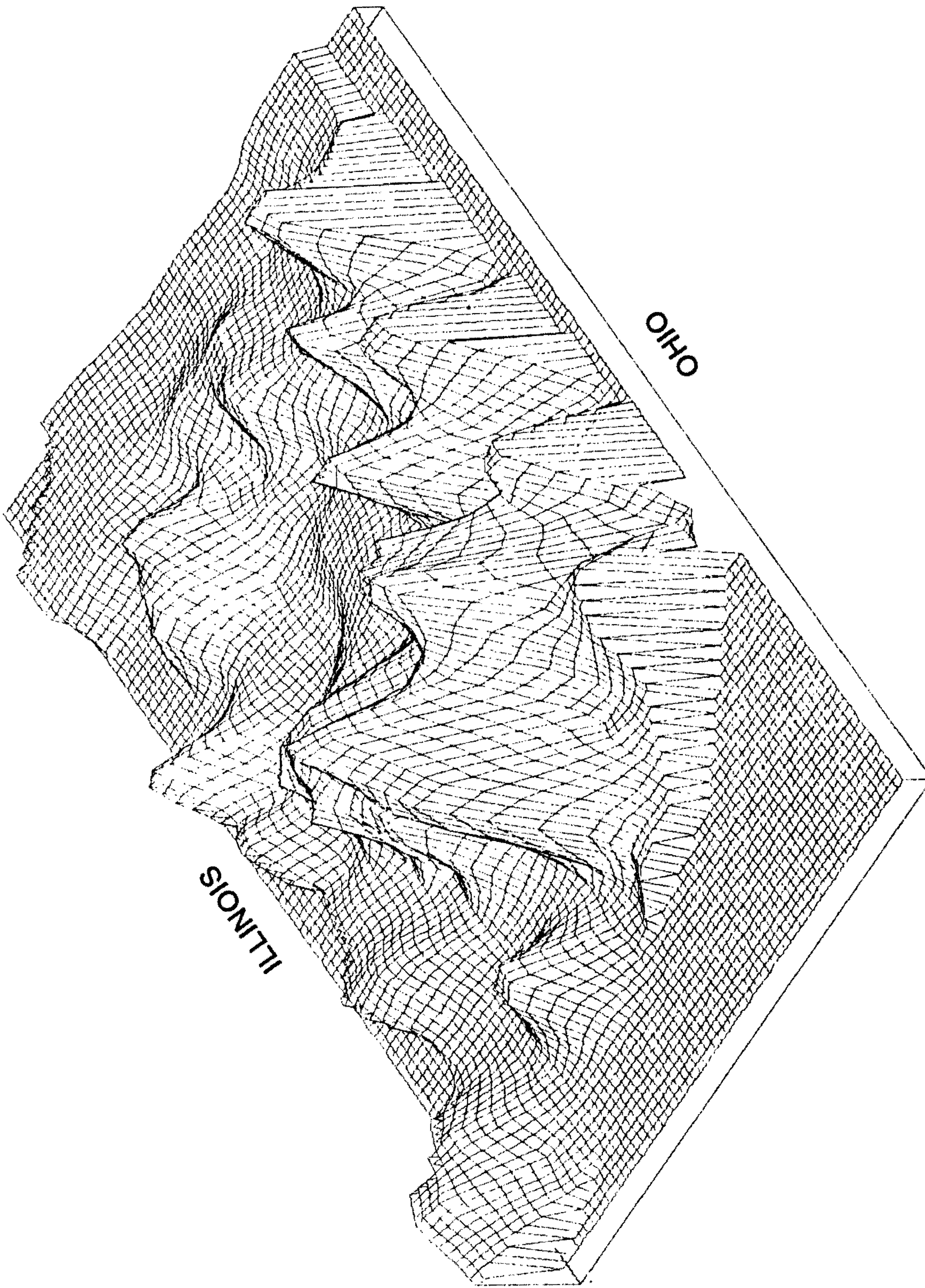


Figure 3. LACV antibody prevalence rates per county (summary of 239 antibody positive samples).



REPORT FROM THE ARBOVIRUS SURVEILLANCE PROGRAM

Division of Laboratories
Illinois Department of Public Health
Chicago, Illinois

For the second consecutive year, no human St. Louis Encephalitis (SLE) cases were identified in Illinois. During the period following the 1975 epidemic of SLE, we have seen an annual decrease in the percentage of juvenile birds, primarily house sparrows (*Passer domesticus*), with HI antibodies to SLE virus (Table 1). When compared with the corresponding number of human cases we are, thus far, reasonably confident of our ability to detect general changes in enzootic SLE virus activity.

However, in the absence of SLE, we continue to see annual recurrence of California Encephalitis (due to LaCrosse virus) in Illinois children. Of the 11 cases confirmed in 1979, the first three were of particular interest because of their early dates of onset, May 31 to June 7. From our experience, it appears that these children were probably infected by transovarially-infected female *Aedes triseriatus*.

Five of the cases were residents of Peoria County where almost 50 percent of all Illinois cases have been identified. In June, 1979, indicator rabbits for HI antibodies to California group viruses (LAC antigen) were placed at 10 sites around Peoria County. They were bled at 17 weekly intervals between June 1 and September 29. HI testing revealed that rabbits at seven sites seroconverted between June 8 and September 8. Comparative neutralization tests performed by Dr. Wayne Thompson at the University of Wisconsin Zoonoses Research Laboratory confirmed that these infections were due to LAC virus and not Jamestown Canyon, Snowshoe Hare, or Trivittatus viruses. In addition to detecting an additional seropositive rabbit, NT antibodies were apparent about 1 week prior to HI antibodies in five of the seven HI positive rabbits.

The rabbit with NT antibodies present on June 8 was located in our Forest Park study area where in 1978 eight strains of LAC were isolated from *A. triseriatus* larvae collected from six of 50 individually-marked treeholes. From April through September, 1979, samples of larvae were again collected from all tagged trees in the park. Preliminary results have yielded nine tentative isolates of LAC from six separate treeholes. Of particular interest to us was the recurrence of virus in four of the treeholes that were positive in 1978.

Testing is also in progress on eggs of *A. triseriatus* collected in ovitraps placed at 15 sites in Peoria County. Some sites were adjacent to rabbits that seroconverted while others were placed in areas where no rabbits or information on virus transmission was available. A similar project emphasizing ovitraps is in progress in forested areas of Cook County (just south and southwest of Chicago) where little evidence of LAC transmission is available.

(Gary G. Clark and Harvey L. Pretula)

Table 1. HI Antibodies to SLE Virus in Juvenile Birds and Humans, Illinois, 1976-1979.

Year	Juvenile Birds*	Confirmed Human Cases **
1976	41/1966 (2.0)	19
1977	55/3425 (1.6)	2
1978	23/3373 (0.7)	0
1979	2/2939 (0.1)	0

* No. positive/No. tested (Percent positive). "Positive" equals 1:20 titer or greater.

** Four-fold or greater rise on simultaneously tested specimens.

REPORT FROM THE VECTOR-BORNE DISEASES DIVISION, CENTER FOR DISEASE CONTROL, FORT COLLINS, COLORADO 80522

Experimental Transmission of Rocio Virus by Mosquitoes

Studies on the experimental transmission of Rocio virus by mosquitoes are continuing in our laboratory with the objectives of (1) screening potential mosquito vectors from the United States in order to help assess the risk of epidemics occurring in this country should the virus be introduced, and (2) obtaining information which may help clarify the role of certain mosquito species in the natural transmission cycle in the epidemic zone (São Paulo State, Brazil). We have evaluated the susceptibility of a variety of mosquito species and strains to infection *per os*, measured virus infection and transmission rates, determined the virus content of infected mosquitoes, and obtained information on the growth pattern of Rocio virus in some of the species and strains. Some of these data are summarized in Table 1.

On the basis of data accumulated thus far, we have classified the mosquitoes tested according to vector potential by assigning them to one of three categories. *Culex tarsalis* from Colorado and Arizona, and *Cx. p. pipiens* from Illinois are relatively efficient experimental vectors. Both speices were readily infected by feeding on infected chicks and high proportions (92% and 71% respectively) were able to transmit virus on the 20th day of extrinsic incubation. Tennessee *Cx. pipiens* ssp. and Argentina *Cx. p. quinquefasciatus* are moderately efficient experimental vectors. Both strains were readily infected (94% to 98%); however, transmission rates were low (36% and 23% respectively). Louisiana *Psorophora ferox*, and *Cx. nigripalpus* and *Cx. opisthopus* from Florida are relatively inefficient experimental vectors. Either infection rates were low or virus did not grow to high titer in those individuals that became infected, or both.

The relative inefficiency of Louisiana *Ps. ferox* as an experimental vector of Rocio virus raises a question about the significance of the single virus isolation from this species in Brazil. However, we are well aware that intraspecific differences in vector efficiency may occur among different geographic strains of *Ps. ferox*. We are planning to assess the vector efficiency of *Ps. ferox* from the Rocio virus epidemic zone in Brazil.

(Carl J. Mitchell, Thomas P. Monath, and C. Bruce Cropp)

Table 1. Rocio Virus Infection Rates in Mosquitoes and Transmission Rates to Chicks

Mosquito Species and Strain	Virus Titer of Infective Meal*	Days Extrinsic Incubation	Infection Rates Ratio**	Infection Rates %	Trans. Rates Ratio***	Trans. Rates %
Cx. tarsalis						
Colorado	6.8-6.9	11	9/10	90	-	-
	6.8-7.5	19-20	10/10	100	Refused to refeed	
Arizona	3.9-4.1	20	2/40	5	1/1	100
	5.1-5.9	20	21/40	53	11/12	92
Cx. p. pipiens						
Illinois	5.9	11	8/10	80	-	-
		20	17/24	71	12/17	71
Cx. pipiens ssp.						
Tennessee	6.6-6.9	13	24/30	80	8/24	33
		20	45/46	98	16/45	36
Cx. p. quinquefasciatus	7.0	13	28/30	93	0/15	0
Argentina		20	30/32	94	7/30	23
Cx. nigripalpus						
Florida	4.2-4.7	14-28	1/61	2	-	-
	8.1-8.2	20	4/4	100	Refused to refeed	
Cx. opisthopus	5.5-5.6	20	1/57	1	-	-
Florida						
Ps. ferox						
Louisiana	4.9-5.9	20	4/97	5	1/3	33
	6.0-6.9	19-20	7/35	20	1/2	50
Ae. aegypti	5.1-5.9	20	1/5	20	0/1	0
Louisiana						

* Pre-and post-feeding titers expressed as log_{10} Vero PFU/ml.

** Number of mosquitoes that contained virus/number of mosquitoes tested.

*** Number of mosquitoes that transmitted virus/number of infected mosquitoes that refed.

INDIRECT FLUORESCENT ANTIBODY TEST FOR YELLOW FEVER SERODIAGNOSIS

Standard diagnostic tests for yellow fever (YF) antibodies (HI, CF, N) are useful, but they require specialized reagents, are not well-suited for performance under field conditions, cannot distinguish between antibodies in the different immunoglobulin classes, and require one or more days to complete.

Recently we evaluated the indirect FA technique as a diagnostic method. Sera were obtained from YF patients during the 1978-1979 outbreak in the Gambia. We also tested sera from vaccinees before and 25 days after immunization with 17D vaccine.

The test employed spot slides of Vero cells infected with YF and heterologous African flaviviruses. Commercially prepared fluorescein-conjugated goat antisera to human IgG and IgM were used. Results were compared with those of HI, CF, and plaque-reduction N tests.

Patients with primary YF infections had high IgM and IgG YF antibodies, without heterologous cross-reactions. A typical example is shown in Table 1A. In this and similar cases, indirect FA test with anti-IgG was more sensitive than CF for the detection of antibody and showed specificity comparable to CF and N tests.

Individuals with prior flavivirus experience had broadly cross-reactive IgG fluorescent antibodies (Table 1B), which appeared to correlate more closely with HI than with other tests. IgM antibodies were present in some, but not all, such cases as late as 4 weeks after onset.

IgM fluorescent antibodies were highly specific, even in cases of YF flavivirus superinfection.

17D vaccinees without prior flaviviral exposures rarely developed detectable IgM or IgG fluorescent antibodies; in 80% of persons with pre-existing heterologous immunity, a rise in IgG antibodies was demonstrated.

The indirect FA test shows considerable promise as a rapid diagnostic test for use during on-site epidemiological investigation of YF epidemics.

(T.P. Monath, C.B. Cropp, C.H. Calisher, D.J. Muth)

Table 1. Serologic Studies of Yellow Fever Patients in the Gambia

A. Primary infection with yellow fever. S_1=2 weeks after onset; S_2=6 weeks.

Test		Titer by Virus													
		YF (17D)		Zika		West Nile		Uganda S		Usutu		Koutango		Banzi	
		S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
IFA	IgM	≥64	64	<4	<4	<4	<4	<4	-	<4	-	<4	-	<4	-
	IgG	128	128	<4	<4	8	<8	<8	<8	<8	<8	<8	<8	<8	<8
HI		≥1280	640	10	10	10	<10	40	20	40	20	10	10	160	80
CF		8	32	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8
N		640	640	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

B. Superinfection with yellow fever. S_1=4 weeks, S_2=11 weeks.

Test		Titer by Virus													
		YF (17D)		Zika		West Nile		Uganda S		Usutu		Koutango		Banzi	
		S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
IFA	IgM	≥128	<8	<8	<8	<8	<8	8	<8	8	<8	16	<8	8	<8
	IgG	256	512	≥256	128	≥256	128	128	16	≥256	32	512	8	512	64
HI		≥1280	640	≥1280	≥1280	≥640	≥640	≥1280	≥1280	≥1280	≥1280	≥1280	≥1280	≥1280	≥1280
CF		32	512	<8	<8	64	8	<8	<8	<8	<8	<8	<8	<8	<8
		320	80	≥320	≥320	<10	<10	160	10	320	80	10	<10	40	10

REPORT FROM THE VIRAL AND RICKETTSIAL DISEASE LABORATORY
CALIFORNIA DEPARTMENT OF HEALTH SERVICES, BERKELEY, CA

ARBOVIRUS SURVEILLANCE, 1979

As usual, our surveillance studies in 1979 were correlated with the work of local mosquito abatement districts (MADs); County Health Departments; the University of California, Berkeley, School of Public Health, Arthropod-Borne Virus Research Unit (UCBSPH,AVRU); private physicians and veterinarians; California Department of Food and Agriculture; and other concerned individuals and agencies. Despite economic limitations on the extent of field and laboratory work which was possible, a useful surveillance program was accomplished. Special assistance by the UCBSPH,AVRU was provided via supplemental field collections and a microbiologist who did much of the mosquito testing and sentinel chicken serologic tests during the summer period.

During 1979, approximately 300 patients throughout California were tested by the State and County virus laboratories for western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE) and other possible causes of encephalitis/meningitis (herpes, mumps, leptospirosis, enteroviruses, etc.). As usual, a selection of those cases for which the etiology could not be determined by standard tests will be subsequently tested by the UCBSPH,AVRU for antibody to other arthropod-borne viruses besides WEE and SLE. There were 12 human brain samples and 2 human cerebrospinal fluid samples tested in suckling mice for arboviruses, but all were negative.

Only one human case of WEE was found in 1979: a 63 year old woman from Sacramento County, who became ill about September 7 and was hospitalized 8 days later because of vomiting, fever, cranial nerve abnormalities, and progressive aphasia and mental changes. Recovery was slow. Only a single serum sample taken October 1 was available for testing. A complement-fixing (CF) WEE antibody titer of 1:32, an indirect fluorescent antibody (IFA) WEE titer of 1:128, and an IgM-specific IFA WEE antibody titer of 1:32 were shown, indicating recent infection, even though complete serologic studies on paired sera could not be done.

Two persons were found to have rather high but stationary levels of SLE antibody by CF, IFA, and neutralization tests, but no SLE-Specific IGM/IFA antibody, thus indicating past infection only (a 41 year old man from Red Bluff and a 51 year old woman from the Sacramento area). A 1-1/2 year old boy hospitalized with meningitis in a Mexicali hospital in July had paired sera and a rectal swab submitted via the Imperial County Health Department. The rectal swab yielded an isolate of ECHO-type 3 virus, but paired sera also showed high levels of SLE-specific CF, neutralizing, IFA, and IFA-IgM antibody, indicating recent SLE infection. It was not clear whether his disease was due to SLE virus, ECHO-3 virus, or both, but SLE virus infection clearly had occurred during the past year in Mexicali.

There were 74 equines tested serologically for WEE during the season, and 18 equine brain samples were tested in suckling mice (no arboviruses were isolated). There were 18 equines considered positive or presumptive-positive for WEE, based on rising or high-stationary CF and IFA antibody titers. Additional tests (hemagglutination-inhibition method) on these equines and the above-mentioned human sera are in progress by the UCBSPH,AVRU, to help complete the serologic studies. The equine cases occurred in the following counties: Sacramento (4), San Joaquin (3), Stanislaus (2), and Imperial, Butte, Shasta, Kern, Yolo, Madera, Solano, Fresno, and Modoc (1 each).

In total, 79,885 mosquitoes (1,812 pools) were tested for viruses, almost entirely representing only 4 species (Culex tarsalis, Culex pipiens complex, Culex peus, and Aedes melanimon). There were 192 virus isolates identified: 113 WEE, 36 Turlock, 29 SLE, 9 Hart Park, 4 California encephalitis group, and 1 Bunyamwera group.

The State Virus Laboratory also participated in a chicken flock serologic surveillance program during 1979, in collaboration with the UCSPH,AVRU involving 15 flocks in the Sacramento Valley, 12 in the San Joaquin Valley, and 4 in Southern California. The birds were bled monthly from June through October, and seroconversions to both SLE and WEE viruses were demonstrated in all 3 regions, by both the plaque-reduction neutralization method and the IFA method, which was being evaluated for the first time for this purpose. The IFA method is rapid and can be performed on a timely basis. Despite some problems with non-specific results at low serum dilutions, which must be worked out, the IFA method appears to be sufficiently sensitive and accurate for use as a serologic surveillance tool in the future.

A special effort was made this year to provide more timely and current information on the results of the surveillance program to interested groups. Besides prompt telephoned reports to the MADS directly involved of positive mosquito pools, human and horse cases, and chicken seroconversions, a weekly summary report was prepared and mailed to all groups (19 issues from June 1 to October 19).

(R.W. Emmons)

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

Detection of Antibodies to California Group Viruses by means of Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA technique has been applied to many virus groups but only recently to arboviruses, e.g. Tick-borne Encephalitis virus (Hofmann *et al.*, 1979), Semliki Forest virus (Oram and Crooks, 1979) and other alphaviruses (Frazier and Shope, 1979). This report describes a modified ELISA test for detecting antibodies to members of the California group of arboviruses.

Virus grown in Vero cell monolayers was concentrated by polyethylene glycol precipitation, inactivated with beta-propiolactone or Tween 80-ether and used as antigen in the test. A preparation of uninfected Vero cell culture was included in the assay as control antigen. Wells of microtitre plates were coated with antigen by overnight evaporation at room temperature. Phosphate buffered saline containing 1% bovine serum albumin fraction V and 0.05% Tween 20 was both diluent and wash fluid in the procedure. The indirect ELISA technique was applied using peroxidase as enzyme and ortho-phenylenediamine as substrate. Human, rabbit and hamster sera, as well as mouse ascitic fluids, were tested. Pre-treatment of sera was not necessary. Antibodies from animal sources were detected with anti-IgG conjugates while those from human sera were assayed against anti-IgG and anti-IgM conjugates. Reference negative and positive samples were included as controls. Blocking tests confirmed the specificity of the reaction.

A comparison of ELISA with haemagglutination-inhibition (HAI) and neutralization (NEUT) tests was carried out with 180 coded human sera. Results (Table 1) showed correlation between ELISA and the other methods at serum dilutions of 1:80 or higher. Some false positive ELISA reactions resulted when sera were tested at 1:40 or lower dilutions. The remaining 115 sera were negative by all three techniques.

Preliminary tests showed that serotypes Snowshoe Hare (SSH), La Crosse (LAC) and Trivittatus (TVT) could be distinguished by ELISA (Fig. 1). Further studies are in progress.

TABLE 1.

SERUM	ELISA POS.	NEUT POS.	NEUT NEG.	HAI POS.	HAI NEG.
1:10	23	0	23	2	21
1:20	7	2	5	4	3
1:40	12	3	9	4	8
1:80	3	3	0	3	0
1:100	7	5	2	6	1
1:200	8	8	0	8	0
1:400	3	3	0	3	0
1:800	1	1	0	1	0

References

Hofmann, H., Frisch-Niggemeyer, W. and Heinz, F. 1979. J. Gen. Virol. 42: 505-511.

Oram, J.D. and Crooks, A.J. 1979. J. Immun. Methods 25: 297-310.

Frazier, C.L. and Shope, R.E. 1979. J. Clin. Microbiol. 10: 583-585.

(P. Glaister, H. Artsob, L. Spence)

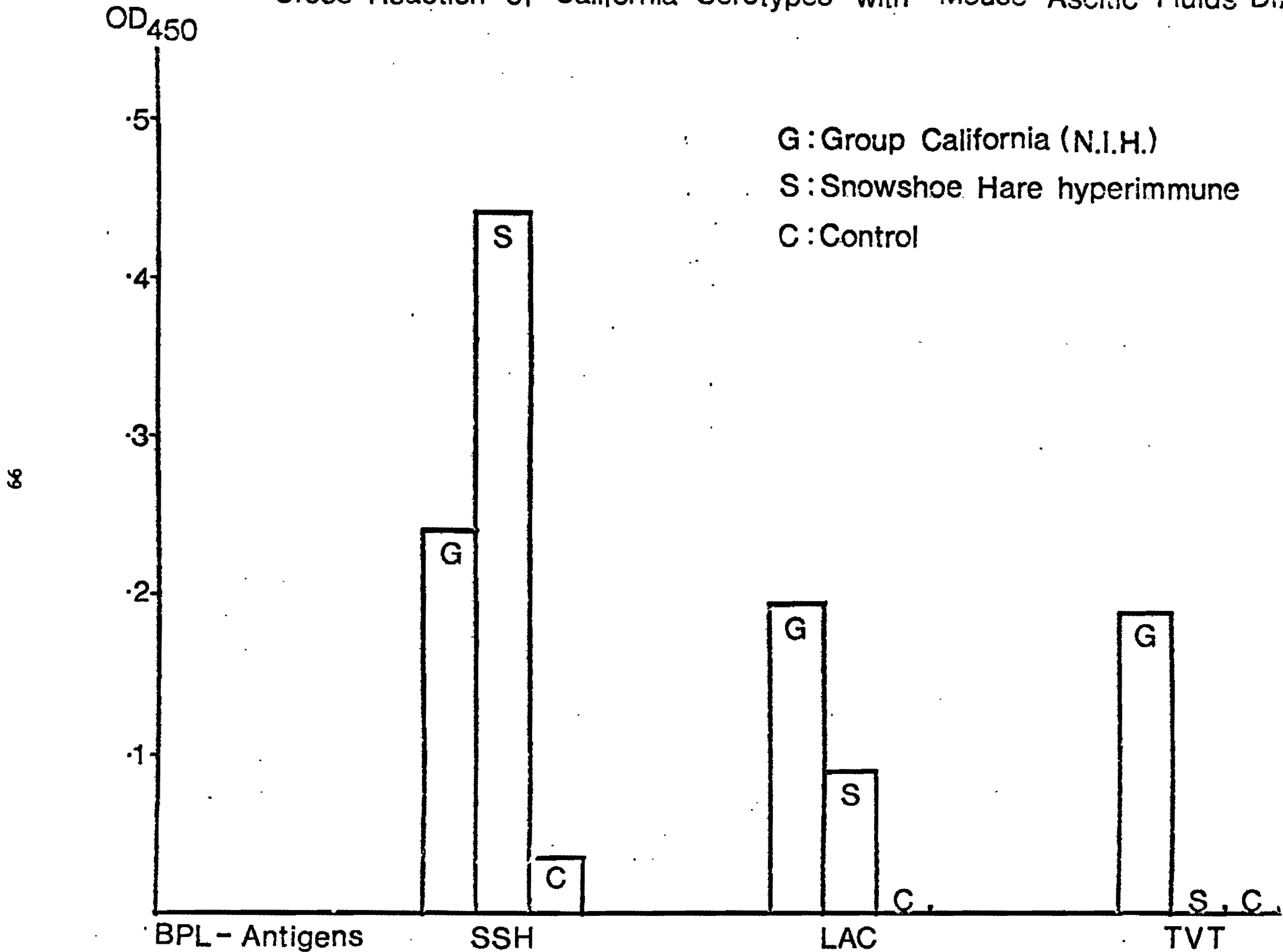


Figure 1

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY, UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, B.C. V6T 1W5, CANADA.

Bunyavirus isolates from Yukon mosquitoes collected during July 1978 (snowshoe hare, SSH strain 78-Y-133 and Northway, NOR strain 78-Y-284), in their second suckling mouse brain passages, were injected intrathoracically into laboratory-bred *Aedes aegypti* mosquitoes in decimal dilutions from 100 to 0.1 PFU for SSH and 10 to 0.01 PFU for NOR. After incubation of mosquitoes at 23 and 32°C for as long as 27 days evidence of virus replication was sought at weekly intervals by examination of head squashes and halves of salivary glands for virus antigen by indirect immunofluorescence, and in replicate halves of salivary glands by infectivity titrations in monolayers of baby hamster kidney (BHK-21) cells by the plaque method. For both SSH and NOR, virus titers were somewhat higher (3.2-3.7 log PFU per gland) at 6 days than after longer periods of incubation (2.1-3.0 log PFU per gland). Rates of visualization of SSH and NOR antigens in head squashes and salivary glands approximated each other at 66 to 90%, whilst rates of detection of virus infectivity were somewhat greater at 80 to 100%. Feeding of 100-1000 PFU NOR virus in blood meals has been followed by virus replication after incubation at 23 and 32°C, in contrast to lack of viral replication after ingestion of smaller virus doses in blood meals.

(D.M. MCLEAN)

REPORT FROM THE PACIFIC RESEARCH UNIT
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Ross River virus infection (epidemic polyarthritis) in American Samoa

In August of 1979, physicians in American Samoa began to observe cases of an illness characterized by headache, myalgia, polyarthralgia and rash. Initially, the disease was thought to be dengue but paired sera submitted to the Center for Disease Control, Fort Collins identified the probable etiologic agent as Ross River (RR) virus.

In mid-December of 1979, a team from the Pacific Research Unit, Honolulu and the Center for Disease Control, Fort Collins went to American Samoa to study the continuing epidemic. Blood specimens for virus isolation were obtained from 16 patients with symptoms compatible with epidemic polyarthritis. One of these samples yielded a virus, subsequently identified as RR. Most of the acute sera from the polyarthritis patients had neutralizing antibodies - which probably explains the low virus recovery rate.

Serum specimens for antibody determinations were collected from a sample of the human population on Tutuila island. Those sera were screened at 1:10 dilution by plaque reduction neutralization test against the Samoan RR virus isolate. The neutralization test results are summarized by age group and sex in Table 1. Overall, 43.8% of the sera had RR virus neutralizing antibodies. All age groups were affected, although the antibody prevalence in males was slightly higher than in females.

A sample of 100 sera, collected from adult residents of American Samoa during a dengue outbreak in 1972, was also examined for RR virus antibody. All of these specimens were negative, suggesting that the virus had not occurred in Samoa during the preceeding 50 or 60 years. The similar antibody rates among the various age groups (Table 1) also support this view.

A survey of mosquito populations was made to assess the distribution and local abundance of Samoan species during a period of active transmission. Ten of the 11 species previously recorded from Tutuila were collected. Larval sites of *Culex annulirostris* (a suspected vector of RR virus in Australia) were found, but the species was not common or abundant at the time and it apparently feeds infrequently on man in Samoa. RR virus is known to replicate in *Aedes aegypti*, but populations of this mosquito were small. By far the most common and abundant mosquitoes were *Aedes polynesiensis* and *Aedes oceanicus* (a member of the *Aedes kochi* group). The former was the most important day-biting mosquito, while *Ae. kochi* group mosquitoes are apparently important nocturnal man-biters. Vector competence of *Ae. polynesiensis* and *Ae. oceanicus* for RR virus is unknown. *Aedes vigilax*, a suspect vector of RR virus in Australia and Fiji, has never been collected in American Samoa.

We suspect that the virus was introduced into American Samoa by an infected person coming from Fiji, where a large epidemic of polyarthritis occurred earlier in 1979. Further studies on the possible mosquito vectors and animal reservoirs of RR virus in Samoa are now in progress.

Until 1979, the known geographic distribution of RR virus was thought to be restricted to Australia, New Guinea and the Solomon islands. The recent epidemics in Fiji and American Samoa indicate that the virus is capable of spreading to new areas and could be introduced into other South Pacific islands.

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and

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Table 1

Prevalence of Ross River virus neutralizing antibody among residents of American Samoa, December 1979

Age group (years)	Male		Female	
	Number tested	Percent positive	Number tested	Percent positive
0-9	24	20.8	25	12.0
10-19	15	66.7	20	45.0
20-29	14	71.4	50	36.0
30-39	19	73.7	27	33.3
40-49	24	50.0	29	48.3
50-59	27	48.2	44	34.1
60-69	35	62.9	14	78.6
≥70	12	33.3	14	21.4
Total	170	52.9	223	36.8

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUT PASTEUR DE NOUMEA - NEW CALEDONIA

Surveillance of dengue

New Caledonia

As previously reported, strains of dengue type 4 were isolated in March 1979, from imported and indigenous cases observed in Noumea. In the past, epidemics were caused alternatively by serotypes 1 and 2 : dengue type 1, in 1943-44, affected allied troops stationed during the Coral Sea battle ; type 2 was responsible of an explosive outbreak from September 1971 to August 1972 ; type 1 was reimported in 1975 and was the lone serotype identified till March 1979. Though the arrival of a new immunological type coincided with the seasonal peak of transmission, it was not followed by a rise of the number of positive (or suspected) cases :

month	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
positive cases	0	1	11	10	5	4	1	0	5	3	1	7

At the fall of November, clinical cases were seen in Thio, on the east coast of New Caledonia. Serological tests confirmed 8/9 suspect cases ; three virus isolations were obtained.

New Hebrides

In September 1979, a dengue infection was suspected in an european man of 45, with signs of meningitis ; after a transfer into Noumea Hospital, eosinophilic meningitis was diagnosed through cerebrospinal fluid examination ; HI tests on paired sera taken at the onset and twelve days later, showed a significant rise from ± 10 to 80, of antibodies reacting with dengue 1 antigen. Patient's serum was toxic for mice and mosquitoes inoculated for virus isolation.

Wallis Island

Clinical cases appeared in September, three years after a small outbreak confirmed by serological tests. Blood samples from 79 patients were examined at the fall of December. On 47 paired sera, results of HI tests may be interpreted as 7 primary responses, 35 secundary responses and 5 non significant. On 32 unique specimens, 5 showed antibodies titers over 1280 and 7 between 160 and 1280. Two strains were isolated by inoculation to baby mice.

Hoorne Islands

Blood specimens from 19 patients were collected in Futuna, during a small outbreak of a dengue-like disease. On 12 pairs of sera, there was no significant result, using dengue 1 antigen, though each sample showed antibodies. Results of HI tests, using Sindbis and Ross River antigens, were also negative excepted for one pair where the titer was 10 for both sera, against Ross River antigen. Four strains were isolated, paralyzing baby mice in five days at the 5th passage. One of them is an alphavirus. Identification pending.

Survey of Ross River virus infections in New Caledonia

Each summer and autumn, Ross River virus is responsible of epidemic polyarthritis in eastern Australia. Anti Ross River virus antibodies were found in people of New Guinea, Solomon Islands and Australia. Recently, about 30 000 cases occured in Fiji, between January and July 1979 ; Many tourists returning to Australia or U.S.A. developed infection at home. The two main vectors, *Aedes vigilax* and *Culex annulirostris* are very common in New Caledonia.

750 serum samples from 527 patients suffering arthritis were screened by HI test using Ross River virus, Sindbis and dengue antigens, with the following results :

	HI Titer (anti Ross River virus)					
1 st serum	< 10	< 10	± 10	+ 10	+ 10	+ 20
2 d serum	< 10	< 10	± 10	+ 10	+ 20	+ 20
Number of patients	214	1	4	2	1	1

Unique sample	< 10	± 10	+ 10	+ 20
Number of patients	295	5	3	1

Cross reactions with Sindbis antigen were seen in 3 cases.

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Ross River virus in Australia. Transplacental transmission of Ross River and Getah viruses has been demonstrated in mice (Fig. 1). In the case of Ross River virus this was associated with slowed weight gain after birth and a high post-partum mortality. We were unable to demonstrate any transplacental transmission with Murray Valley encephalitis virus although infection of mice, during pregnancy, with this virus was associated with significant post-partum mortality if infection took place after establishment of a functional placenta (10th day post conception).

Studies of cell mediated immunity to RRV in man have demonstrated a strong T lymphocyte proliferative response on re-exposure to viral antigen *in vitro*. In epidemic polyarthritis patients peak lymphoproliferation in response to viral antigen occurred during the first 8-10 weeks post onset of symptoms. Levels dropped after that but significant reactivity could still be detected in many patients nine months after onset of symptoms. Natural Killer cell activity in the peripheral blood of polyarthritis patients was depressed for the first 15-20 weeks post onset of symptoms after which it returned to normal levels. Natural Killer (NK) cell activity remained depressed for longer intervals in those patients in which symptoms persisted. Levels of NK cell activity returned to normal about the same time as the patient became free of symptoms.

Forty-seven epidemic polyarthritis patients were also HLA typed at the A, B and C loci. There was no difference between the frequency of these antigens in polyarthritis patients and the normal Australian population.

Studies on the persistence of IgM antibody to Ross River virus in polyarthritis patients has shown IgM antibody to be detectable for up to 3 years in one case. However data obtained from 44 other cases in which the IgM did disappear suggested a persistence of approximately 14 weeks post onset of symptoms.

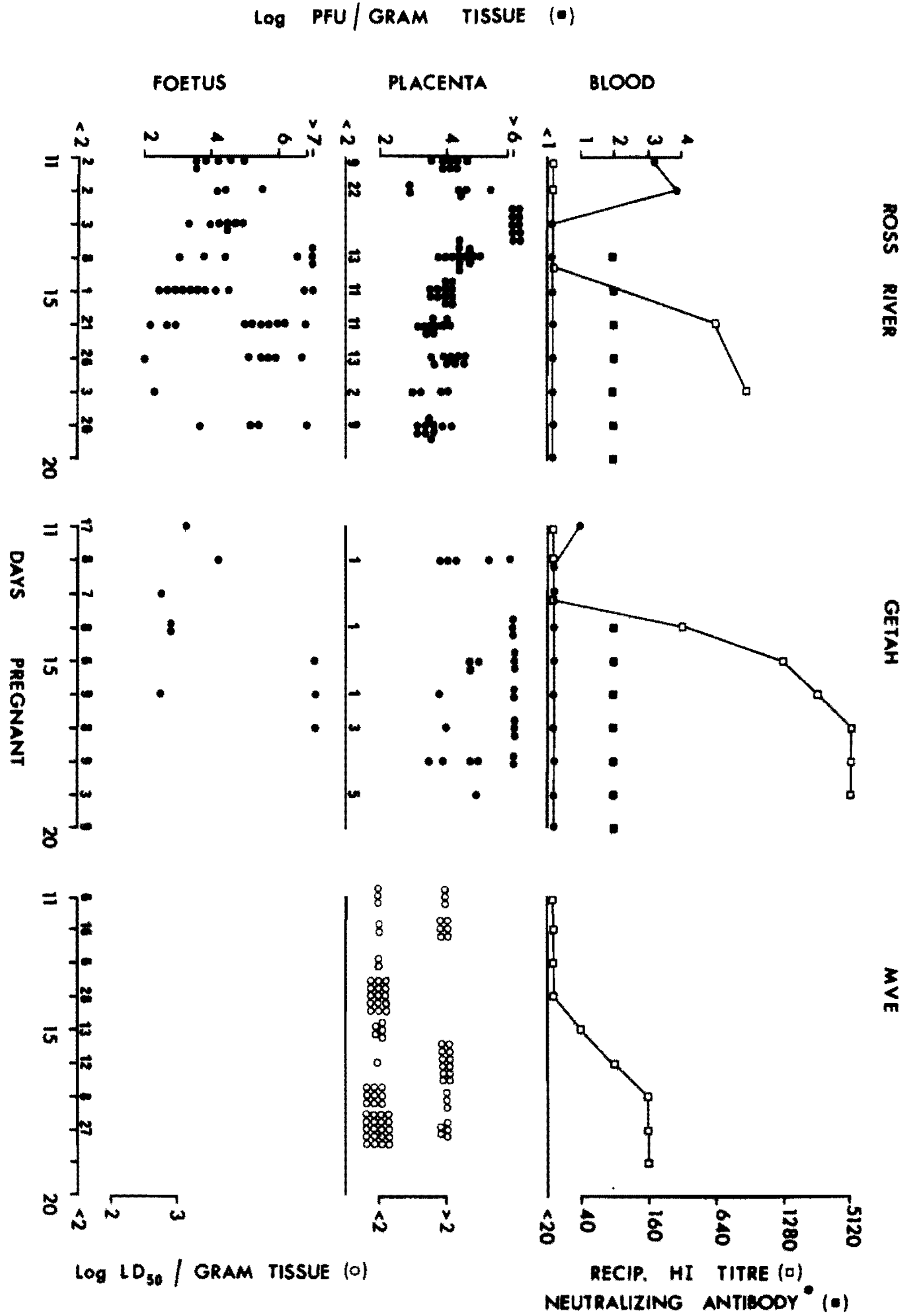
Ross River virus in Fiji. A comparison of HI antibody titres to Ross River virus in sera collected before and after the recent epidemic showed a pre-epidemic incidence of 13% (HI titre >1/20) and a post epidemic incidence of between 58 and 92 percent. The median HI titre pre epidemic was 1/20 and post epidemic 1/160. Neutralization tests on post-epidemic sera showed strong reactivity against Ross River virus but little or none against Getah, Sindbis, Chikungunya or Bebaru viruses.

The pattern of clinical symptoms of polyarthritis occurring principally in adults, in particular adult women, seen in Australia was also found in Fiji. In addition, several Australian tourists contracting polyarthritis in Fiji were studied on return. The cytology of joint effusions, lymphocyte function, persistence of anti Ross River virus IgM and clinical symptoms were all similar to that seen in polyarthritis patients infected with Ross River virus in Australia.

To date maternal and cord blood from 368 pregnancies have been examined for IgM antibody to Ross River virus. Of these, 11 cord bloods contained anti Ross River virus IgM suggestive of *in utero* infection. None of the IgM + babies showed any gross abnormalities.

(John Aaskov)

Figure 1. Transplacental transmission, in mice, of Ross River and Getah but not Murray Valley encephalitis virus.



REPORTS FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF WESTERN AUSTRALIA, PERTH, WESTERN AUSTRALIA, AUSTRALIA.

A. "A Survey of the Pilbara Region of Western Australia following the first known human infection with Murray Valley encephalitis in that Region"

In April, 1978 the first case of human encephalitis caused by infection with Murray Valley encephalitis virus (MVE) was recorded in the Pilbara Region of Western Australia at Port Hedland (see Arbovirus Information Exchange, September, 1978, p. 171). This was the first direct evidence of the activity of flaviviruses outside the Kimberley Region in this State. Previously only alphavirus (Ross River virus) infections were known. The timing of the case fits closely with the known seasonality of activity for MVE in the Kimberleys (Table 1, p. 103, Arbovirus Information Exchange, September, 1979). Following a brief epidemiological investigation of this case, it appeared that MVE had only a very short transient period of activity in the Pilbara (5% of humans tested by HI were positive whilst 60 to 90% were positive from localities in the Kimberleys; sentinal chickens showed similar drops in positivity ratios between the regions).

In 1979 it was decided to survey the whole Pilbara in April to determine (1) What were the parameters of mosquito populations present at this time of the year and (2) How widespread was the activity of MVE in 1978? The localities visited are presented in the accompanying map. Mosquito surveys were conducted in most localities and sentinel animals (chickens and horses) were bled where possible. All serological samples were age-graded and a history of individual movements was kept. In fact, the horses were found to have such varied histories that the interpretation of their serological results is not as clear cut as for the chicken sera.

The results of entomological investigations are presented in Table 1. Although only 30% of the collections are identified, some observations are possible. *Culex annulirostris*, the major vector of MVE was the dominant species in all localities sampled (including Karratha and Roebourne where the proportions were noted even though the samples are not yet sorted). Over 70% of all mosquitoes so far identified are of this species. The most interesting observation is that the majority of breeding sites for *Culex annulirostris* are in the untended overflows and seepages from the municipal sewage treatment lagoons. All these sites are suitable for control by the physical maintenance of levee banks and by instigating drainage procedures.

These sewage lagoons are also important in that they form the largest network of permanent water bodies in the arid Pilbara. As such, they are a focus for migrating birds. In fact, the largest aggregations of waterfowl are found on these treatment lagoons (some have even been classed as fauna sanctuaries to protect the bird life). It is apparent that these lagoons fulfil the major prerequisites for the temporary circulation of arboviruses - high mosquito populations in close association with high numbers of vertebrate hosts. It is also clear that, in the Pilbara, these conditions occur in intimate association with the major population centres.

None of the mosquitoes collected during this investigation have been processed for virus isolation. In 1978, the survey of Port Hedland yielded one arbovirus strain - a single strain of the Anopheles A/B group - PH.88.

Table 2 shows the results of H.I. surveys of chicken sera collected from various localities in 1979. Although some increases in positivity

are evident with increasing age, the differences are not significant (χ^2) and probably reflect the small sample sizes. There were no localities where the sample size of different age groups was sufficient to allow detailed comparisons. The differences between localities may reflect differences in the intensity of local transmission.

The main valid observations from these data are that chickens less than 1 year old were all negative, whilst those 1 year and older were between 50% to 90% positive. Thus MVE was active in the Pilbara in 1978 but absent in 1979. The second fact to emerge is that every locality sampled showed that chickens were positive to MVE by H.I. test indicating that MVE was active throughout the Pilbara.

Table 3 gives the results of the serology done on the horses from various localities in the Pilbara. Again there is high positivity by both H.I. and N.T. against both MVE and/or Kunjin viruses. The movements of these animals are so varied that no information can be obtained other than the crude positivity ratios. However, these data reinforce the earlier conclusions from the serology on sentinel chickens.

This year we plan to do a transect study south from the Pilbara to determine the southern limit of the activity of MVE in 1978.

The conclusions one draws from these surveys is that whilst the conditions prevailing during the season of peak transmission would appear conducive to the activity of MVE, the activity is not apparent every year. The exceptional weather patterns in the Kimberley and Pilbara during 1978 allowed the movement of MVE into the Pilbara where it underwent an explosive dissemination.

(P. LIEHNE, A. WRIGHT, N.F. STANLEY, D. BRITTEN, P. PIHU, H. SAMBRAILO, W. JOLLEY).

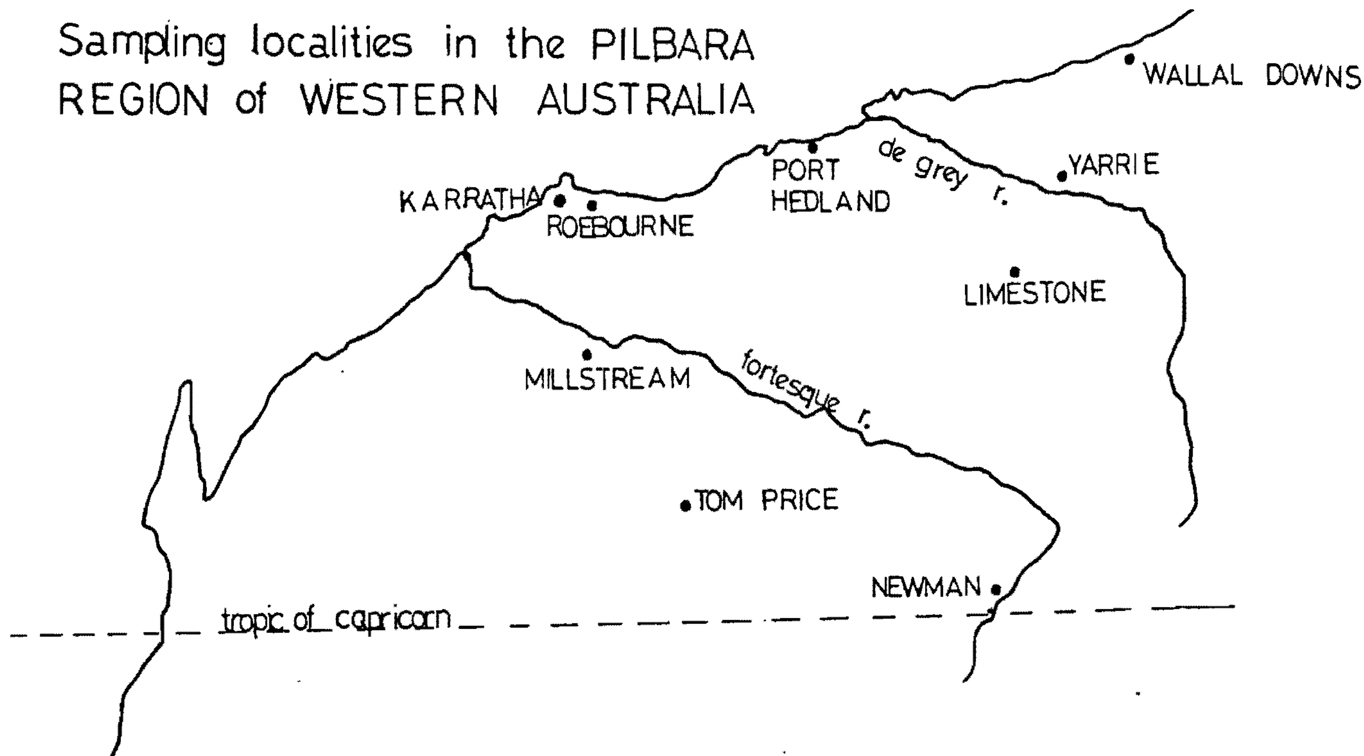


TABLE 1. MOSQUITO COLLECTIONS IN THE PILBARA IN MARCH/APRIL, 1979.

Species	Tom Price	Newman	Marble Bar[1]	Port Hedland	Karratha	Roebourne	Millstream	Totals
Culex annulirostris	125	1652	340	1202			375	3694
Cx. fatigans	36	215	15	21				287
Cx. australicus		28	1	2			3	34
Cx. bitaeniorhynchus							129	129
Aedes tremulus	1	11	4	2				18
Ae. normanensis	2	2		4				8
Ae. pseudonormanensis		119						119
Ae. eidovoldensis		10						10
Ae. vigilax				16				16
Ae. bancroftianus/sp,#85		255	1					256
Ae. alternans		8						8
Anopheles amictus	1	19						20
An. annulipes	16	142	3	1			14	176
TOTAL IDENTIFIED	181	2461	364	1248			521	4775
UNIDENTIFIED, UNSORTED	≈280		≈430	≈400	≈7200	≈275	≈100	≈8685

[1] Marble Bar township is 8 km west of Limestone Station.

TABLE 2:

SEROLOGICAL SURVEYS OF SENTINEL FOWLS IN THE PILBARA COLLECTED IN APRIL 1979. TESTED BY HI FOR FLAVIVIRUS ANTIBODY (AGAINST MVE AND KUNJIN VIRUSES)

Locality	TOTAL TESTED	AGE GROUP <1 year	1-2 yrs.	2-3 yrs	3-4 yrs	>4 yr	TOTAL +VE	%
TOM PRICE	28	0/9	7/19				7	25
ROEBOURNE	13			6/11	1/2		7	54
YARRIE STATION	14	0/4				9/10	9	64
LIMESTONE STATION	17	-	7/12		5/5		12	70
WALLAL STATION	12				9/12		9	75
MILLSTREAM STATION	21		1/1	1/3	13/16	1/1	16	76
TOTAL +VE		0/13	15/32	7/14	28/35	10/11		
% +VE		0%	47%	50%	80%	91%		

TABLE 3.

FLAVIVIRUS (MVE, KUNJIN) SEROLOGY (HI AND NT) OF SENTINEL HORSES BLED IN THE PILBARA IN APRIL 1979

LOCALITY	HI [1]		NEUTRALIZATION [2] [3]			
	NO. TESTED	% +VE	NO. TESTED	% +VE MVE	% +VE KUN	% +VE BOTH
NEWMAN	14	93	12	9	25	66
YARRIE STATION	6	83	6		15.8	50
LIMESTONE STATION	5	60	5			60

[1] HI +ve sera are >20, positivity to either MVE or KUNJIN is recorded as positive

[2] Neutralizations carried out against both MVE and KUNJIN viruses at two serum dilutions (1:10 and 1:20)

[3] Neutralization results presented as % of total tested +ve only against MVE; those +ve only against KUNJIN and those +ve against both viruses.

B. Dry Season Mosquito Captures at Impounded Water Bodies in the Kimberley Region of Western Australia, 1978 - 1979

There are basically three significant impounded water bodies in the Kimberley region of Western Australia; Lake Kununurra and Lake Argyle at the Ord River study area, and 17 mile Dam near Camballin in the West Kimberley. Locations are shown on the accompanying map. 17 mile Dam and the Diversion Dam forming Lake Kununurra were built at approximately the same time (both in 1961) and the margins of the lakes thus formed are heavily vegetated although the successions of plants at both lakes have not yet fully stabilised. These two sites differ in two ways. Firstly, the water level of Lake Kununurra is kept relatively constant for irrigation purposes by manipulation of the water imput from Lake Argyle upstream and output from the Diversion Dam gates. In contrast the 17 mile Dam water level, although controlled to a certain extent for irrigation purposes, is more dependent on the seasonally variable flow of the Fitzroy River. Secondly the margins of Lake Kununurra are the natural levee banks of the Ord River which are breached in several places to form extensive shallow swamplands. In contrast, the margins of 17 mile Dam are a combination of man-made earth walls and shallow natural slopes. The combined effect of these differences is that of the two lakes 17 mile Dam has a greater seasonal variation in waterline both horizontally and vertically, and the vegetation differs considerably as a result.

Lake Argyle was much more recently formed by the construction of the Main Ord Dam in 1972; the lake itself filled for the first time in 1974. As can be seen from the map Lake Argyle is vastly larger than the other two lakes. Apart from the dam wall its margins are natural - steep rocky mountain faces to the west, and gentle slopes regularly scarred by creek beds to the east and south. The water imput to the lake is strictly dependent on the seasonal monsoonal rains which vary considerably from year to year. Water output is via a man-made, but uncontrolled, spillway and three huge pipes at the dam wall itself. The water output from these pipes can be controlled by their closure. The considerable seasonal variation in water level and the recent formation of the lake itself have meant that marginal vegetation has barely started to establish, let alone stabilise. Submerged growth of weed is extensive in places however, effectively preventing predation of mosquito larvae by the various fish present.

The tabulated results show several features of note:

i) The reduced species diversity at each of the three Lake Argyle sites compared to the other two sites. This is probably attributable to the relative lack of larval habitat diversity at Lake Argyle which in turn is a result of its recent formation.

ii) The considerable similarity between the three Lake Argyle sites, again probably reflecting the lack of larval habitat diversity.

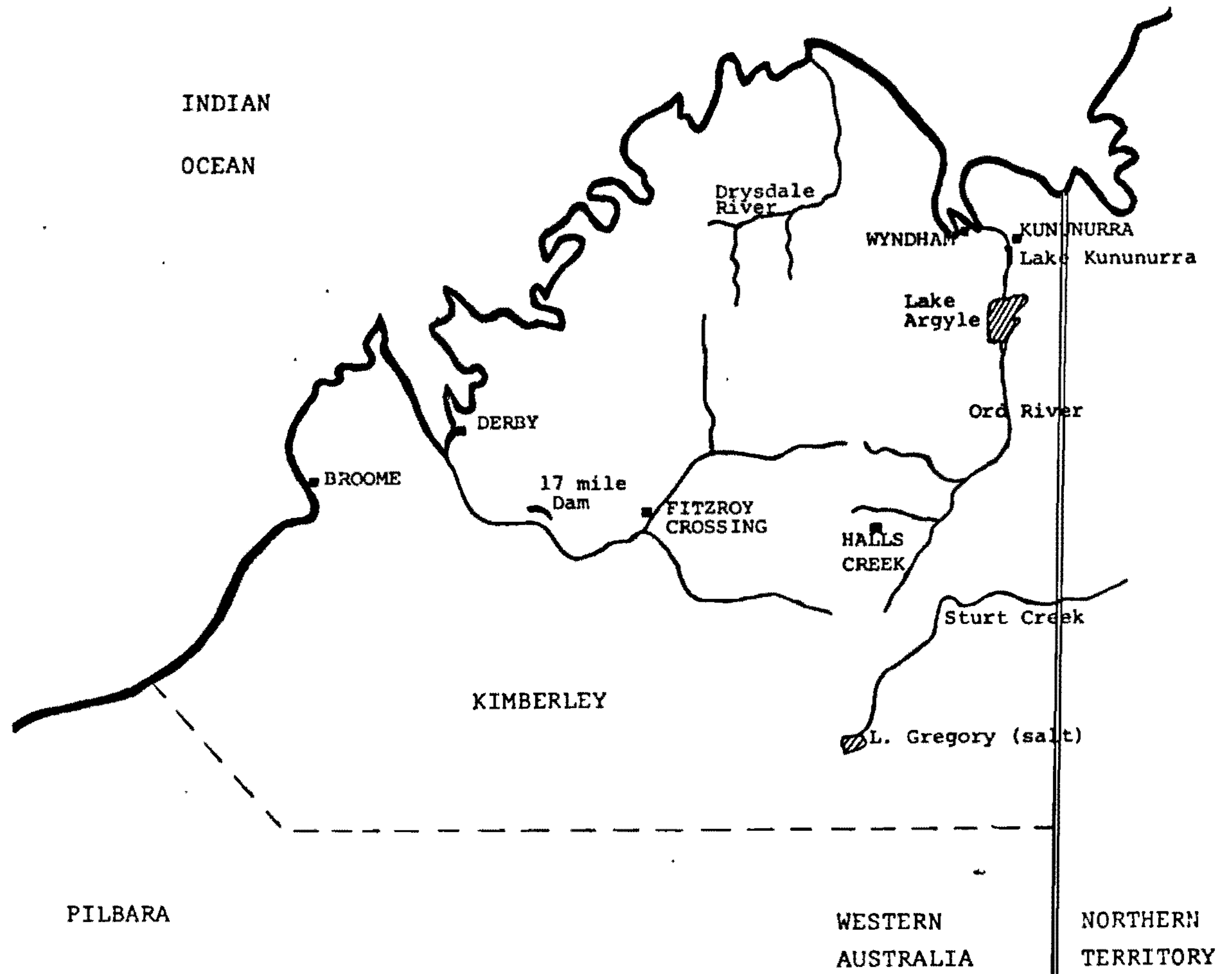
iii) The greater species diversity at Lake Kununurra compared to that at 17 mile Dam. This is probably attributable to the greater water level stability at Lake Kununurra and the greater species diversity in the Ord River drainage area in general, when compared to that of the Fitzroy River drainage area. This difference in turn if probably partially attributable to the fact that the Ord River drainage area has a slightly wetter, monsoonal wet season (15-50" per annum) than that of the Fitzroy River drainage area (10-30" per annum).

iv) The change in dominance from *Anopheles* species (especially *An. annulipes*) at Lake Argyle to the MVE vector *Cx. annulirostris* at Lake Kununurra

and 17 mile Dam. This again can be attributed to the relative suitabilities of the three lakes as larval habitats; *Cx. annulirostris* larvae are nearly always found in shallow freshwater with marginal emergent vegetation, especially grasses, whilst *Anopheles* larvae are generally not so vegetation-dependent.

It should also be noted that 1978 was the first time that mosquitoes of any species were captured in considerable numbers at Lake Argyle, and that mosquito larvae of any species were first noted there in 1976. Furthermore, Lake Argyle waterfowl numbers in the dry season have been observed to increase from mere hundreds in 1976 to up to about 80,000 in 1979. Hence it would seem that ecological stabilisation at Lake Argyle is well under way and warrants our continued monitoring in the future.

(A. WRIGHT, P. LIEHNE, D. BRITTEN, N.F. STANLEY)



Dry season mosquito captures[1,3] at impounded water bodies in the Kimberley region of Western Australia, 1978 - 1979.

Species	SITE				
	Lake Kununurra	17 Mile Dam Camballin	N.E. Lake Argyle	E. Lake Argyle	S.W. Lake Argyle
Aedes (Finlaya) notoscriptus	1 (0.01)	-	-	-	-
Aedes (Macleaya) tremulus	6 (0.03)	3 (0.02)	6 (0.18)	-	-
Aedes (Mucidus) alternans	5 (0.03)	-	-	-	-
Aedes (Neomelaniconion) lineatopennis	7 (0.04)	-	-	-	-
Aedes (Ochlerotatus) normanensis	13 (0.07)	3 (0.02)	2 (0.06)	-	1 (0.01)
Aedes (Ochlerotatus) vigilax	42 (0.24)	-	-	-	1 (0.01)
Aedes (?Subgenus) sp. #160[2]	-	-	1 (0.03)	-	-
Aedeomyia catasticta	413 (2.35)	6 (0.03)	58 (1.74)	39 (0.52)	166 (2.40)
Coquillettidia xanthogaster	90 (0.51)	-	-	-	-
Mansonia (Mansonioides) uniformis	19 (0.11)	-	-	-	-
Culex (Culex) annulirostris	10,746 (61.16)	12,230 (61.32)	187 (5.62)	886 (11.82)	623 (8.99)
Culex (Culex) fatigans	178 (1.01)	-	-	-	-
Culex (Culex) starckeae	-	8 (0.04)	-	6 (0.08)	12 (0.17)
Culex (Culex) ?Normanton sp.[2]	-	28 (0.14)	-	1 (0.01)	-
Culex (Culiciomyia) pullus	14 (0.08)	-	-	-	-
Culex (Lophoceraomyia) fraudatrix annulata	1 (0.01)	-	-	-	-
Tripteroides (Rachionotomyia) punctolateralis	-	1 (-)	-	-	-
Anopheles (Anopheles) bancroftii	561 (3.19)	15 (0.08)	-	-	-
Anopheles (Cellia) amictus	827 (4.71)	274 (1.37)	239 (7.18)	3,054 (40.76)	2,784 (40.17)
Anopheles (Cellia) annulipes	4,645 (26.44)	7,378 (36.99)	2,835 (85.16)	3,508 (46.82)	3,343 (48.24)
Anopheles (Cellia) hilli	2 (0.01)	-	-	-	-
Anopheles (Cellia) novaguinensis	-	-	1 (0.03)	-	-
TOTALS	17,570	19,946	3,329	7,493	6,930
TRAPPING EFFORTS	29	43	11	18	12
AVERAGE PER TRAP	605.9	468.5	302.6	416.3	577.5
CAPTURE DATES	8.7.78 - 28.7.78	30.7.79 - 3.8.79	29.7.78 - 31.7.78	22.8.79 - 27.8.79	16.7.78 - 17.7.78

1. Numbers captured with percentages of totals in brackets.

2. Recognised as distinct species but as yet unnamed and undescribed.

3. Adult female mosquitoes.

(All mosquitoes captured by EVS/CO_2 light trap).

REPORT FROM THE VIROLOGY AND ENTOMOLOGY DEPARTMENTS,
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1. Isolates of Japanese Encephalitis Virus in Indonesia

There have been previous reports of the isolation of Japanese encephalitis virus (JE) from West Java (Van Peenen, et al., 1975, Trans. R. Soc. Trop. Med. & Hyg., 69: 477-479 and Van Peenen, et al., 1975, J. Med. Ent., 12: 573-574). We have recently isolated viruses which we have **preliminarily** identified as most closely resembling JE.

The origin, species and date collected of the mosquito pools yielding isolates are given in Table 1. The isolates were made in Vero or BHK cells inoculated with suspensions of the triturated mosquito pools. Identification of the isolates was performed using a microneutralization test using PS cells and reference mouse ascitic fluids. The results of the microneutralization tests are presented in Table 2.

One of the JE isolates was obtained from a pool of female Cx. tritaeniorhynchus mosquitoes collected in W. Lombok. This isolate may be interesting because of Lombok's location directly east of Wallace's Line. Data on the mosquito species collected in Lombok are presented in Table 3.

We are now in the process of preparing hyperimmune ascitic fluids to these isolates so that further characterization may be attempted.

(T. G. Ksiazek, J. G. Olson, V. H. Lee, Ratna Tan, Soeharyono, S. Nalim)

2. Prevalence of Dengue Antibodies in Bali

Our studies of fever in Bali indicate that group B arboviruses are important causes of fever in both inpatient and outpatient populations. Several strains of dengue virus representing 2 serotypes (DEN-1 and DEN-3) have been isolated from patients with influenza-like illnesses. Despite the occurrence of dengue fever cases, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) are extremely rare in Bali.

Jakarta, on the other hand, experiences many DHF/DSS cases each year. The absence of DHF/DSS in urban areas of Indonesia where dengue viruses are being transmitted has been observed by others (Dengue Newsletter 5(2): 21, Nov, 1979). Several reasons have been suggested for the occurrence of dengue fever without DHF/DSS including differences in vector competence, differences in virulence among the viruses and what could be called the "iceberg phenomenon". Serious dengue occurs in only a small percentage of the individuals infected with dengue virus. Many infections are subclinical and many more result in influenza-like illnesses. If the infection rate in the population was sufficiently low then DHF/DSS cases would be very few or non-existent.

We conducted a survey for dengue HI antibodies in a population resident in Kuta, Bali in which dengue fever is responsible for about 40% of fevers among outpatients. The prevalence of antibody in most age groups is lower than for Jakarta (Figure 1). The age adjusted prevalence is lower for Kuta (76%) than for Jakarta (86%) ($p < 0.02$).

The lower infection rate in Bali, as evidenced by the lower prevalence of antibody, may partially account for low frequency of reported DHF and DSS cases. However, a more likely reason for the low number of reported DHF/DSS cases in Bali is the small population at risk. In Jakarta there are nearly 2 million urban children under 15 years of age while in Bali there are only about 36,000.

If Denpasar, the principal urban area of Bali, had the same incidence rate of DHF/DSS as Jakarta we would expect about 14 suspect cases each year. This estimate is based on the observed incidence of suspect DHF/DSS reported in Jakarta during 1977 and 1978. During 1977 and 1978, 22 and 21 cases, respectively, were reported in Bali.

(J. G. Olson, T. G. Ksiazek, A. Sie, R. Mulya and S. Leimena).

3. Lack of Rift Valley Fever (RVF) HI Antibodies in Animal Sera from Indonesia.

Sera collected from monkeys in S. Kalimantan (Borneo) and sheep and goats from Java were recently tested for RVF HI antibodies. The monkey sera were also tested for CHIK and DEN-1 HI antibodies. The results of the tests are presented in Tables 4 and 5.

The 46 monkey sera tested had only three sera which had titers of 1:10. We feel the 1:10 titers probably represent non-specific inhibition of hemagglutination or weak cross reaction with other related viruses. The monkeys had no CHIK HI antibodies. However, 71% of the monkeys tested had DEN-1 HI antibodies.

The goat and sheep sera were negative for RVF HI antibodies.

The high prevalence of DEN-1 HI antibodies indicates that one or more flaviviruses are infecting the *P. cristatus* population of S. Kalimantan.

(T. G. Ksiazek, J. G. Olson, A. Sie, Soeharyono).

4. Use of the Immune Adherence Hemagglutination Test for Typing Dengue Isolates

The use of mosquito inoculation coupled with immunofluorescence for the isolation of DEN viruses (Kuberski and Rosen, 1977, AJTM&H, 26: 538-543) have been useful techniques for isolation and identification of DEN viruses in our laboratory. However, our laboratory does not

routinely use the CF test and we have found it rather laborious to perform on a periodic basis. Recent descriptions of the immune adherence hemagglutination (IAHA) test (Lennette and Lennette, 1978, J. Clin. Microbiol, 7: 282-285 and Wong, et al, 1978, J. Clin. Microbiol., 7: 6-11) as a suitable substitute for the CF test have lead us to compare the IAHA with the CF test in a limited series of DEN virus identifications. The viruses used in the comparison were isolates obtained from human patients in Jakarta, Indonesia by the Laboratory Biomedis, Ministry of Health and our own laboratory.

The results of the comparison of the CF and IAHA are contained in Table 6.

We have found the IAHA test is easier to perform, can be performed in a shorter amount of time, and is slightly more sensitive than the CF test. The identification by both techniques agree well with one exception in the limited series performed. Both the IAHA and the CF test have shared our difficulty: many antigens prepared have shown some anti-complementary activity in both tests inspite of those of only male mosquitoes.

Tesh's recent description of the use of the C6/36 clone of Ae. albopictus cells for isolation and the use of culture supernatant as a CF antigen (Tesh, 1979, A J T M & H, 28: 1053-1059) has stimulated us to explore this as a system to be used in conjuction with the IAHA test for identification of DEN isolates.

(T. G. Ksiazek, A. Sie, Soeharyono).

5. Bali Fever Study

Paired sera were collected from 120 hospitalized patients at Wangaya Hospital, Denpasar, Bali. 64 of these patients had 5 days of fever or less when they sought medical treatment and 56 had more than 5 days of fever. From an additional 21 patients only a single blood specimen was obtained. From each patient a rectal swab and blood specimen was cultured for pathogenic bacteria. Each pair of sera was tested for antibodies to group A and B arboviruses, influenza viruses and rickettsiae.

Laboratory diagnoses were made on 39 (61%) of the 64 patients with 5 days of fever or less. Patients with longer durations of fever upon admission had a greater frequency of laboratory diagnoses 44/56 (79%). Of the 21 patients from whom a single blood specimen was collected, only 5 (24%) had laboratory diagnoses were made.

Table 7 shows the relative importance of bacterial and viral agents as causes of fever in inpatients. Viral causes were more frequent among patients with fevers of short duration (28%) than among those with longer histories of fever (16%). Bacterial causes were more frequent among patients with long duration or fever. Several patients' had serologic tests indicating infection with viruses but also had bacteriologic studies indicating possible bacterial causes. A single patient had evidence that Rickettsia tsutsugamushi caused his illness and 4 patients (6%) had evidence of bacterial and viral infections.

In patients with fevers of longer duration bacterial causes were more frequent (53%) than viral ones (16%). Several patients (7%) had evidence of bacterial and viral infection and two patient's illnesses were caused by rickettsiae, one by *R. tsutsugamushi* and another by *R. mooseri*. Table 8 shows the relative frequency of bacterial, viral and rickettsial agents diagnosed in outpatient populations seeking treatment at 2 health clinics in Bali. Bacterial studies were limited to blood cultures for salmonellae. Clearly, fevers of viral etiology were more frequent causes of fever among the outpatients than the inpatients.

Tables 9 and 10 show the frequencies of specific etiologic agents associated with the fevers in inpatients and outpatients respectively. *Salmonella typhi* was the pathogen most frequently recovered from inpatients. Serologic tests incriminated flaviviruses and influenza viruses (Table 9) as the next most common causative agents. Influenza viruses and flaviviruses were the most frequently diagnosed agents responsible for fevers in outpatients (Table 10). The salmonellae were also responsible a considerable amount of illness in this population.

(S. L. Leimena, R. Mulia, J. G. Olson, T. G. Ksiazek and R. Tan).

Table 1. Virus isolates from Indonesian mosquitoes.

Isolate	Orig.M. Pool #	Spec. type	Date coll.	Geographic source
JKT Ar.log 451*	3428	*Cx. tritaeniorhynchus*	16 May 78	Lombok
657*	4026	*Cx. tritaeniorhynchus*	26 Oct 78	Kapuk
745*	4114	*Cx. tritaeniorhynchus*	26 Dec 78	Kapuk
792*	4161	*Cx. tritaeniorhynchus*	8 Jan 79	Kapuk
811*	4180	*Cx. tritaeniorhynchus*	8 Jan 79	Kapuk
813*	4182	*Cx. fuscocephalus*	8 Jan 79	Kapuk
788*	4157	*Cx. tritaeniorhynchus*	8 Jan 79	Kapuk

* most closely resembles JE in preliminary characterization

Table 2. Results of Microneutralization tests of isolates from Indonesian mosquito pools.

HMAF	ISOLATE* 451	657	745	792	811	813	788
JE	1:10	1:120	1:40	1:40	1:40	1:40	1:20
MVE	-**	1:10	1:10	-	-	-	-
SLE	-	1:10	-	-	-	-	-
WN	-	-	-	-	-	-	-
KUN	-	-	-	-	-	-	-
DEN-2	-	-	-	-	-	-	-
TMU	-	-	-	-	-	-	-

Isolates were also tested against the following HMAFs and found to have neutralizing titers of < 1:10: DEN-1, DEN-3, DEN-4, LGT, SEP, ZIK, YF, BEB, EEE, GET, RR, SAG, SIN, VEE, WEE, WHAT, BAK, BAT, BUN, ING, UMB, and normal MAF.

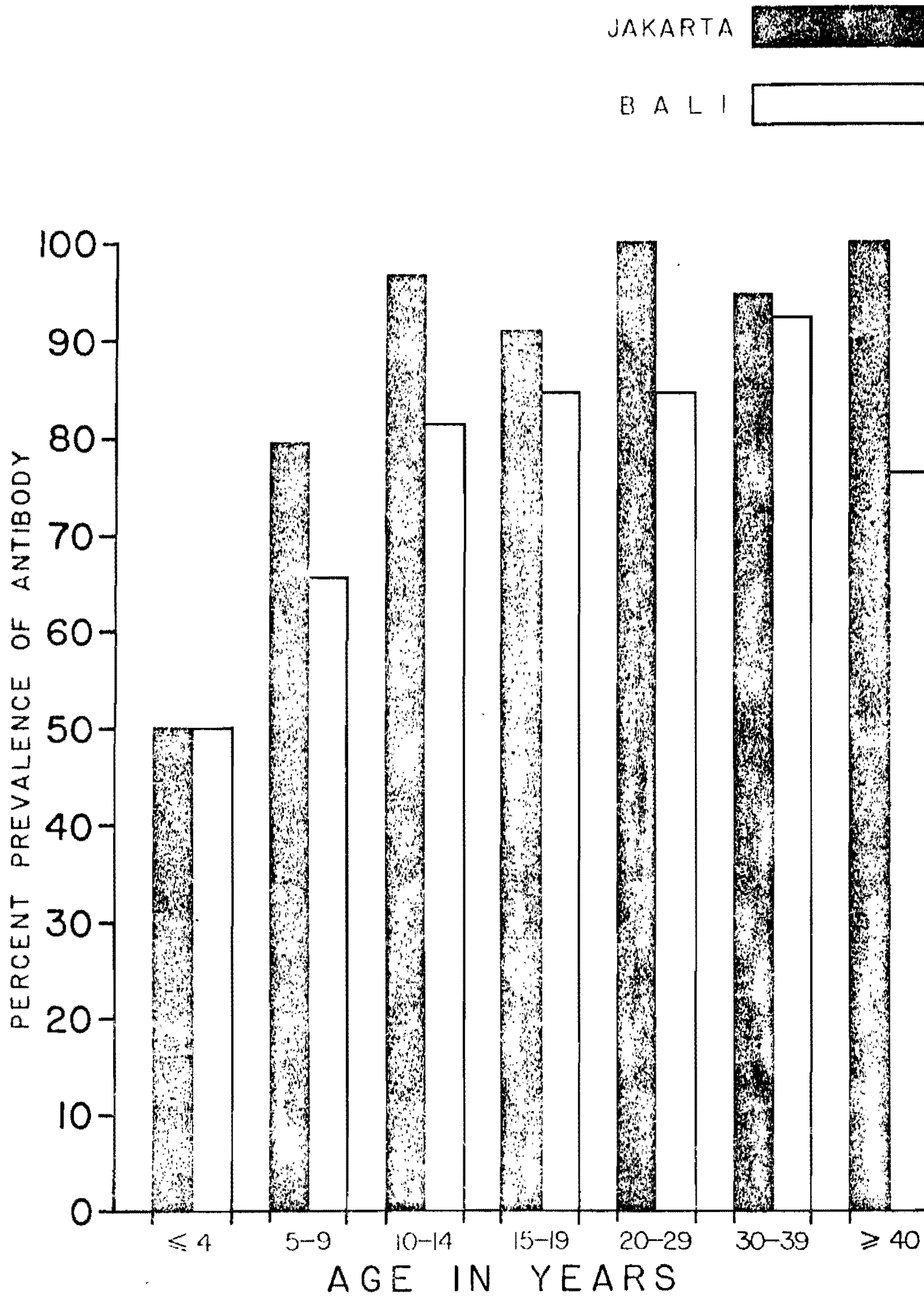
* Virus was used at a dose of 1.5 to 2.5 $\log_{10}$ $TCID_{50}$

** * - = < 1:10

Table 3. Arthropods collected in Lombok, May 1978.
(Combined data from west and east Lombok)

Species	No. (% mosquitoes)		No. pools
Culicoides spp	89100		91
Cx. annulus	7621	(50.8)	156
Cx. tritaeniorhynchus	2758	(18.4)	60
Cx. gelidus	528	(3.5)	14
Cx. *pipiens quinquefasciatus*	42	(0.3)	3
Cx. bitaeniorhynchus	30	(0.2)	2
Cx. spp.	35	(0.2)	2
Anopheles vagus	878	(5.9)	23
An. annularis	706	(4.7)	19
An. tesselatus	167	(4.1)	8
An. barbirostris	144	(1.0)	9
An. spp	131	(0.9)	3
Aedes lineatopennis	528	(3.5)	14
Ae. (Finlaya) spp.	373	(2.5)	12
Ae. vexans	218	(1.5)	9
Ae. spp.	798	(5.3)	15
Mosquitoes	15,607	(100)	349
Total	104,057		440

Figure 1. Prevalence of dengue type 1 hemagglutination inhibiting antibody* by age in residents of Jakarta and Bali, Indonesia.



* Titers ≥ 1:20 were considered positive.

Table 4. Results of test for Arbovirus (DEN-1, CHIK, & RVF) HI Antibodies in monkeys from South Kalimantan.

	ANTIGEN		
	DEN-1	CHIK	RVF
<u>P</u>. <u>cristatus</u>	32/45 (71)*	0/45	3/45**(7)
<u>M</u>. <u>fascicularis</u>	0/1	0/1	0/1
GMT	22.3	5.0	5.9

* Pos/tested (%). Positive was taken as a titer of ≥ 1:10.

** All three positives had a titer of 1:10.

Table 5. Results of HI tests for Rift Valley fever antibodies in sheep & goats slaughtered in Jakarta.

	< 1:10	≥ 1:10	1:10
Goats	24	0	24
Sheep	30	0	30
TOTAL	54	0	54

Table 6. Comparison of virus identification of DEN isolates by CF and IAHA tests.

Isolate No.	CF					ID	IAHA					ID
	D_1	D_2	D_3	D_4	JE		D_1	D_2	D_3	D_4	JE	
510	256	128	128	128	32	D_1	320	80	160	160	< 40	D_1
529	128	256	64	64	64	D_2	160	640	80	< 40	40	D_2
717	32	32	64	< 32	32	D_3	160	80	1280	< 40	< 40	D_3
684	64	256	32	32	64	D_2	320	640	160	320	160	D_2
538	32	< 32	64	< 32	< 32	D_3	160	80	320	< 40	40	D_3
686	128	256	64	64	64	D_2	320	1280	160	80	80	D_2
530	64	256	64	64	64	D_2	160	640	80	< 40	80	D_2
512	128	256	64	32	64	D_2	160	320	80	160	40	D_2
694	128	64	64	< 32	< 32	D_1	320	80	40	< 40	< 40	D_1
517*	128	64	64	< 32	< 32	D_1	160	640	80	80	80	D_2
513	128	256	128	32	64	D_2	160	320	160	80	40	D_2
528	128	256	64	128	64	D_2	320	640	< 40	< 40	40	D_2
710	64	256	32	< 32	32	D_2	160	320	80	80	80	D_2
638	128	256	64	64	64	D_2	160	640	80	160	40	D_2
509	64	256	32	< 32	< 32	D_2	160	1280	80	160	40	D_2
637	128	256	64	64	64	D_2	80	320	80	80	80	D_2
715	64	128	< 32	32	32	D_2	160	320	80	40	< 40	D_2
696	128	256	64	64	64	D_2	160	1280	80	40	80	D_2
535	64	64	256	< 32	64	D_3	160	80	640	160	40	D_3
709	64	256	32	< 32	32	D_2	160	640	160	160	80	D_2
536	64	64	256	32	32	D_3	160	80	640	40	< 40	D_3
537	64	64	256	< 32	32	D_3	160	80	640	80	40	D_3
642	128	256	64	64	64	D_2	160	1280	160	160	80	D_2
546**	-	-	-	-	-		160	320	80	80	40	D_2
DEN-1	256	64	64	< 32	< 32		160	< 20	< 20	< 20	-	
DEN-2	128	512	128	128	64		320	320	< 20	< 20	-	
DEN-3	128	32	128	< 32	< 32		40	40	160	< 20	-	
DEN-4	32	< 32	< 32	128	< 32		< 20	20	20	160	-	

* IAHA and CF did not agree

** CF test did not have titers indicating lack of antigen.

Table 7. Frequency of bacterial, viral and rickettsial agents as causes of fever among inpatients admitted to Wangaya Hospital, Denpasar, Bali 1978 - 1979.

	patients with 5 days of fever or less on admission		patients with history of more than 5 days of fever on admission	
	no. positive * / no. tested	(%)	no. positive / no. tested	(%)
bacterial causes	16/64	(25)	30/56	(53)
viral causes	18/64	(28)	9/56	(16)
rickettsial causes	1/64	(2)	1/56	(2)
etiologic agent uncertain	4/64	(6)	4/56	(7)
no etiologic agent identified	25/64	(39)	12/56	(21)

* etiologic agent isolated from blood or feces and or diagnostic increase (4 fold) in antibody titer from acute to convalescent phase.

Table 8. Frequency of bacterial, viral and rickettsial agents as causes of fever among outpatients visiting Kuta and Ubud Health Clinics, Bali, 1978 - 1979.

	patients with history of 5 days of fever or less		patients with history of more than 5 days of fever	
	no. positive / no. tested	(%)	no. positive / no. tested	(%)
bacterial causes	5/56	(9)	1/8	(12)
viral causes	27/56	(48)		
rickettsial causes	2/56	(4)		
etiologic agent uncertain	3/56	(5)		
no etiologic agent identified	19/56	(34)	7/8	(88)

Table 9. Frequency of laboratory diagnoses by etiologic agent among inpatients of Wangaya Hospital, Denpasar, Bali, 1978-1979.

Bacteria:		53
Salmonella typhi		30
Sal. paratyphi A		2
Sal. enteritidis	B	3
	C	3
	D	-
	E	3
	F	1
Aeromonas hydrophila		1
Shigella flexneri		1
Vibrio cholerae		2
Staphylococcus aureus		1
Staph. epidermiditis		2
Enterobacter cloacae		2
Escherichia coli		1
Pseudomonas sp.		1
Viruses:		23
alphaviruses		2
flaviviruses		16
influenza viruses		5
Rickettsiae		2
Rickettsia tsutsugamushi		1
R. mooseri		1
evidence of infection with more than one etiologic agent		11

Table 10. Frequency of laboratory diagnoses by etiologic agent among outpatients of Kuta and Ubud health clinics, Bali, 1978-1979.

Bacteria:	5
Salmonella typhi	2
Sal. paratyphi A	2
Sal. choleraesuis	1
Viruses:	25
alphaviruses	1
flaviviruses*	11
influenza viruses	13
Rickettsiae:	2
Rickettsia tsutsugamushi	1
R. mooseri	1
evidence of infection with more than one etiologic agent **	5

* includes 2 dengue-1 and 1 dengue-3 strains.

** 2 patients had serologic evidence of infection with influenza virus and flavivirus and 2 patients showed evidence of infection with influenza virus and *R. mooseri* and 1 patient showed evidence of infection with flavivirus and *R. mooseri*.

REPORT FROM THE DEPARTMENT OF VIROLOGY,
U.S. COMPONENT, ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES,
BANGKOK, THAILAND

1. COMPARISON OF THE MOSQUITO INOCULATION TECHNIQUE WITH CELL CULTURE TECHNIQUES FOR ISOLATING DENGUE VIRUSES FROM THAI CHILDREN

Recently we have carried out studies designed to compare the mosquito inoculation assay to cell culture methods for isolating dengue viruses, and to identify the component(s) of the blood of dengue patients which yield the maximum number of virus isolations. Plasma, platelets and leukocytes fractions were obtained from blood of confirmed dengue patients at the Children's Hospital during 1978, and separated according to the protocol in Figure 1.

Blood fractions were assayed for virus by the mosquito inoculation technique employing _Tx. splendens_, and in LLC-MK2 cells by the direct and delayed plaque assay or by cocultivation in an infectious centers assay. Eight or more _Tx. splendens_ were inoculated intra-thoracically with each of the undiluted cellular specimens and a 1:5 dilution of plasma, 0.85 ul per mosquito. After 14 days incubation at 32°C, mosquitoes were stored at -70°C. Head squashes of individual mosquitoes were examined for virus by the direct fluorescent antibody technique and corresponding thorax-abdomens were tested for virus by the direct and delayed plaque assay. The amount of inoculum was 0.3 ml per cell culture. Viruses were identified by the plaque reduction neutralization test using monospecific dengue virus types 1, 2, 3 and 4 antisera prepared in rhesus monkeys. Cell culture assays were performed at 35°C.

Isolation rates for dengue viruses from plasma and cellular components of the blood of dengue patients by the mosquito inoculation, and the direct and delayed plaque assay are shown in Table 1.

Differences in the virus isolation rates obtained by the mosquito inoculation and the cell culture assays from the different blood fractions were analysed according to the HI antibody titers of dengue patients. Isolations of dengue viruses from plasma exclusively by the mosquito inoculation technique appeared to be related to the acute HAI antibody titers; the geometric mean antibody titer for patients from whom virus isolations were obtained from the plasma fractions by both techniques was 1:33 while the titer was 1:352 for patients from whom viruses were isolated only by the mosquito inoculation technique. Differences in the isolation of viruses from platelets and polymorphonuclear fractions by the two techniques were not related to antibody titers. Isolation of dengue viruses from mononuclear cell fractions appeared to be related to antibody titer of the patient, but the pattern was opposite to that observed for plasma fractions (Table 2). Four virus isolations were obtained only by the mosquito inoculation technique from mononuclear cell fractions of patients with an HI antibody less than 10 (in most cases), while eight isolations obtained only by the assay of mononuclear cells were from patients with high fixed antibody titers.

Sixty-nine adherent and nonadherent cell fractions were tested for virus by the infectious center assay in LLC-MK2 cells. The rate of isolation of dengue viruses by this technique as compared to the mosquito inoculation and the direct and delayed plaque assay in these specimens is presented in Table 3. Isolation rates for adherent and non-adherent cell fractions were higher than those observed for other fractions assayed by either the mosquito inoculation or the direct and delayed plaque assay, but were similar to those obtained for mononuclear cell fraction by each of the other techniques.

Of the total of 37 strains isolated in 1978, 0 were D1, 32 D2, 1 D3, and 4 D4. The few isolates that were not D2 did not appear to differ from the D2 strains with regard to their isolation characteristics.

Overall isolation rates were strongly correlated with both the day of illness on which the blood specimen was obtained and the concurrent homologous HI antibody titer (Figure 2). Specimens obtained on or after day 6 of illness, or with an HI titer of ≥ 640 were unlikely to yield a virus isolate.

(Submitted by D.M. Watts, A. Nisalak and D.S. Burke)

2. ISOLATION OF FLAVIVIRUSES FROM PLANTATION WORKERS IN CENTRAL MALAYSIA

During 1978-1979 the AFRIMS Department of Virology performed over 2,000 HAI serologies in support of studies on pyrexia of unknown origin in palm oil and rubber plantation workers in Central Malaysia conducted by the Kuala Lumpur US Army Medical Research Unit (KL-USAMRU). Almost all patients had flavivirus HAI antibodies in both acute and convalescent serum specimens. Although final tabulation of the coded HAI serology results is not yet available to this department, overall approximately 15% of patient developed a four fold or greater rise in serum antibody titer to the representative flavivirus antigen (Tembusu) used. The actual virus type or types responsible for these infections remained unknown. All patients in the KL-USAMRU PUO study had an acute blood specimen frozen in liquid nitrogen at the bedside on admission to the study; therefore, acute specimens from the known flavivirus sero-responders were available to us for virus isolation attempts.

A list of 84 patients were drawn up who fulfilled the following criteria: (1) 8x or greater rise in HAI titer against flavivirus antigen, (2) acute blood obtained with 7 days of onset of illness (3) at least 0.5 ml of acute blood frozen in liquid nitrogen remaining. The acute blood specimens for isolation were shipped frozen from Kuala Lumpur to Bangkok, thawed, diluted 1:4 in RPMI 1640, and disrupted by sonication. Aliquots of each specimen were then processed for viral isolation by (1) the delayed plaque method on LLC-MK2 cells by standard methods (2) by inoculation onto C6/36 A. albopictus cells and passage of the tissue culture supernatant fluid onto LLC-MK2 cells on day 7, (3) by intrathoracic inoculation into Tx. splendens mosquitoes with detection of mosquito infection

by (a) examination of head squash preparation for direct fluorescence with fluorescent conjugated, broadly flavivirus reactive, pooled convalescent serum from Dengue Hemorrhagic Fever patients (b) plaquing of thorax-abdomen suspensions onto LLC-MK2 cells and (c) blind passages of the thorax-abdomen suspensions through C6/36 cells for one week with subsequent assay of the supernatant fluid by the direct plaque assay method on LLC-MK2 cells (4) by intracerebral inoculation of 1 to 2 day old suckling mice with observation for sickness or death; if no illness was observed the mice were challenged on day 14 with 100 LD50 of virulent D-2 intracerebrally and observed for another two weeks. In liters showing even slight signs of sickness or death 1/2 of the liter was blind passed through at least another two mouse passages at two weeks intervals, with titration of pooled brains from the final mouse passage in LLC-MK2 cells by the direct plaque method 14 days after inoculation.

The overall scheme of diagnostic maneuvers is presented in Figure 3. Separate personnel performed the mouse inoculation and challenge, the mosquito inoculation and head squash fluorescent assay, and the tissue culture passages plaquing; all results for a given method were completed without knowledge of results obtained by other personnel using other methods.

A total of 21 confirmed virus isolates were made from the 84 acute blood specimens assayed by all seven methods. All confirmed isolates were made by more than one of the diagnostic methods used and produced a passable plaque forming agent. An additional seven specimens were thought to be positive for virus based on preliminary assay results; in six of these the results of only one assay system was questionably positive and no plaque forming agent was isolated, and in the seventh results of two independent methods were questionably positive but no plaque forming agent was isolated. Results are presented in tabular form in Table 3. The 21 confirmed virus isolates have been identified by the plaque reduction neutralizing antibody method in LLC-MK2 cells. Nineteen isolates are dengue type 3 and two are dengue type 2. Although the range of diagnostic methods used was reasonably broad, no other flaviviruses were isolated, only dengue.

All 21 of the confirmed isolates were recovered by the mosquito inoculation technique, and all 21 were detected by both fluorescent staining of head squash preparations and passage of thorax-abdomen suspensions onto LLC-MK2 cells. The delayed plaque method on LLC-MK2 cells detected only 43% of the confirmed positives, while passage through C6/36 cells before direct plaquing on LLC-MK2 cells detected only 24%. As a check for the possibility that an unusually insensitive line of LLC-MK2 cells was used, a new line which had been frozen and stored five years ago at this laboratory was brought out and tested. Aliquots of the twelve frozen blood specimens which were positive by mosquito inoculation but negative by LLC-MK2 cells and assayed for virus by the delayed plaque method. Only two of the specimens originally negative on LLC-MK2 cells were positive for transmissible plaque forming virus on the new cells.

In this trial the mosquito inoculation technique was clearly superior to the LLC-MK2 cell culture technique in detecting D3 in frozen whole blood specimens from febrile Malaysian adults. This finding contrasts with our concurrent (and more extensive) experience in the detection of dengue viruses (usually D2) in plasma and leukocytes of children with hemorrhagic fever in Bangkok; in the latter population we find the two techniques to be of roughly equal sensitivity.

(submitted by D.S. Burke; D.M. Watts and A. Nisalak)

TABLE 1 Dengue Virus Isolations from Plasma and Cellular Components of the Blood of Dengue Patients

Blood Fractions	Virus Assay Technique					
	Plaque Assay	(%)	*Tx. splendens*	(%)	Total[1]	(%)
Plasma	18/116	(16)[2]	24/116	(21)	25/116	(22)
Mononuclear Cells	20/103	(19)	21/103	(20)	28/103	(27)
Polymorphonuclear Cells	11/71	(15)	11/71	(15)	14/71	(20)
Platelets	09/64	(14)	10/64	(16)	12/64	(19)

1. Number of virus isolations obtained by both assay techniques
2. Number of virus isolations/number of fractions tested

TABLE 2 Isolation of Dengue Viruses from Mononuclear Cell Fractions of Dengue Patients by the Mosquito Inoculation Technique and Cell Culture in Relation to Concurrent Hemagglutination Inhibition Antibody and Neutralizing Antibody Titers

Patient Number	Disease	Technique: Mosquito Assay	Technique: Plaque Assay	Concurrent Homologous Antibody Titers: HI	Concurrent Homologous Antibody Titers: PRNT-50
D78-017	4	+	ND	1280	ND
D78-026	6	+	0	5120	ND
D78-050	3	+	0	10	0
D78-069	2	+	0	10	ND
D78-091	5	+	0	10	ND
D78-118	2	+	0	10	ND
D78-009	4	0	+	320	ND
D78-014	4	0	+	2560	ND
D78-044	4	0	+	20	32
D78-074	6	0	+	5120	ND
D78-077	5	0	+	2560	640
D78-108	6	0	+	5120	ND
D78-025	4	+	+	1280	ND
D78-051	5	+	+	320	040
D78-078	5	+	+	160	190
D78-099	6	+	+	10	035
D78-112	5	+	+	10	0
D78-114	2	+	+	10	ND
D78-117	3	+	+	10	130
D78-132	4	+	+	160	ND
D78-133	2	+	+	10	350
D78-135	2	+	+	10	ND
D78-136	4	+	+	1280	ND
D78-145	5	+	+	160	ND
D78-157	5	+	+	10	ND
D78-159	4	+	+	640	640
D78-168	5	+	+	80	ND
Total		17	20		

Virus positive = +
Virus negative = 0

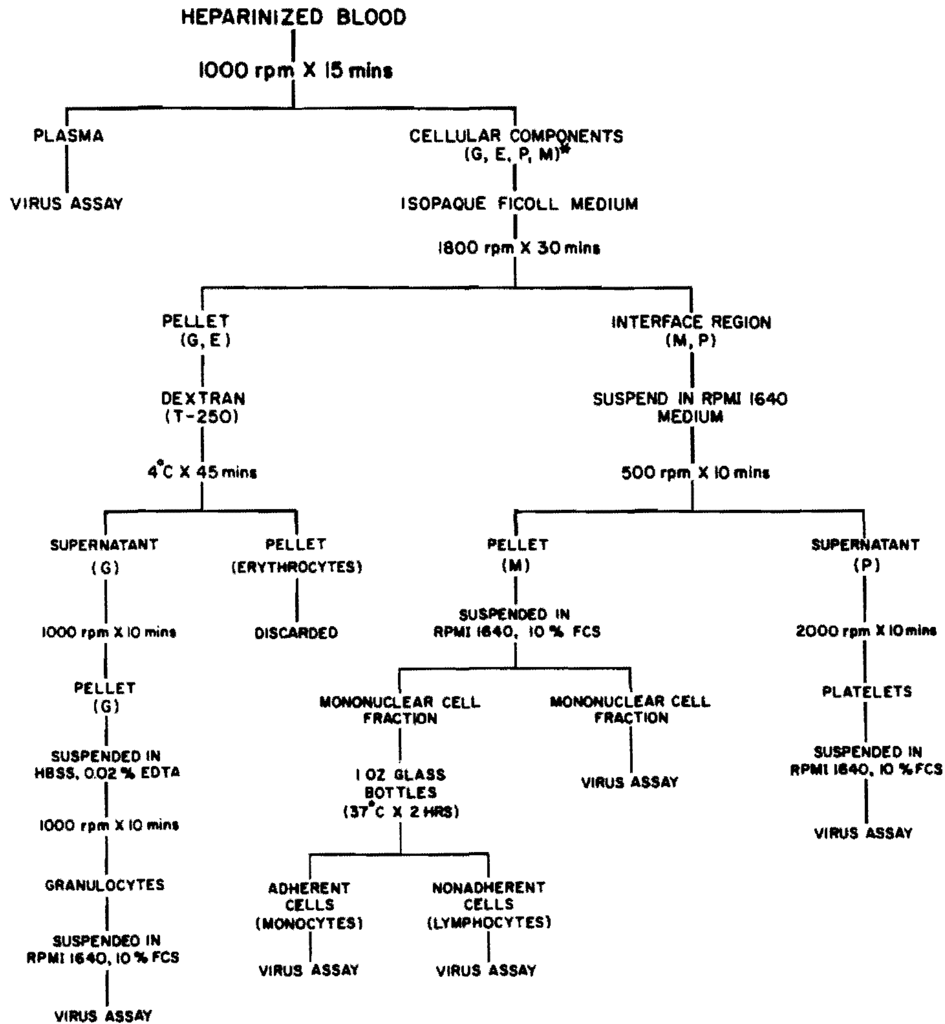
TABLE 3 Flavivirus Isolates

AFRIMS Number	Tissue Culture Isolation: LLC/MK2 Delayed	Tissue Culture Isolation: Pass C6/36 LLC-MK2 Direct	Mosquito Inoculation: FA No.(+)/ Tested	Mosquito Inoculation: Direct Plaque Thorax-Abdomen on LLC-MK2 Confirmed	Mosquito Inoculation: Thorax-Abdomen C6/36→Direct Plaque on LLC-MK2 Cells	Suckling Mouse Inoculation: Virulent Virus Challenge: No. Protection/No. Challenged	Suckling Mouse Inoculation: Sickness or Death
1724/79	TNTC	0	4/4	50	10.7×10^2	3/6	0
1725/79	36	0	2/4	74	4.3×10^2	5/9	+
1728/79	0	0	2/4	138	2.4×10^2	8/11	0
1729/79	0	0	4/4	TNTC	9.9×10^2	3/10	0
1730/79	88	0	4/4	TNTC	7.8×10^2	6/11	0
1731/79	0	0	2/4	122	10×10^2	6/9	0
1733/79	TNTC	0	4/4	49	5.5×10^2	8/11	0
1734/79	0	0	2/4	169	5×10^2	2/12	0
1739/79	30	0	4/4	TNTC	2×10^3	10/11	+
1754/79	0	0	3/4	79	8×10^3	3/12	0
1755/79	0	0	4/4	TNTC	1.2×10^3	3/11	0
1756/79	72	3×10^2	4/4	TNTC	0	12/12	0
1757/79	33	0	2/3	106	2.2×10^3	10/12	+
1758/79	0	0	4/4	TNTC	1.4×10^3	8/9	0
1762/79	0	0	2/4	109	3×10^3	3/11	0
1765/79	60	7×10^2	4/4	58	4.1×10^3	7/13	0
1775/79	0	0	2/4	106	9×10^2	2/15	0
1784/79	0	7×10^1	2/4	TNTC	11.4×10^2	2/12	0
1785/79	0	$11 \times 10_2$	4/4	TNTC	24.5×10^2	1/12	0
1787/79	1.5×10^2	4.6×10^2	4/4	TNTC	0	2/12	0
1800/79	0	0	4/4	TNTC	3×10^2	4/6	+
			UNCONFIRMED**				
1748/79	0	6×10^2	0/4	0	0	0/12	0
1752/79	0	0	0/4	0	0	2/5*	0
1771/79	0	0	0/4	0	0	3/9*	0
1773/79	0	0	0/4	0	0	ND	+*
1778/79	0	1.8×10^2*	0/4	0	0	2/7*	0
1797/79	0	0	0/4	0	8×10^2*	0/11	0
1805/79	0	0	0/4	0	0	ND	+*

* Unconfirmed positive result

** An additional 56 specimens were negative in all seven assay systems

Figure 1. **Separation and isolation of plasma and cellular components of the blood of dengue patients.**



*G = GRANULOCYTES, E = ERYTHCCYTES, P = PLATELETS, M = MONONUCLEAR.

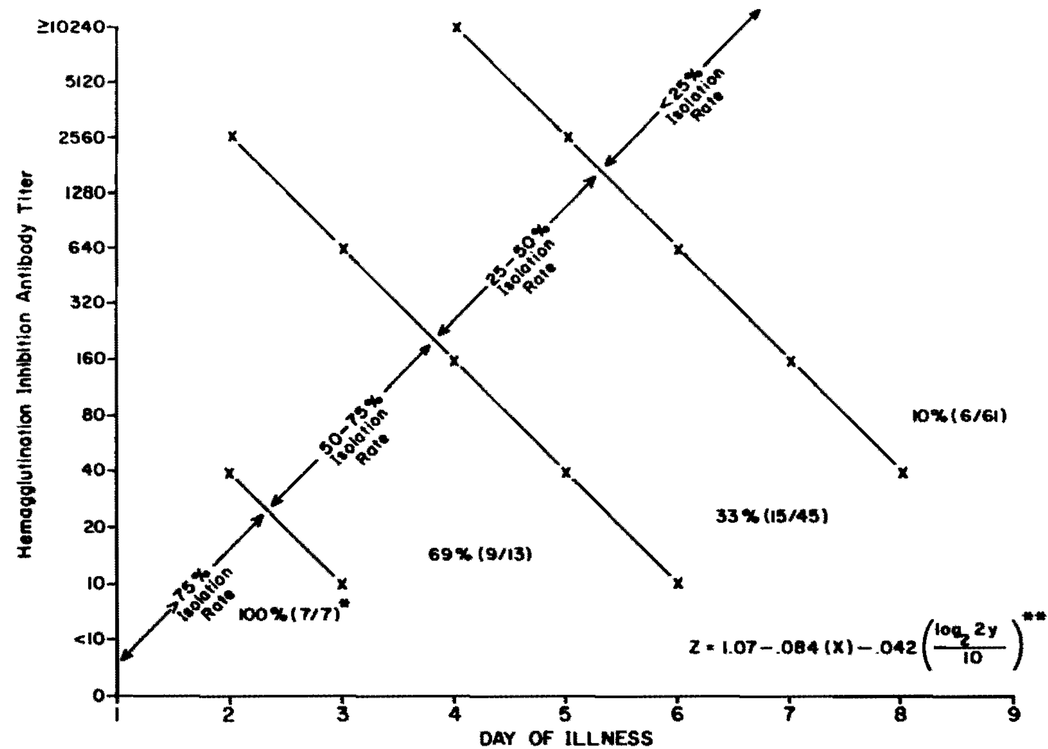


Figure 2 Dengue virus isolation rates in comparison to predicted rates determined by multiple linear regression analysis of actual virus isolation data in relation to the day of disease and the hemagglutination inhibition antibody titers of dengue patients

* actual virus isolation rate

** z = probability of isolating virus
x = day of illness
y = hemagglutination inhibition antibody titer

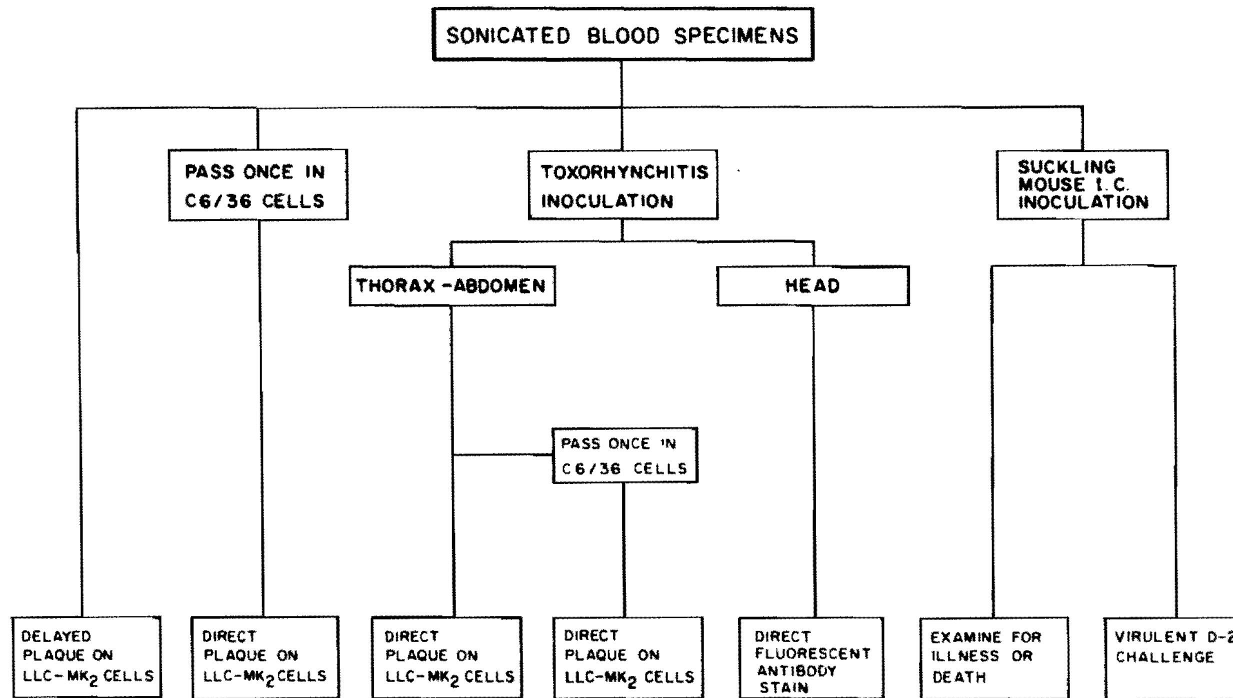


Figure 3 Diagramatic scheme of techniques used to detect flaviviruses in blood specimens from febrile Malaysian adults

REPORTS FROM DEPARTMENT OF MICROBIOLOGY,
KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Inoculation of mouse-adapted dengue virus into "nude" mice.

A mouse-adapted strain of DEN-1 virus, Mochizuki, was used throughout. When 4-week-old nu/nu and nu/+ mice were injected ip with 10^7 PFU virus, final mortality ratio of nu/+ mice was about 40% and all deaths occurred 5 to 11 days post inoculation. In the case of nu/nu mice, mortality ratio within 2 weeks post inoculation was about 45%. However, smaller number of infected nu/nu mice were paralyzed after 3 weeks or later, so that the cumulative mortality ratio at 4 weeks was 60%. While the differences in mortality ratios were not significant, the average survival time of dead mice was longer in nu/nu than in nu/+.

The infected nu/nu mice transiently produced anti-DEN Ig M antibodies in the early stage of infection, but the titers were lower than those by nu/+ mice. No Ig G production was shown in the nu/nu mice, while the same antibodies were clearly demonstrated in the nu/+ mice 3 weeks after inoculation.

As for the distribution of DENV in the body of infected mice, the highest virus titers, usually about 10^8 PFU/g, were revealed in the brain. The skeletal muscle and heart tissue were the second for the virus contents, showing 10^4 PFU/g or more. The infective virus was detected in the lymph nodes, lung, liver, spleen, and kidney, however, their titers varied from one sample to another. A general tendency was noted that the titers of lymph nodes were higher than those of spleen. Viremia was not clear. There was no definite difference in the distribution of DENV between nu/nu and nu/+ mice, except for the cardiac tissues in which the virus contents in nu/nu were higher than those in nu/+.

By the fluorescent antibody method, the specific DEN-1 antigens were detected in the neurons and also in the skeletal muscle fibers, cells in the connective tissues of skeletal or cardiac muscle, and the Kupffer cells of the liver.

No hemorrhagic changes were noted either in the infected nu/nu or nu/+. Necrosis of the skeletal and cardiac muscle fibers was often seen. The Kupffer cells exhibited acidophilic hyaline degeneration, sometimes containing small vacuoles in the cytoplasm. These changes showed certain resemblance to those seen in the liver of fatal cases of dengue hemorrhagic/shock syndrome.
(Investigations conducted by H.Hotta, I.Murakami, K.Miyasaki, Y.Takeda, and H.Shirane)
(Reported by S.Hotta)

REPORT FROM THE DEPARTMENT OF VIROLOGY AND RICKETTSIOLOGY, NATIONAL INSTITUTE OF HEALTH, SHINAGAWA, TOKYO 141, JAPAN

Human overt Japanese encephalitis in Japan in 1979

Eighty four human cases were confirmed or determined as presumptive deaths in whole Japan in 1979. It includes 25 deaths showing mortality 29.8%. Among survivals of 59 cases, sequelae were observed in 30 cases. Out of totally 84 cases, 46 including 11 death were male and 38 including 14 deaths were female. Except presumptive death of 6 cases, all were confirmed by serological tests.

Japanese encephalitis (JE) cases were reported mostly from the western half part of Japanese Island particularly from Kyushu district. In fact, 57 cases including 15 deaths were from Kyushu district comprising 67.9% of total. It was marked that 35 cases including 11 deaths occured in one prefecture (Kumamoto).

Cases from aged population was again emphasized. Forty seven including 18 deaths were of over 60 in their ages. This comprises 60 % of total. Mortality was higher in aged patients and lower in younger ones. Thus, mortality of over 60 was 18/47 (38.3%), 10 to 59 ages 7/29 (24.1 %) and less than 9 ages 1/8 (12.5 %). Most of cases did not have vaccination history.

(A. Oya)

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

Evidence of Japanese encephalitis infection in mules

One hundred and thirty-one mule sera were collected from the eastern Himalayan region of India for search of arbovirus antibodies. Haemagglutination-inhibiting (HI) antibodies against one alfavirus, Chikungunya (CHIK) and three flaviviruses namely dengue type-2 (D_2), Japanese encephalitis (JE) and West Nile (WN) were searched for. Results showed that 10.7% of the sera had antibody against CHIK antigen while Group B antibodies were present in 69.5% of the sera. Antibodies against the individual antigens, namely D_2, JE and WN were 21.4, 68.7 and 50.4 per cent respectively (Table-I). Neutralization test (NT) was performed with 50 sera having HI antibodies to both JE and WN antigens. The test was performed in suckling mice intraperitonially using heat inactivated (56°C for 30 minutes) undiluted sera with 100 to 150 LD_{50} dose of JE and WN viruses respectively, and the test was considered positive when 50% or more of the inoculated animals were protected upto 21 days or above. Results are shown in Table-II.

Table-I

Result of HI test of mule sera against the following arbovirus antigens

Group A	Group B			Total Group B
CHIK	D_2	JE	WN	
$\frac{14^a}{131^b}$ (10.7%)	$\frac{28}{131}$ (21.4%)	$\frac{90}{131}$ (68.7%)	$\frac{66}{131}$ (50.4%)	$\frac{91}{131}$ (69.5%)

a = Number of positive
b = Number of tested

Table-II

Result of neutralization test with JE and WN viruses of 50 HI positive sera

Virus	Positive
JE	41 (82%)
WN	12 (24%)

COMMENTS

The results of HI and N tests of mule sera collected from the eastern Himalayan region, India, indicate the predominating role of JE infection in them.

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Fatal encephalitis in a child caused by dengue virus - based on serological findings.

A male child, aged 5½ years, a resident of Calcutta was admitted in the Medical College Hospital, Calcutta with a history of fever for 15 days and drowsiness for 7 days. Relevent Clinical findings on admission were:

Fever - 99°F, Pulse - 120 per minute, regular; respiration - 24 p.m., regular; Neck rigidity - absent, Kernig's sign - negative, Planter reflex - extensor.
Liver - just palpable.
Two days after the admission, the patient gradually passed into coma till death on the 38th day of illness. Fever persisted throughout the illness (100°-106°F) with occassional convulsions.

Laboratory clinical findings:

a) Peripheral blood count:

W.B.C - 11,000/C.cm., Neutrophil - 84%, Lympho - 16%.

b) C.S.F. - clear fluid, tension - normal, Protein - 20 mgm%, Sugar - 80 mgm%, chloride - 580 mgm%, cells - 4 cell/c.mm (lympho) bacteriological culture - negative.

Results of other examination like urine, stool, skiagram of chest and mantoux test - No abnormality.

Virological findings:

No virus could be isolated from the first blood sample (15th day of illness) in either suckling mice or in tissue culture (VERO or human amnion). Serological (HI & CF) tests were done against chikungunya (Chik) and three flavivirus antigens namely dengue type 2 (D_2), Japanese encephalitis (JE) and West Nile (WN), and the results are shown in the table below :

	Day of illness	Chik		D_2		JE		WN	
		HI	CF	HI	CF	HI	CF	HI	CF
1st sample	15	<20	<4	40	4	40	4	20	4
2nd sample	24	<20	<4	5120	128	160	8	40	4

Neutralization test revealed significant rise of neutralizing antibody with D_2 virus only (from 1/20 to 1/640).

Note:- This is the second case of dengue encephalitis reported from this department, the first one was reported in 1966 (Ref: Ind.Jour.Med.Res. (1969), 57, 1616).

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REPORT FROM THE VIRUS RESEARCH CENTRE,
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NAIROBI, KENYA.

MARBURG VIRUS DISEASE - NAIROBI

On 24 Jan, 80, a 29 year old male physician working in the intensive care unit of Nairobi Hospital, Nairobi, Kenya, developed an illness characterized by fever, malaize, myalgia, joint pains, dryness of the mouth, persistent right upper quadrant tenderness, diarrhoea and vomiting. No rash was noted. Laboratory findings included a progressive leukopenia, presence of atypical monocytes, occult blood in the stool and elevated transaminases and alkaline phosphotase. A liver scan suggested that surgery was indicated and on the 12th day of illness a laparotomy was performed with unremarkable results and a liver biopsy taken.

The condition of the patient worsened and a viral disease was suspected. Paired serum samples from 30 Jan, 80, and 6 Feb, 80, were sent to CDC, Atlanta, who reported back 34 hours later that the paired sera showed a rise from zero to 1: 256 against Marburg virus over the 7 day period whilst no antibodies were detectable against Ebola, Lassa, Crimean-Congo or Rift Valley fever viruses. The patient is now recovering.

A less severe second, as yet unconfirmed, case may have occurred in a nurse who assisted the patient in treating a European who had been working in western Kenya, who entered Nairobi Hospital on 15 Jan, 80, suffering from severe shock and haemorrhaging. This man died within a few hours of entering hospital.

Virus isolation attempts and serological investigations are underway at CDC, Atlanta, and the Institute for Virology, Johannesburg, South Africa on material from the patient and suspected contacts. Over 100 people are under passive or active observation.

ISOLATION OF O'NYONG-NYONG VIRUS FROM *ANOPHELES FUNESTUS* IN WESTERN KENYA.

The alphavirus o'nyong nyong (ONN) caused a major arbovirus epidemic in East Africa between 1959 and 1962. The virus, which was transmitted by *Anopheles funestus* Giles and *Anopheles gambiae* Giles *s.l.* occurred in epidemic form on the Kano Plain in western Kenya in 1961. Serological evidence suggested that ONN transmission continued to occur but declined (Bowen, et al., 1973; unpublished observations, MRC Project Kisumu). Between 1969 and 1975 isolation attempts were made on nearly 325,000 mosquitoes, 3,600 wild or domestic vertebrate samples and 989 sera from febrile patients (Johnson et al., 1977, 1977a, 1978) but ONN virus was not isolated.

In a follow-up study conducted in June, 1978, on the rice growing scheme at Ahero on the Kano Plain, 21,809 mosquitoes were captured by light trapping over a 6 day period. A single isolate was recovered from a pool of 100 *Anopheles funestus*. The isolate, KsMP-172, caused only sporadic sickness or death in IC inoculated suckling mice and has continued to do so through 16 suckling mouse brain passages. KsMP-172 virus formed clear plaques in BHK cells and less clear plaques in Vero cells. Micro-neutralization tests using BHK cells and NIH arbovirus grouping fluids indicated that the isolate was an alphavirus. Results of cross-neutralization tests with chikungunya (S-27 strain), ONN (Ahero strain), Semliki Forest (NgMP-4 strain) and KsMP-172 viruses and antisera produced in mice indicate that KsMP-172 is similar or identical to ONN virus (Table).

Investigations are underway to attempt to determine whether ONN virus activity is increasing on the Kano Plain and if this increase may indicate an impending epidemic.

REFERENCES

Bowen et al., (1973). Trans. Roy. Soc. Trop. Med. Hyg. 67: 702-709.
Johnson et al., (1977). Trans. Roy. Soc. Trop. Med. Hyg. 71: 512-517.
Johnson et al., (1977a). Trans. Roy. Soc. Trop. Med. Hyg. 71: 518-522.
Johnson et al., (1978). J. Med. Virol. 2: 15-20.

TABLE

INDICES OF NEUTRALIZATION OF 10^5 BHK cell PFU OF RELATED VIRUSES BY HOMOLOGOUS OR HETEROLOGOUS MOUSE ANTISERUM DILUTED 1: 20.

VIRUS \ ANTISERUM	KsMP 172	ONN	SEMLIKI	CHIKUNGUNYA
KsMP 172	<u>3</u>	3	0	2.5
ONN	3	<u>3</u>	0.5	2.5
SEMLIKI FOREST	0	0	<u>2.7</u>	1.2
CHIKUNGUNYA	0.4	0.3	0.2	<u>3</u>

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REPORT FROM THE ARBOVIRUS RESEARCH UNIT
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I. Studies on Certain Tickborne Viruses

A. Seroepidemiology of Thogoto virus

Thogoto (THO) virus (Bunyaviridae: ungrouped) presents a potential threat to human and animal health in Egypt. THO virus was isolated in Egypt in 1970 from *Hyalomma a. anatolicum* ticks from camels in the Imbaba camel market near Cairo but no serological survey had been made until the present study was undertaken.

THO virus was first isolated in 1960 from the ticks *Boophilus decoloratus* and *Rhipicephalus* spp. from cattle in Thogoto Forest near Nairobi, Kenya (Haig et al. 1965). THO isolates have been reported from 7 tick species (4 genera) in Africa: *B. decoloratus* (Nigeria, Central African Republic, Cameroun, Uganda), *Hyalomma anatolicum anatolicum* (Egypt), *Rhipicephalus appendiculatus* (Kenya). *R. simus* (Kenya), and *R. evertsi* (Kenya, Ethiopia). [Strains from *R. bursa* and domestic animals in Sicily, Italy, previously ascribed to THO virus, are now considered to represent a separate virus in a THO serogroup.] (Srihongse et al. 1974).

THO virus was also isolated from humans, cattle, and camels in Nigeria. Moore et al. (1975) reported that the isolates from human cases were associated with serious optic neuritis and with fatal meningoencephalitis. Neutralizing antibodies were detected by Haig et al. (1965) in cattle (12/53), sheep (3/47), and goats (1/15) from Rift Valley, Kenya, and cattle (15/15) from Entebbe, Uganda.

In the first serological survey to determine the presence of THO virus in Egypt, 2,646 sera (455 from humans, 2,191 from domestic animals and *Rattus rattus*) were investigated by the hemagglutination-inhibition (HI) and complement-fixation (CF) tests. No antibodies to THO virus were detected in sera from pigs and horses (Table 1). HI antibodies were detected in 0.44 to 10.48% (avg 6.3%) of the other sera. CF antibodies were lower prevalence and positive percentages. HI titers were 1:10 to 1:40. CF titers were chiefly 1:4, with a few 1:8. The 2 HI positive human sera were from a male and a female aged 30 and 35 years, respectively, from Sharqiya Governorate in the southeastern sector of the Nile Delta; the HI titers were only 1:10.

The earlier isolation of THO virus from camel-infesting *Hyalomma a. anatolicum* from near Cairo (Williams et al. 1973) provided the first evidence for the presence of this virus in northern Africa and the only evidence for the association of THO virus with *Hyalomma* ticks. The present serological data show that in Egypt this virus also infects humans and other domestic animals (cattle, camels, sheep, goats) reported to be serologically and/or virologically positive in Sub-Saharan Africa, as well as the donkey, domestic buffalo, and commensal rat (*Rattus rattus*), which have not previously been recorded in THO epidemiology. No studies have been made in Africa or elsewhere of the veterinary importance of this virus. However, the THO isolates from human cases presenting in Nigeria with serious optic neuritis and fatally-ending meningoencephalitis (Moore et al. 1975) point to this agent as a distinct threat to human health.

Two of the 6 tick species associated with THO virus south of the Sahara also occur in Egypt: *Boophilus annulatus* and *Rhipicephalus simus*. In addition, *Amblyomma variegatum* and *R. evertsi* are sometimes introduced with imported cattle and camels and the former species is also found in Egypt on northward-migrating birds. *Hyalomma a. anatolicum*, one of the most common members of the Egyptian tick fauna, is distributed from Bengladesh to West Africa.

The role of B. annulatus, H. a. anatolicum, R. simus, and other Rhipicephalus species in disseminating THO virus among the human population and the domestic and wild animal fauna is in need of investigation in Upper and Lower Egypt and in the Eastern, Western, and Sinai deserts. Extending these studies to the rich tick faunas of the Sudan and of Arabia would be useful epidemiologically. Four or more different tickborne viruses of medical/veterinary importance have been isolated from each of these tick species in Africa and/or Eurasia.

Differences in dynamics of appearance and disappearance of HI and CF antibodies to THO virus probably account for the higher prevalence rate in the HI test (Table 1). In sera from Sicily, Albanese et al. (1971) employed the HI test and in sera from Kenya and Uganda, Haig et al. (1965) employed the neutralization test. However, Moore et al. (1975) considered that existing techniques are probably inefficient for detecting antibodies to THO virus (and to Dugbe virus).

B. Antibodies against Bhanja virus

In a previous report, we discussed the presence of antibodies to Bhanja (BHA) virus in sera from the sheep, buffalo, cow, goat, and camel. This finding provided the first evidence for BHA virus circulation in Egypt and North Africa. BHA has not been isolated in Egypt but certain tick species from which it has been isolated elsewhere exist here: Hyalomma marginatum, H. truncatum, Amblyomma variegatum, and Boophilus annulatus.

In the HI test, the prevalence rate for BHA virus in Egypt is 4 times higher than in the CF test (Table 2). For THO virus, the prevalence rate in the HI test is 6 times higher than in the CF test (Table 1). Thus, these 2 viruses behave more or less similarly in respect to dynamics of appearance and disappearance of HI and CF antibodies.

C. Comparative prevalence rates of tickborne viruses of potential public health importance

In a previous report, we indicated the presence in Egypt of CF antibodies agains 4 tickborne viruses associated with human diseases: Dugbe (DUG), Crimean-Congo hemorrhagic fever (CCHF), Bhanja (BHA), and Tettnang (TET). To compare prevalence rates, we examined certain animal sera for CF antibodies against these 4 viruses, together with Thogoto (THO) virus antibodies.

CF antibodies against these viruses were detected in sera from the sheep, buffalo, cow, and goat (Table 3). Sera from the pig, donkey, and camel were positive for 1, 2, and 3 viruses, respectively. The highest percentage of overall positive reaction was for DUG, followed by CCHF and BHA, and lastly THO and TET viruses. The comparative serological findings for THO and BHA viruses (stated above) lead us to suspect that the percentages of prevalence would be higher than those shown in Table 3. This subject is now being investigated.

II. Rift Valley fever virus sero-epidemiology

In October 1977, a Rift Valley fever (RVF) outbreak occurred in Sharqiya Governorate, in the Nile Delta northeast of Cairo. The disease erupted suddenly and presented mainly as an acute febrile, dengue-like illness of humans. Severe cases complicated by hemorrhage, jaundice, meningoencephalitis, and retinitis were also encountered. This epidemic represents the first incidence of RVF virus in Egypt and North Africa. In Kenya, Uganda, South Africa, and elsewhere in Africa, RVF has been considered to be an enzootic disease with occasional epidemic extension into the human population. The outbreak in Egypt, on the contrary, appeared first in humans, and in an explosive manner, and was also unique in the relatively numerous severe complicated cases and in the fact that the first reports were of fatal cases with diffuse hemorrhage and liver failure.

Human cases were initially observed on 28 September 1977 at Inshas, Sharqiya. The disease spread rapidly to more than 21 villages in Sharqiya and in a few weeks to Qalyubiya and Giza Governorates of the Nile Delta. Later (about 11 December 1977), cases appeared in the Nile Valley of middle Egypt (Minya and Asyut Governorates). By the end of December, the human epidemic ceases. Official data showed about 18,000 cases, with 598 fatalities, a C.F.R. of 3.3%. In 80% of fatalities with hemorrhagic manifestations, a pre-existing advanced bilharzial hepatosplenomegaly was observed. The 0.2% C.F.R. of the disease among military personnel possibly reflects the younger age, better health, accurate reporting, and early medical care for this group. Morbidity in the civilian population of most communities reached a peak within 2-3 weeks. The longest period of common disease within a community was 6 weeks and the highest incidence rate was 21.5%. RVF attacked all ages and both sexes with a higher incidence in adult males.

In late June 1978, human RVF cases reappeared in Sharqiya Governorate, but in different localities. The virus identified as RVF was isolated from 28 patients, 1 sheep, and 2 engorged Culex pipiens mosquitoes. More than 200 human RVF cases were officially reported from late June 1978 to the end of 1978. The predominant clinical picture was a febrile syndrome, with few fatal cases exhibiting hemorrhages or liver failure. Clinical RVF in animals appeared in 3 new Governorates (Damietta, Kafr El Sheikh, and Gharbiya) which were believed to have been free of the disease in 1977.

In 1979, a nation-wide serological survey of human and animal sera was undertaken. Sera exhibiting HI antibodies (titers 1:20 or more) and/or CF antibodies (titers 1:8 or more) were considered positive. Table 4 shows the results of the human survey. RVF antibodies were detected in 21 of the 25 surveyed Governorate, with an overall prevalence rate of 5.5%. In Lower Egypt (north of Cairo) the coastal Governorates (Matruh, Alexandria, Damietta, Port Said, Ismailia, and Suez) exhibited a lower positive incidence (0.9%) than the inland Governorates of the Nile Delta (6.1%); the highest prevalence was in Sharqiya and Qalyubiya Governorates (15.5% and 6.0%, respectively) where the epidemic of 1977 first started in the first Governorate and quickly spread to the second. For Upper Egypt (south of Cairo), Assiut Governorate (19.1%) showed the highest prevalence rate (Fig. 1). The relatively high incidence among the human sera from Sinai (7.0%), surveyed for the first time, corresponded to the HI antibody incidence encountered in the surveyed animal sera from Sinai (Table 5).

References

Albanese, M.; G. Di Cuonzo; G. Randazzo; S. Srihongse and G. Tringali. (1971). Survey for arbovirus antibodies in domestic animals of western Sicily. Ann. Sclavo, 13(5):641-647.

Darwish, M. A.; Z. E. I. Imam; and F. Omar. (1978). Seroepidemiological study for Rift Valley Fever virus in humans and domestic animals in Egypt. J. Egypt. Pub. Hlth. Assoc., 53:153-162.

Haig, D. A.; J. P. Woodall and D. Danskin. (1965). Thogoto virus: A hitherto undescribed agent isolated from ticks in Kenya. J. Gen. Microbiol., 38(3):389-394.

Moore, D. L.; O. R. Causey; D. E. Carey; S. Reddy; A. R. Cooke; F. M. Akinkugbe; T. S. David-West and G. E. Kemp. (1975). Arthropod-borne viral infections of man in Nigeria, 1964-1970. Ann. Trop. Med. Parasit., 69(1):49-64.

Srihongse, S.; M. Albanese, and J. Casals. (1974). Characterization of Thogoto virus isolated from ticks (Rhipicephalus bursa) in Western Sicily, Italy. Am. J. Trop. Med. Hyg., 23:1161-1164.

Williams, R. E.; H. Hoogstraal; J. Casals; M. N. Kaiser and M. I. Moussa. (1973). Isolation of Wanowrie, Thogoto, and Dhori viruses from Hyalomma ticks infesting camels in Egypt. J. Med. Ent., 10(2):143-146.

(Medhat A. Darwish and Imam Z. Imam)

Table 1. Antibodies to Thogoto virus in sera from Egypt

Sera	No. tested	HI antibodies No.	HI antibodies %	CF antibodies No.	CF antibodies %
Human	455	2	0.44	ND*	-
Horse	16	0	0	0	0
Pig	120	0	0	0	0
Donkey	61	2	3.28	0	0
Sheep	562	48	8.54	12	2.14
Goat	646	52	8.05	3	0.46
Buffalo	124	13	10.48	2	1.61
Cow	421	40	9.50	7	1.66
Calf	18	0	0	1	5.56
Camel	78	1	1.28	1	1.28
Rattus	145	9	6.21	ND	-
Total	2,646	167	6.31	26	0.98

*ND = Not done.

Table 2. Antibodies to Bhanja virus in sera from Egypt

Sera	No. tested	HI antibodies No.	HI antibodies %	CF antibodies No.	CF antibodies %
Human	433	0	0	0	0
Horse	16	0	0	0	0
Donkey	61	2	3.28	1	1.64
Sheep	531	22	4.14	5	0.94
Goat	646	21	3.25	8	1.24
Buffalo	124	9	7.25	6	4.84
Cow	421	12	2.85	3	0.71
Calf	18	3	16.66	0	0
Camel	78	14	17.94	3	3.85
Pig	120	10	8.33	0	0
Rattus	145	16	11.03	ND*	-
Total	2,593	109	4.2	26	1.00

*ND = not done.

Tabld 3. Complement-fixing antibodies against 5 tickborne viruses in animal sera

Sera	No.	DUG		CCHF		BHA		THO		TET	
	tested	No.	%	No.	%	No.	%	No.	%	No.	%
Sheep	356	16	4.5	4	1.12	3	0.84	8	2.25	6	1.68
Buffalo	310	20	6.45	24	7.74	15	4.84	6	1.93	12	3.87
Cow	337	12	3.56	4	1.19	4	1.19	7	2.1	8	2.37
Goat	325	12	3.69	5	1.54	6	1.85	3	0.92	2	0.61
Camel	110	5	4.54	2	1.82	6	5.45	-	-	-	-
Pig	190	-	-	-	-	5	2.63	-	-	-	-
Donkey	100	-	-	-	-	2	2.0	2	2.0	-	-
Total	1,728	65	3.76	39	2.26	41	2.37	26	1.50	28	1.62

Table 4. Serological study on human sera from Egypt (1979) for RVF virus antibodies

Locality	No. tested	Positive sera	
		No.	%
A. Lower Egypt (Total)	16,045	900	5.6
1. Qalyubiya	1,014	61	6.0
2. Minufiya	2,874	55	1.9
3. Gharbiya	1,491	45	3.0
4. Sharqiya	4,100	635	15.5
5. Daqahliya	1,877	24	1.3
6. Kafr el Sheikh	1,142	37	3.2
7. Beheira	1,990	28	1.4
Total inland Governorates	14,488	885	6.1
8. Matruh	0,180	0	0
9. Alexandria	0,273	0	0
10. Damietta	0,270	8	2.9
11. Port Said	0,270	0	0
12. Ismailia	0,419	3	0.7
13. Suez	0,145	4	2.7
Total coastal Governorates	1,557	15	0.9
B. 14. Cairo	0,091	3	3.3
C. Upper Egypt (total)	9,914	526	5.3
15. Giza	1,421	42	2.9
16. Faiyum	0,518	15	2.9
17. Beni Suef	0,869	10	1.2
18. Minya	1,383	53	3.8
19. Asyut	1,445	276	19.1
20. Sohag	1,758	22	1.3
21. Qena	0,198	10	5.0
22. Aswan	1,544	97	6.3
23. Wadi el Gedeed	0,712	1	0.1
24. Red Sea	0,066	-	-
D. 25. Sinai	0,313	22	7.0
Total for Egypt	26,363	1,451	5.5

Table 5. Serological study on animal sera from Sinai, Egypt (1979) for RVF virus HI antibodies

Animal species	No. tested	Positive sera No.	Positive sera %
Sheep	120	22	18.3
Goat	511	63	12.3
Camel	25	2	8.0
Donkey	4	0	0
Rodent	67	6	8.9
Total	727	95	13.0

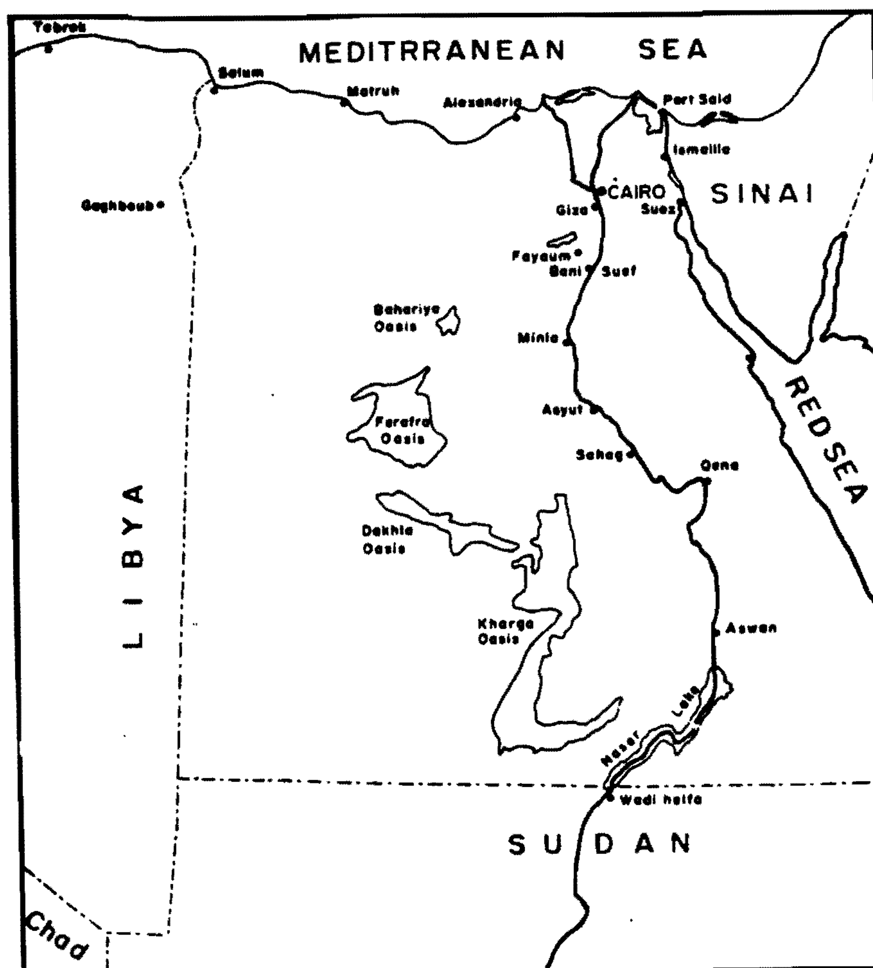

FIGURE 1.

REPORT FROM WHO COLLABORATING CENTRE FOR ARBOVIRUS REFERENCE AND RESEARCH, INSTITUTE OF VIROLOGY, BRATISLAVA, CZECHOSLOVAKIA

Morphology of the tick bite and virus transmission by vectors

The most common vectors of viruses and rickettsiae in Central Europe are the ticks of genera Ixodes, Haemaphysalis and Dermacentor. The transfer of virus occurs during feeding. Either the vector becomes infected from the viremic host or vice versa. The time of attachment varies in different tick species. An example of short-feeding ticks are the larvae of H. inermis, which become attached for 1 hour or the nymphs and adults of Argas persicus, which are fed for 10-15 min. On the other hand, females Ixodes ricinus are slow feeding for up to 10 days.

The salivary glands of ticks consist of 4 types of alveoli; females reveal 3 types. The first type is agranular and has an osmoregulatory function in the exchange of water and salts. The alveoli of types II and III contain up to 9 cell kinds. They secrete chemotactic substance, prostaglandins, enzymes /nonspecific esterase, acetylcholin esterase, phosphatase, protease/ and proteoglycans. The latter form the "cement" which surrounds the inserted mouthparts. Alveoli of type II contain the "b" cells and alveoli of type III the so-called "e" cells, which take part in cement secretion and reveal PAS positive granules in their cytoplasm.

The sucking of Haemaphysalis inermis larvae lasts an average 1 hour. The insertion site is difficult to find even in serial sections. No cement formation can be seen. In contrary, the nymphs form cement, clearly visible around the chelicera. The sucking of nymphs lasts for about 3-4 hours; the mouthparts

become deeply inserted into the upper corium. The sucking of the female _Haemaphysalis inermis_ shows a similar picture. During the feeding, which procedes for 5-7 days, vasodilatation, slite infiltration with macrophages and polynuclear leukocytes develops in the deep corium layer.

During the feeding of _Dermacentor reticulatus_ larvae superficial cement surround the chelicerae and the hypostom. The mouthparts are inserted deeply into epidermis, but they do not reach the corium in the upper corium, vasodilation and mononuclear and polynuclear infiltration can be seen. Vasodilatation may due to pharmacodynamic substance and histamin-blocking agent presented in the tick´s saliva. Fully fed larvae _D. marginatus_, which became infected on viremic mice, revealed the antigen of the tick-borne encephalitis virus /TBE/ in the oesophagus, in the suboesophageal ganglion and in some alveolar cells in the salivary glands. Only a few gut cells were affected. Brightly fluorescing granules were observed in the lumen of Malpighian tubuli, which were not positive in controls stained with the conjugate only. We consider the penetration of TBE virus from the oesophagus to the tick "brain" as the possible route of primary infection. After metamorphosis, viruliferous nymphs showed virus titres up to $10^4 LD_{50}/0.03$ ml of suspension when tested in suckling mice. Transfer of virus into the host body was confirmed by induction of antibodies to TBE in the blood of mice /titres from 1:4 to 1:16 respectively/ used for the feeding of the nymphs. TBE antigens were seen in serial sections of viruliferous nymphs mainly in the epidermal columnar cells and in a few gut cells. The immunofluorescence findings were essentially same also in viruliferous _I. ricinus_ females. It is probable that the columnar epidermal epithelium might be the site of the virus survival during metamorphosis.

During feeding the TBE virus is deposited into the host lymphatics and transported to the regional lymph nodes. In the vicinity of the insertion site scarce fluorescing fibrocytes can be seen indicating the area where the virus had been deposited.

The mouthparts of the *Ixodes ricinus* female penetrate deeply into the corium and subcutis. Within 4 hours, the chelicerae are surrounded by PAS positive structureless substance, which also shows positive reaction for collagen. There is no distinct internal cement formation, because the carbohydrate protein secretions of the alveolar glands is mixed with the collagen in the internal layer of the newly formed conus. The outer cell-rich layer of the conus contains the nuclei of the proliferating fibrocytes. The conus is surrounded by a dense inflammatory infiltrate, which contains many polynuclear leukocytes. Within 36-72 the mononuclear phagocytes predominate. The epidermis occasionally proliferates inwards in the direction of the insertion site. The half-engorged females when stained in serial sections by fluorescent antibody method showed specific fluorescence of the TBE antigen /s/ at 36 and 60 hours in the columnar epidermal cells.

The different distribution of TBE virus in primoinfected larvae on one hand and in viruliferous nymphs and females on the other hand may be important for viral persistence.

J. Rajčáni and J. Nosek

REPORT FROM THE MEDICAL INSTITUTE OF ENVIRONMENTAL HYGIENE, ARBOVIRUS LABORATORY*, AUF'M HENNEKAMP 50, D-4000 DUESSELDORF, WEST GERMANY

Inactivation of Dengue-2 Virus by Heat, Formalin and ß-Propiolactone

The dengue-2 virus strain, employed for our experiments, was derived through the courtesy of Prof. KUNZ, Vienna. The virus was propagated in 3 day old babymice (NMRI-race). Infected mice died at the 5th to the 7th day (average 6th day). The brains of the 6th to the 8th mousepassages, carried through in our institute, were pooled and the infective dosis was adjusted to 1.6×10^7 PFU/ml before each inactivation.
We examined the following three comparable inactivation methods, following the methods of WHITE et al. (1971) for the most part:

a) <u>heat inactivation</u>: incubation for 72 h at 41°C

b) <u>ß-propiolactone inactivation</u>: incubation for 96 h at 4 to 6° C with final concentration of 0.16% ß-propiolactone

c) <u>formalin inactivation</u>: incubation for 96 h at 22° C with final concentration of 0.05% formalin, followed by storing at 4 to 6° C.

During the first 24 hours every 4 hours and then every 12 hours we took samples from the virus pool for titration of the residual infectivity.
First we titrated the subsequent samples in Verocell cultures under methylcellulose overlay (in CO_2- incubator) to find the zero point of the inactivation curve. We found the following zero points of the three different inactivating treatments:

a) heat: after 12 hours
b) ß-propiolactone: after 16 hours
c) formalin: after 24 hours.

We respected a safety factor of 4 hours. That means the time of inactivation was extended for 4 hours, to make sure that there would not be any residual infectivity during our immunization experiments. Besides this all vaccines were tested for infectivity by direct inoculation of 0.2 ml into Verocell cultures and of 0.02 ml into each of a litter of 3 day old NMRI-mice (average of 10 mice per litter).

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*) Formerly VACCINATION CENTER (LANDESIMPFANSTALT)

14 day old NMRI-mice were immunized by a series of five inoculations of inactivated virus suspensions. The mice were vaccinated on day 1 and 4 by injections of 0.2 ml by subcutaneous route and on day 21, 24 and 27 by injection of 0.25 ml intraperitoneally, following BRANDT et al. (1967) who found this method optimal for antibody production in mouse ascitic fluid. Each of the immunization groups consisted of a litter of NMRI-mice (8-13 mice per litter).

All animals were challenged intracerebrally with 100 mice LD_{50} of the 8th mousepassage 14 days after the last immunization. After the challenge test the mice were observed upto day 31, to find the rates of survival, mainly between day 11 and 31 after challenge day. Normally mice die during an intervall of 8 or 9 days (7-10 days), if they were infected with dengue-2 virus at an age of 14 days.

Results: 9 of 12 (75%) mice inoculated with heat inactivated virus survived the period from day 11 to 31, no one of 9 (0%) in the ß-propiolactone group and 4 of 12 (33.3%) mice in the formalin group in contrast to 2 of 13 (15.4%) control mice. All survival rates above 62% were significant at the p=0.05 level. It means that only the heat inactivated virus produces a sufficient protection in mice against challenge with live dengue-2 virus.

In another experiment with the three inactivated vaccines, we added FREUND's adjuvant in equal volumes to the antigen and inoculated on day 1 and 4 0.4 ml subcutaneously with FREUND's complete adjuvant and 0.5 ml on day 21, 24 and 27 intraperitoneally with the incomplete adjuvant. The challenge test was the same as above.

Results: In the heat inactivated vaccine group 5 of 9 (55.5%) mice survived during 31 days, in the formalin group 4 of 9 (44.4%) and in the ß-propiolactone group 5 of 12 (41.7%), while all of the control mice (0%) died. In this experiment only the heat inactivated virus produced a significant protection (more than 46% survival rate at p= 0.05 level).

In a third experiment we added aluminiumhydroxid [$Al(OH)_3$] in the same way as FREUND's adjuvant to the inactivated virus suspensions. The schedule of immunization was the same as above.

Results: 2 of 9 (22.2%) mice immunizied with heat inactivated virus plus $Al(OH)_3$ survived the period of 31 days, in the formalin group 6 of 9 (66.7%) in the ß-propiolactone group only 1 of 9 (11.1%). 2 of 13 (15.4%) control mice survived the challenge test. If 62% of mice survive the challenge test, there is a significant rate of surviving mice at a p=0.05 level. Only the survival rate in the formalin group is significant.

These examinations show that an inactivation of dengue-2 virus by heat (41°C for 16 hours) produces a good protection in mice against a challenge with live dengue-2 virus. The combination of heat inactivated virus material with FREUND's adjuvant as well as the combination of formalin inactivated virus with aluminiumhydroxid proved suitable for vaccination, too.

A semiquantitative IgG- and IgM determination by radial immundiffusion in agargel confirmed these results. The mice of groups with significant survival rates showed higher IgG and IgM-titers in comparison with the other experimental groups.

There are only few results of inactivation experiments with togaviruses in the literature which may be compared with our own. WHITE et al. (1971) demonstrated an analogous immunological protection of a heat inactivated vaccine of Eastern Equine Encephalomyelitis Virus. But they found a better efficacy of the ß-propiolactone inactivation in comparison with the formalin inactivation. Our formalin inactivated virus was of higher immunogenic potency, but only significant after addition of aluminiumhydroxid. MUSSGAY et al. (1973) found a better efficacy of the formalin vaccine of the Semliki Forest Virus when they compared it with the ß-propiolactone inactivated virus.

Literature:

1) W.E. BRANDT; E.L. BUESCHER and F.M. HETRICK (1967): Production and Characterization of Arbovirus Antibody in Mouse Ascitic Fluid Am. J. Trop. Med.Hyg. 16, 339-347.

2) O.R. EYLAR and C.L. WISSEMAN, Jr. (1975): Thermal Inactivation of Type 1 Dengue Virus Strain Acta Virol. 19, 167-168.

3) M. MUSSGAY and E. WEILAND (1973): Preparation of Inactivated Vaccines against Alphaviruses Using Semliki Forest Virus-White Mouse as a Model. I. Inactivation Experiments and Evaluation of Double Inactivated Subunit Vaccines Intervirology 1, 259-268.

4) A. WHITE, S. BERMAN and J.P. LOWENTHAL (1971): Inactivated Eastern Equine Encephalomyelitis Virus Vaccines Prepared in Monolayer and Concentrated Suspension Chick Embryo Cultures. Appl.Microbiol. 22, 909-913.

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A new method for virucide testing

The major problems for virucide testing are :

- Rapid removal of excess disinfectant
- Toxicity of disinfectant for tissue cultures
- Necessity of high virus titers
- Difficulties to detect low residual viral activity
- Exact time of contact between virus and disinfectant

In order to solve these problems we developed the virus virucide floating technique.

A block 2% agarose of about one square cm is cut and mounted on the edge of a glass slide. A well known quantity of virus suspension is put on the surface of the agarose and allowed to dialize and evaporate until the surface is completely dry; this usually takes about 10 minutes. Two drops of formvar solution are then added and left for a few seconds, and excess formvar is removed by keeping the slide vertically against blotting paper. When the film is dry, the edges of the block are cut with a sharp knife. By dipping the block gently into the disinfectant solution a film, containing the virus, is released and floats on the surface of the disinfectant solution. This moment of virus - disinfectant contact is noted. After a well-known time of contact the virusfilm is removed from the disinfectant by picking up the film with a sealed Pasteur pipette. Excess disinfectant still present is removed by gently touching the film against the side wall of a tube. The film is transferred into 1 ml tissue culture medium and disrupted with the Pasteur pipette to bring residual virus particles free in solution. This solution is inoculated into tissue culture to determine residual infectious virus. In control experiments the disinfectant was replaced by tissue culture medium.

Results obtained by this method are represented in

Fig. 1 and Fig. 2

The virus AnY 1444 (an orbivirus) is extremely resistant to 4% formaldehyde.

The advantage of this method can be summarized as follows :

1. The virus to be tested must not be a high titered one.
2. High concentrations of disinfectants highly toxic for tissue cultures can be used.
3. Perfect control of contact time between virus and disinfectant.
4. Rapid and simple removal of excess disinfectant.
5. Possibility to measure very low residual viral activities.
6. The method simulates as much as possible the in-use conditions of the disinfectant. The virus was inoculated onto a surface, allowed to dry, and then treated with the product, according the directions for use.

The method is limited by the chemical resistance of the formvar sheet to the disinfectants. Ethanol can not be tested.

(G. van der Groen, A.A. El Mekki, and S.R. Pattyn)

Measurement of antibodies to Ebola, Marburg and Lassa virus in human sera from East-Zaire and Rwanda

Eighty human sera, obtained in 1979, in the Eastern part of Zaire (Kasongo) and 190 human sera obtained in 1977 in Rwanda (Kigali area), were screened for Ebola, Marburg and Lassa antibodies by the indirect immunofluorescence antibody test (IFA).

Gamma and U.V. irradiated mixed antigen slides with Ebola, Marburg and Lassa infected Vero cells (CDC 801486) kindly provided by the Center for Disease Control, Atlanta, Georgia, U.S.A. were used.

Positive sera were further tested on single antigenslides.

Sheep antiserum against human immunoglobulin G conjugated with fluorescein isothiocyanate (Wellcome lot No. K. 7095) was used at a dilution of 1:80, counterstained with Evans blue in a final concentration of 0.2 percent (w/v).

The sera were tested at a dilution of 1:16.

All sera were negative for Marburg and Lassa antibodies.

Ten percent of the sera from Kasongo (East-Zaire) were positive for Ebola antibodies, all the others were negative.

These results indicate that Ebola virus is endemic in the Eastern part of Zaire and more widespread as thought previously (1).

References

- G. van der Groen & S.R.Pattyn (1979) Measurement of antibodies to Ebola virus in human sera from N.W. Zaire, Ann.Soc. belge Méd. Trop., 59, 87-92.

(G. van der Groen and S.R. Pattyn)

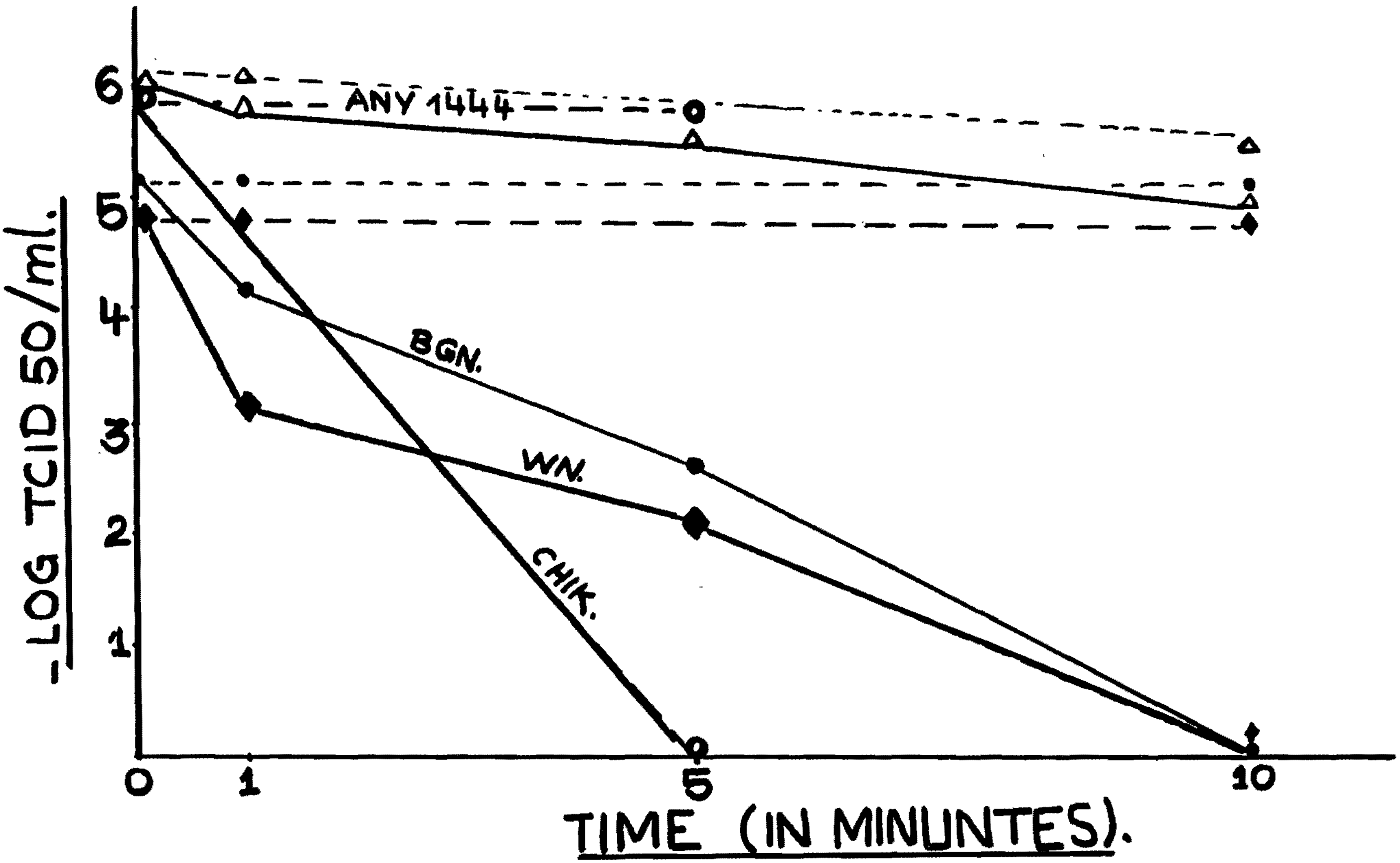

Fig. 1. EFFECT OF 4% FORMALDEHYDE ON SOME ARBOVIRUSES.

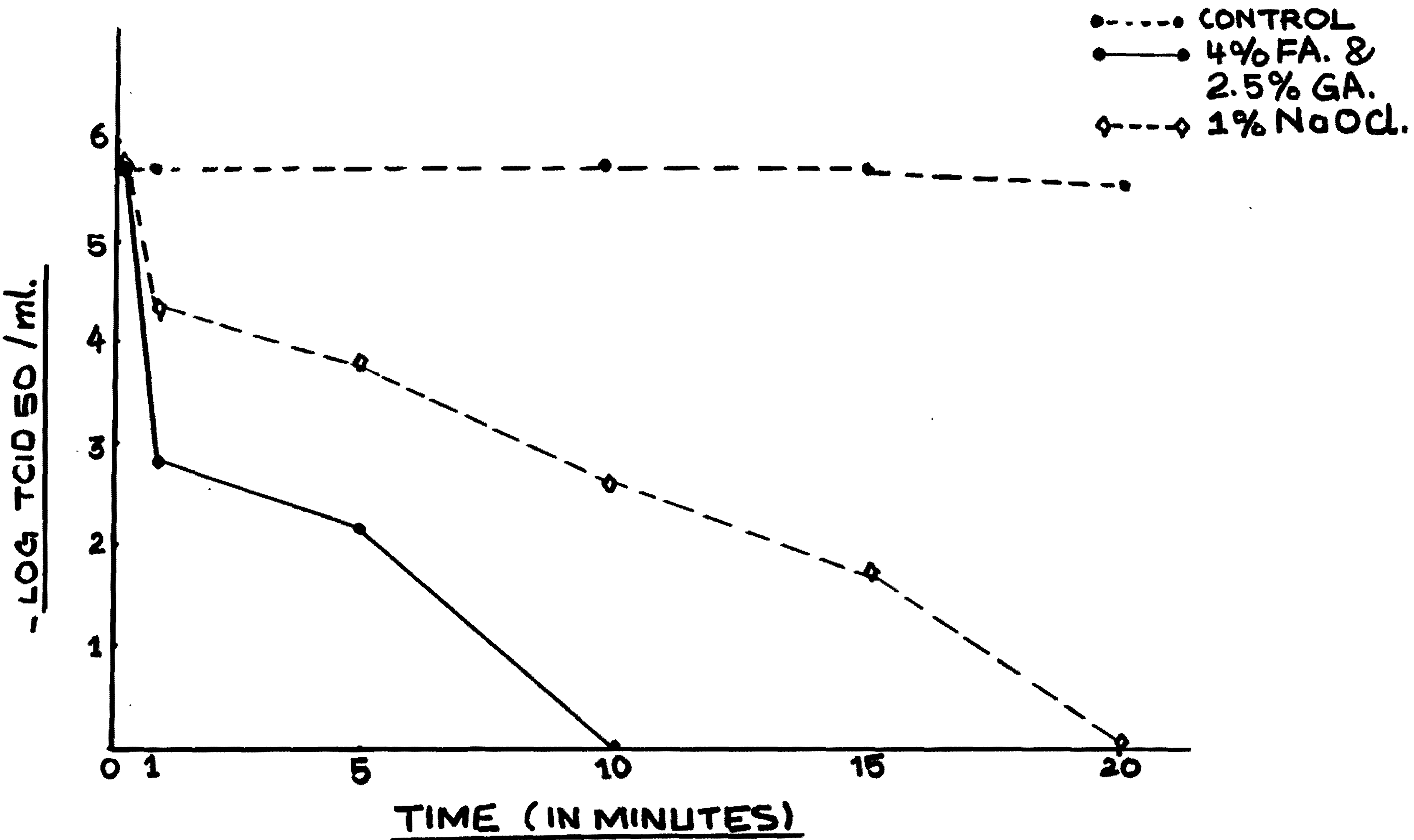

FIG. 2. EFFECT OF DIFFERENT DISINFECTANTS ON VSV.

REPORT FROM ARBOVIRUS LABORATORY,UNIT OF VIRAL ECOLOGY

INSTITUT PASTEUR , PARIS , FRANCE

Two yellow fever virus strains have been isolated in Paris from two fatal human cases imported from Senegal, in November 1979.In both cases, virus was isolated from the blood. No virus was recovered from liver tissue.

In the first case, the virus was easily isolated by intracerebral inoculation of the serum to suckling mice, and, after a second passage, all suckling mice becamse sick on the 6th day. The serum was also inoculated by intrathoracic route to *Toxorhynchites amboinensis* mosquitoes. Ten days later the *Toxorhynchites* were ground and inoculated to suckling mice which were sick on the 8th day. The virus recovered from the brains was identified as yellow fever virus.

In the second case, the isolation attempts were conducted as in the first case, but the virus was isolated *only* from *Toxorhynchites* and not by direct passages on suckling mice. The strain was also identified as yellow fever virus.

In both cases, clinical features and hispathological aspects in the liver were characteristic of yellow fever. The two patients were european tourists and came back from a two weeks journey in Senegal. They were not vaccinated against yellow fever.

These observations could indicate that *Toxorhynchites* intrathoracic inoculation is a more sensitive technique than intracerebral inoculation of suckling mice for yellow fever virus isolation.

(C. HANNOUN, F. RODHAIN, X.JOUSSET).

REPORT FROM THE VIRUS LABORATORY, FACULTY OF MEDICINE, BREST, FRANCE

SEROLOGICAL EVIDENCE OF ARBOVIRUS INFECTIONS IN SMALL MAMMALS IN SPAIN

1. METHODS

During entomological survey in Spain, 386 small mammals (rodents, insectivora, carnivora and cheiroptera) were trapped alive and their blood collected on clotting papers. Specimens were obtained from 15 different places in the North and the South of the country, at the beginning of 1978 and 1979.

Serosurvey was carried out by IH or CF tests using micromethods and the following antigens : Sindbis, West Nile, Tick-borne encephalitis (european type), dengue type 2, Wesselsbron, Calovo, Tahyna, Uukuniemi, Bhanja and Tribec viruses. Non specific inhibitors were removed by aceton.

2. RESULTS

No antibody was found against Sindbis, Wesselsbron, Calovo and Tribec viruses. On the other hand, positive results were observed against West Nile, Tick-borne encephalitis and dengue type 2 viruses for flaviviruses, Tahyna in the California encephalitis group and two bunyaviruses : Bhanja and Uukuniemi. The IH titers ranged from 20 to 160 ; multiple and monospecific reactions were both observed.

The percentages of positive reactions were quite the same in the North and in the South of Spain, but in the northern part of the country, positive reactions were encountered against all the above mentionned antigens, while in the southern part all but one positive reactions concerned Tahyna virus.

Finally, positive reactions were encountered almost entirely in two species of rodents, ***Mus spretus*** and ***Apodemus sylvaticus***, with an unique positive reaction for West Nile virus in ***Crocidura russula***, an insectivora. No antibody was found in small carnivora and cheiroptera.

3. CONCLUSIONS

Results of our serosurvey on small mammals correlated well with those of FILIPE et al (1969-1977), previously obtained on domestic animals and men, in Portugal and the North-Western part of the Spain.

[To be published in detail in : Bull. Soc. Path. exot., Paris.]

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- H. LAUNAY and J.C. BEAUCOURNU, Medical Entomology, Faculty of Medicine, Rennes, France.

SPECIES \ PLACE	NORTHERN SPAIN			SOUTHERN SPAIN			TOTAL (%)	Antigens concerned by positive
	Huesca	Terruel	Zamoza	Jaen	Ronda	Badajoz		
Rodents :								
M. spretus	13/95*	0/3	-	0/27	4/12	4/13	21/150 (14 %)	WN, D_2, TBE-TAH-UUK-BHA
A. sylvaticus	0/55	1/7	5/14	0/45	6/21	0/2	12/44 (8.3 %)	TAH
P. duodecimocostatus	0/6	0/8	-	0/1	0/14	0/17	0/46	
M. arvalis asturianus	-	0/4	-	-	-	-	0/4	
R. rattus	-	-	-	0/1	-	-	0/1	
Insectivora :								
C. russula	1/13	0/7	0/2	0/8	0/1	-	1/31	WN
T. occidentalis	-	-	0/2	-	-	-	0/2	
Carnivora :								
M. nivalis	0/1	0/1	0/1	-	-	-	0/3	
Cheiroptera :								
Pipistrellus sp.	-	0/5	-	-	-	-	0/5	
Total	14/170	1/35	5/19	0/82	10/48	4/32	34/386 (8.8 %)	

* Positive / Tested

REPORT FROM THE SPECIAL PATHOGENS REFERENCE LABORATORY, PUBLIC HEALTH LABORATORY SERVICE CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH, PORTON DOWN, SALISBURY, WILTSHIRE, ENGLAND

TWO HOSPITAL-ASSOCIATED OUTBREAKS OF CONGO/CRIMEAN HAEMORRHAGIC FEVER IN THE MIDDLE EAST

Two quite separate outbreaks of Congo/Crimean haemorrhagic fever occurred in Iraq and Dubai in the second half of 1979. The first episode took place in September 1979 in Yarmouk Hospital, Baghdad when a young woman died from a severe bleeding disease. Shortly afterwards a doctor and a nursing auxiliary, both of whom had cared for the patient, developed a similar disease and both died. Shortly after that other unassociated cases of a similar illness were reported from other areas of Iraq. Specimens from some of these later patients and pools of Hyalomma anatolicum ticks collected in areas where the cases died were processed for virus isolation attempts in suckling mice, guinea pigs and CER, Vero and BHK_{21} cell lines.

An isolate was obtained in suckling mice which by the fourth passage produced a uniformly fatal illness - mice dying between days 5 - 7. This 4th-passage mouse brain material produced obvious cytopathic effects in BHK_{21} cells.

A sucrose-acetone extracted antigen prepared from 4th passage infected mouse brain was tested at a 1/10 dilution against a broad range of arbovirus antisera as well as several NIH polyvalent mouse ascitic fluids. Indirect immunofluorescence tests were carried out against Lassa, Marburg and Ebola antisera. The only positive result was against the Polyvalent Congo antisera. The sucrose-acetone extracted antigen was then tested by complement fixation against the following Congo antisera - AR 10200, AR 7620, 3010 and SE 65 - with the following results:

CFT. Results. Reciprocal of the antibody titre tested against a 1/10 dilution of the SA antigen prepared from the acute blood 12 isolate

Congo Antigens / Congo Virus Antisera	Acute blood 12 antigen	AR 10200	AR 7620	3010	SE 65	Poly Congo	Serum Control
AR 10200	1024	1024					<4
AR 7620	2048		≯1024				8
3010	512			512			<4
SE 65	2048				1024		4
Poly Congo	256					ND	
Antigen Control NMS	<4	<4	<4	<4	<4	-	

2 units of C^1 in the test

In BHK_{21} cells the isolate was identified by indirect immunofluorescence as Congo using the same specific Congo antisera - AR 10200, AR 7620, 3010 and SE 65.

The second episode occurred in the El Rashid Hospital, Dubai. The index case died in the Casualty Department following cardiac arrest and severe bleeding. Five hospital staff - 2 doctors, 2 nurses and an interpreter - all of whom had attempted to resuscitate the patient later developed a severe haemorrhagic illness. The 2 nurses died.

Congo virus was isolated from post-mortem liver of one nurse using the techniques shown above.

Convalescent sera from one recovered case in Baghdad and three recovered patients in Dubai all had Congo antibodies in their sera detected by immunofluorescence at titres between 1 : 128 and 1 : 256.

Three isolates have been made from *Hyalomma* tick species collected in Iraq and Dubai but have not yet been identified.

E T W BOWEN

D I H SIMPSON

REPORT FROM THE EDWARD GREY INSTITUTE OF FIELD ORNITHOLOGY AND THE UNIT OF INVERTEBRATE VIROLOGY, SOUTH PARKS ROAD, OXFORD, ENGLAND.

Tick-borne viruses from seabird colonies

The above named institutions are collaborating on a project to determine what viruses are associated with seabirds. The following is a preliminary report on the isolation of viruses from *Ixodes uriae* (and one bird) collected from seabird colonies in the British Isles. All the isolations were initially made by intracerebral inoculation of 2 day old Swiss mice. We are currently performing neutralisation and complement fixation tests in order to classify these viruses.

St. Abb's Head, Berwick. Four virus isolations have been made from material collected at this site. The first three isolations (Table 1) were made by Dr H Reid (Moredun Institute, Edinburgh). The ticks were collected from crevices in rocks where guillemots nest; the kittiwake was a juvenile and appeared ill. The viruses replicate in BHK, Vero, XTC (*Xenopus laevis*), MRC-5 (human embryonic lung), and calf testis cells. The effect of various physico-chemical conditions on virus infectivity (Table 2) was measured by plaque assay in Vero cell cultures. The viruses were sensitive to sodium deoxycholate, chloroform, and pH 3.0; isolate (2) was sensitive to ether whereas isolates (1) and (3) were relatively resistant. Electron microscopic examination of infected cell cultures revealed particles characteristic of orbiviruses: densely-stained cores 32nm in diameter each surrounded by an outer shell giving an overall diameter of 64nm in diameter. Infected BHK cell cultures also contained two types of tubules, 32 and 64nm in diameter; the smaller tubules were often associated with densely-staining granular areas which resembled viral matrices. Virions budding at the plasma membrane were observed in BHK cell cultures infected with isolates (1) and (3). Cultures infected with isolate (4) also contained particles resembling bunyaviruses. These were oval, long axis 100nm, and had a closely adherent envelope.

Isle of May, Firth of Forth. *I.uriae* were collected from ledges on which guillemots nest. The two virus isolates from these ticks multiply in Vero and BHK cells: one isolate produced a cytopathic effect whereas the other did not. Their morphology was characteristic of orbiviruses.

Shiant Islands, Outer Hebrides. *I.uriae* were collected from puffins and shags (and the hands and arms of the collectors). The virus isolates produce a cytopathic effect in Vero and BHK cells. Infected Vero cells contained characteristic orbivirus particles and particles resembling a bunyavirus.

(P.A. Nuttall, K.A. Harrap, C.M. Perrins & H.W. Reid)

Table 1. Source of the virus isolations

Collection Site	No of isolate	Collection date	Material	Virus
St. Abb's Head	1	17.12.74	22 ♂ *I.uriae*	orbivirus
Berwick	2	27.07.75	10 engorged nymphs *I.uriae*	orbivirus
	3	27.07.75	Kittiwake brain and blood	orbivirus
	4	27.09.79	20 engorged nymphs *I.uriae*	bunyavirus and orbivirus
Isle of May	5	2.07.79	10 engorged ♀	orbivirus
Firth of Forth	6		5 ♂ *I.uriae*	orbivirus
Shiant Islands	7	1.07.79	3 engorged ♀ *I.uriae*	orbivirus and bunyavirus
Outer Hebrides	8		9 ♂ *I.uriae*	orbivirus

Table 2.

Sensitivity of virus isolates to sodium deoxycholate (SDC), ether, chloroform and pH 3.0.

No. of isolate*	log$_{10}$ p.f.u./ml infectivity reduction				
	SDC[1]	ether[2]	chloroform[3]	chloroform (3h)[4]	pH 3.0[5]
1	>7.54	0.80	4.89	1.05	>6.74
2	>7.70	6.04	5.00	>3.48	>5.65
3	>7.18	1.71	5.38	3.20	>6.54
6				2.54	
7				1.99	

* Refer to table 1.

1 0.5% (w/v) SDC for 1 hour at 37°C.

2 50% ether for 18 hours at 4°C.

3 50% chloroform for 18 hours at 4°C.

4 50% chloroform for 3 hours at 4°C.

5 14mM HEPES pH 3.0 (compared with HEPES pH 7.4) for 3 hours at 4°C.

REPORT FROM THE SECTION OF ARBOVIRUSES
LABORATORY OF VIROLOGY
NATIONAL INSTITUTE OF HEALTH
LISBON - PORTUGAL

The organization of a Section of Arboviruses in the Laboratory of Virology in the National Institute of Health of Lisbon was an old aim from the Direction of the Institute and the Laboratory of Virology. In 1979, Dr. Armindo R. Filipe left the Institute of Tropical Medicine and changed to the National Institute of Health where a new laboratory of arboviruses has been in organization since then.

Meanwhile, the program to study the prevalence of the tick-borne viruses in Portugal has been reorganized in an interdisciplinar perspective - medi cal and veterinary - in order to evaluate the situation of the tick-borne diseases of veterinary importance.

In 1971, the Dhori virus was isolated in Portugal from *Hyalomma marginatum* ticks. The work already done with Dhori virus, original strain and Vidiguei ra strain isolated in Portugal, have shown differences in the virulence for the mice between the two strains of Dhori virus. Research work is now in progress to study the expression of virulence and genetic markers with this virus.

Several research work on the morphogenesis of Dhori virus (Vidigueira strain) is now being carried out in our laboratory. The virus was inoculated in Vero, BHK-21 cells and suckling-mice. The observations with electron microscope done by Dr. Maria Francisca Avillez (research worker in charge of virus electron microscopy) have shown that virus particles were pleomorphic, round or oval and with dimensions between 74 nm and 94 nm.
The morphologic data is not enough to classify a virus, however it seems to us that Dhori virus is a Bunyaviridae. Research work on the structure and biochemistry of this virus (original and Vidigueira strain) will try to confirm the results already obtained with electron microscopy.

From *Rhipicephalus sanguineus* ticks, collected in July 1977 from sheep of Vila Viçosa, a small town at 200 km east of Lisbon, were isolated 2 viruses pathogenic for suckling mice. These viruses now at the 3 pas. kill the new born mice after 4 days of i.c. inoculation. However, adult mice are not killed by the same agent. These viruses are waiting further characterization studies.

(Armindo R. Filipe)

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

Dengue in Puerto Rico, 1979-80

During 1979 there were 856 cases of suspected dengue reported to the San Juan Laboratories, compared with 12,324 in the epidemic year of 1978. Half of these were reported during the first quarter, and represent the tail of the dengue-1 epidemic. Serological confirmation of dengue etiology was obtained from 83/311 pairs of sera submitted; the confirmation rate fell from 41% for patients with onset in January to around 10% for those with onset in April through July, 1979, then rose to an average of 38% over the last 5 months of the year. Virus isolations were made from 6 patients with onset of illness in October, November and December, 1979, and all were identified as dengue-1. Three of these patients resided in the metropolitan San Juan area, and the others in municipalities in the center of the island. Two more isolations of dengue-1 virus have been made from metropolitan area residents with onset of illness in January 1980.

Aedes aegypti population indices

Modified New Jersey traps were operated at eight locations in five cities. Weekly collections from these traps ranged from 0 to 9 female *A. aegypti* per trap day during the last 3 months of 1979. Breteau indices remained high during the last 3 months of 1979, the average for three cities being 28.5.

Dengue in Hispaniola, 1979-80

The contract with PAHO for dengue surveillance in this Caribbean island produced specimens from 240 patients from Haiti with onset of illness from August through November 1979, including paired sera from seventeen patients. Five of these were laboratory confirmed as dengue cases, with onset in August, September and November, and four were recent cases. Only two of the remainder were antibody negative (aged 16 and 24 years).

Monthly household surveys of 100 houses in Port-au-Prince revealed *Aedes aegypti* indices as follows:

	House Index	Breteau Index	Container Index
1979 Aug	38%	61%	9.5%
Sep	47%	74%	9.1%
Oct	36%	68%	9.5%

	House Index	Breteau Index	Container Index
1979 Nov	30%	67%	10.5%
Dec	27%	34%	5.9%
1980 Jan	26%	43%	5.8%

These levels of infestation are clearly adequate for transmission of dengue.

From the Dominican Republic, no specimens have been received from patients since May 1979, and no cases reported since July (cumulative total for 1979 was 167). However, in spite of the damage and disruption of services caused by Hurricanes David and Frederick in September 1979, monthly household surveys for A. aegypti of 200 houses in Santo Domingo continued, and gave the following results:

	House Index	Breteau Index	Container Index
1979 Aug	22.5%	32.5%	8.9%
Sept	6.5%	6.5%	2.7%
Oct	13.0%	19.5%	3.4%
Nov	39.6%	52.5%	23.2%
Dec	23.5%	69.5%	9.9%
1980 Jan	15.0%	31.0%	3.6%

These levels are also high enough to support transmission of dengue. The hurricanes temporarily reduced breeding.

Dengue in Mexico, 1979-80

Official reports from Mexico during 1979 indicated that there had been outbreaks of dengue-like disease in the southern and eastern states of Quintana Roo, Yucatan, Chiapas, Oaxaca and Veracruz. There was serological confirmation for the cases along the border in Chiapas and Quintana Roo. Recent reports of cases have come from the states of San Luis Potosi in October and November 1979, and Tamaulipas (Tampico and neighboring towns) in November 1979 through January 1980. Laboratory confirmation is pending on these cases. At the end of February 1980, a team from CDC visited Mexico City, Merida (Yucatan) and Tampico (Tamaulipas) at the invitation of the Mexican Health Department, carried out household surveys for mosquito infestation and dengue antibody rates, and obtained acute and convalescent specimens from cases of dengue-like disease. These studies have resulted so far in the isolation of one strain of dengue type 1 virus from Merida.

(J.P. Woodall, R.H. López-Correa, R. Craven, C.G. Moore, G.E. Sather and G. Kuno. San Juan Laboratories, Center for Disease Control, GPO Box 4532, San Juan, Puerto Rico 00936).