ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 40 March 1981

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Roy W. Chamberlain, Editor
Margaret L. Hopping, Secretary

ANNOUNCEMENT AND EDITORIAL COMMENTS

I wish to announce that I intend to retire during the coming summer and will no longer be the editor of the Arthropod-Borne Virus Information Exchange. However, the Info-Exchange will remain in good hands; Dr. Adrian Chappell has kindly consented to take over the job. I am certain you will give him the same excellent support you have given me.

The Info-Exchange has truly come of age. It is now 21 years old. It was started in 1960 by Dr. Telford Work and remained under his editorship until 1972. Dr. Work, currently Professor of Infectious and Tropical Diseases, School of Public Health, University of California at Los Angeles, was responsible for most of what the Info-Exchange is today--the format, the types of reports, and above all, the encouragement of full international cooperation.

Future reports should be addressed to:

Dr. Adrian Chappell
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Center for Infectious Diseases
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Atlanta, Georgia 30333, U.S.A.

Don't forget, the deadline for issue No. 41 is September 1, 1981.

Best regards to all. It has been a pleasure serving you.

Roy W. Chamberlain

Roy W. Chamberlain, Sc.D.

RECENTLY PUBLISHED BOOKS OF INTEREST TO THE ARBOVIROLOGIST

In case you are not aware of some of the recently published books of interest to the arbovirologist, the following list is presented.

St. Louis Encephalitis. Thomas P. Monath, editor. American Public Health Association, Inc., Washington, D.C., 679 pp., 1980. Library of Congress No. 79-53721; International Standard Book No. 0-87553-090-7.

This book's 16 chapters by 23 contributors covers virtually all aspects of St. Louis encephalitis virus--its history; its physical, biochemical, antigenic and pathological characteristics; its epidemiology and epizootiology; the clinical disease and diagnoses; and prevention and control. Dr. William C. Reeves provided an apt foreword. This book will be the SLE bible for some time to come.

The Togaviruses; Biology, Structure, Replication. R. Walter Schlesinger, editor. Academic Press, Inc., New York/London, 687 pp., 1980. Library of Congress No. 79-6783; International Standard Book No. 0-12-625380-3.

This book has 21 chapters which include an introduction; antigenic characteristics and classification; medical significance; virus-host interactions; immunological aspects; epidemiology and arthropods as hosts and vectors, in nature and experimentally; morphology and morphogenesis; molecular virology; genetics and physiology; growth in cultured arthropod cells; and consideration of non-arbo togaviruses for completeness. In all, 24 scientists have contributed their expertise.

Rhabdoviruses, Volumes I, II, III. David H. L. Bishop, editor. CRC Press, Inc., Boca Raton, Florida, 1980. Library of Congress No. 79-20575; International Standard Book Nos. 0-8493-5913-9 (Vol. I), 0-8493-5914-7 (Vol. II), and 0-8493-5915-5 (Vol. III).

Practically every aspect of the large and diverse family Rhabdoviridae is considered in these three volumes. The various chapters are authored by recognized world experts. Vectors, hosts, biochemistry, pathogenicity and pathogenesis--all these facets and many more are included. As Dr. Bishop states in his foreword, the goal of this series was to provide an overview of rhabdovirology suitable for students, teachers and research workers alike.

Virology in Health Care, by Donald M. McLean. Williams and Wilkins, Baltimore/London, 286 pp., 1980. Library of Congress No. 80-10343; International Standard Book No. 0-683-05864-9.

This well-written and comprehensive little book covers a wide range of viruses and viral infections, but as would be expected of Dr. McLean, whose life-long interest in arboviruses is well known to all of you, the chapter on arboviruses (Togaviridae and Bunyaviridae) was given special touches. Highly recommended reading.

Hagan and Bruner's Infectious Diseases of Domestic Animals, Seventh Edition, by James H. Gillespie and John F. Timoney. Cornell University Press, Ithaca, New York, 851 pp., 1981. Library of Congress No. 80-15937; International Standard Book No. 0-8014-1333-8.

Many of you are undoubtedly familiar with previous editions of this respected textbook of veterinary diseases. In this new edition, deliberate efforts were made to revise and update the portions dealing with arboviruses and arboviral infections. Unfortunately, some room for improvement still remains. For example, there is a tendency to perpetuate relatively unimportant information in historical treatment of some of the viruses (implied role of assassin bugs and chicken mites in perpetuation of WEE), a few mispellings are found (e.g., of Wesselsbron and Middelburg), some mistatements are made because of outdated reference material (louping ill of sheep and spring-summer encephalitis of man attributed to the same virus), and there is an error in systematics (placement of California encephalitis virus with the flaviviruses). However, these faults are relatively minor and do not detract significantly from the value of this excellent textbook.

The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

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REPORT FROM THE SUBCOMMITTEE ON EVALUATION OF ARTHROPOD-BORNE STATUS (SEAS) FOR 1980

During 1980, 18 newly registered viruses and one previously reviewed virus were evaluated by this subcommittee.

These viruses and their sources are as follows:

1. Connecticut. Group Sawgrass - Connecticut, tick.
2. Highlands J. Group A - Florida, bird.
3. Peaton. Group Simbu - Queensland, *Culicoides*.
4. Agua Preta. Ungrouped - Brazil, bat.
5. Altamira. Group Changuinola - Brazil, phlebotomine.
6. Caninde. Group Changuinola - Brazil, phlebotomine.
7. Crimean Hemorrhagic Fever. Group CCHF-USSR, man.
8. Gurupi. Group Changuinola - Brazil, phlebotomine.
9. Ife. Ungrouped. Nigeria, bat.
10. Jamanxi. Group Changuinola - Brazil, phlebotomine.
11. Ourem. Group Changuinola - Brazil, phlebotomine.
12. Toscana. Group Phlebotomus fever - Italy, phlebotomine.
13. Turuna. Group Phlebotomus fever - Brazil, phlebotomine.
14. Aroa. Group B - Venezuela, hamster.
15. Lednice. Group Turlock - Czechoslovakia, mosquito.
16. Sal Vieja. Group B - Texas, rodent.
17. San Perlita. Group B - Texas, rodent.
18. Virgin River. Group Anopheles A - Arizona, mosquito.

In the absence of adequate supporting data, 13 of the 18 agents are considered "possible" arboviruses, 3 are "probable" arboviruses and two, Highlands J (related to Western Encephalitis virus) and Crimean Hemorrhagic Fever virus, are true arboviruses.

One virus, Wad Medani, a Kemerovo group tick agent from the Sudan, was upgraded to the "probable" category on the basis of multiple isolations from various genera (4) of ticks, experimental viremia in domestic animals and infection of ticks feeding on such animals and wide distribution of the virus from Asia and Africa to the West Indies.

The subcommittee continues to provide useful assistance in the editing of submitted data relating to newly registered viruses.

One member, Dr. Albert Rudnick, resigned during the year.

Thomas H. G. Aitken
Chairman

October 1980

THGA:cb

P.S. A subcommittee meeting was held 3 Nov. 80 at Rivermont Ranch, Cleveland, S.C. (O.R. Causey home) with 3 members present. During the course of Am. Soc. Tr. Med. Hyg. meetings, 5-7 Nov. in Atlanta, talks were held with 2 additional members.

Statement from the Subcommittee on Evaluation of Arthropod-borne Status (SEAS)

This subcommittee (and possibly much of the arbovirus community at large) is becomming increasingly concerned with the paucity of information relating to arthropod susceptibility as well as other virus characteristics on new registrations. This observation is not intended to deter registrants from submitting a new agent for cataloguing since a primary aim of the working catalogue is to inform other scientists of the existence of new viruses. Rather, SEAS hopes that the comments which follow will stimulate early submission of data essential to classify a virus as arthropod-borne.

In 1975 when the 2nd edition of the Arbovirus Catalogue was issued, 359 viruses were registered (p. 12). Of these 359 viruses, 42% were acknowledged true or probably true arboviruses. A large group of viruses, 51%, were considered possible arboviruses and the remainder were probably not arboviruses. Since that time, SEAS members have evaluated 70 additional

agents as follows: 1976 (8 agents), 1977 (11), 1978 (17), 1979 (16) and 1980 (18). Each year a plea has been expressed for contributors to be more conscientious in their registrations, however the information continues to be fragmentary. Of the 70 new agents registered during the past 5 years, 76% can only be considered possible arboviruses, to a large extent through absence of experiments involving arthropods.

The criteria for evaluating arboviruses are clearly set forth on page 10 of the Catalogue as well as in the Arbovirus "Info-Exchange" No. 23 (Oct. 1972) and No. 26 (Mar. 1974); the latter report also encourages workers to enhance their new virus registrations with suggestions for vertebrate and invertebrate experimentation.

With a little bit of extra effort on the part of the registrant, it should be possible to determine if a new agent can produce a viremia in one or more experimental animals. Many virus laboratories now have access to mosquito colonies. Even if animal experimentation is not convenient, practicable or possible, rudimentary tests can be performed by inoculating mosquitoes with virus or by feeding them on blood-virus pledgets or droplets and then assaying the salivary glands after a suitable incubation period; indeed, such mosquitoes may also be induced to transmit virus by in vitro feeding on capillary meals or blood droplets. However if such investigations are out of the question, it should be possible to forward the agent to a government laboratory where suitable facilities might be available for entomological studies. Another registration weakness concerns evidence for virus filtration, and some new viruses fail to be studied for resistence or sensitivity to chemicals, etc. The usefulness of the ACAV and its Catalogue could be greatly enhanced by more care taken in the preparation of virus registrations. Contributors are reminded of the instructions for preparing registrations which were published in the "Info-Exchange" No. 29 (Sept. 1975).

Thomas H.G. Aitken, Chairman SEAS

The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1980 ANNUAL REPORT ON THE CATALOGUE OF ARTHROPOD-BORNE AND SELECTED VERTEBRATE VIRUSES OF THE WORLD*

by

THE SUBCOMMITTEE ON ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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 1980, 168 mailings of Catalogue material were being made. During the year, 3 addresses were dropped and 5 new participants were added to the mailing list. At the end of the year, 173 mailings of Catalogue material were being made, including 62 within the U.S.A. and 111 to foreign addresses. Distribution by continent was: Africa 19, Asia 20, Australasia 7, Europe 36, North America 74, and South America 17.

Abstracts and Current Information: A total of 819 abstracts or references were coded by subject matter and distributed to participants during 1980. Of this total, 624 were obtained from Biological Abstracts, 194 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 1 from current journals, personal communications, or other sources. A total of 12,756 references or units of information have been issued since the start of the program.

Registration of New Viruses: Twelve new viruses were registered during 1980. As of December 1979, the Catalogue contained 423 registered viruses. With the acceptance of twelve new virus registrations during 1980, the total number of registered viruses increased to 435 as of December 1980. The viruses registered during 1980 are listed below:

Virus Name	Recommended Abbreviation	Country	Source	Antigenic Group
Connecticut	CNT	U.S.A.	Ixodid ticks	SAW
Highlands J	HJ	U.S.A.	Bird	A
Peaton	PEA	Australia	Culicoides	SIM
Agua Preta	AP	Brazil	Bat	
Altamira	ALT	Brazil	Phlebotomus sp.	CGL
Caninde	CAN	Brazil	Phlebotomus sp.	CGL
Ife	IFE	Nigeria	Bat	
Jamanxi	JAM	Brazil	Phlebotomus sp.	CGL
Gurupi	GUR	Brazil	Phlebotomus sp.	CGL
Ourem	OUR	Brazil	Phlebotomus sp.	CGL
Toscana	TOS	Italy	Phlebotomus sp.	PHL
Turuna	TUA	Brazil	Phlebotomus sp.	PHL

These registered viruses were isolated between 1960 and 1979. HJ was isolated in 1960, GUR in 1961, OUR in 1962, CAN in 1963, AP in 1964, IFE in 1971, TOS in 1972, JAM in 1973, ALT in 1975, PEA in 1976, and CNT and TUA in 1979.

Highlands J (HJ) virus was evaluated as an Arbovirus by the Subcommittee on Evaluation of Arthropod-Borne Status (SEAS)*, Toscana was evaluated as Probable Arbovirus while all others were evaluated as Possible Arboviruses.

None of these viruses have been isolated from man nor have they been reported to produce disease in man.

* 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.

Antigenic Grouping: Previously published data have shown that there are repeatable low-level HI cross-reactions between the Anopheles A serogroup and two serogroups of the Bunyamwera Supergroup (1). This information has been reviewed by SIRACA** and this subcommittee has decided that the data justifies the inclusion of the Anopheles A serogroup in the Bunyamwera Supergroup (2). Likewise, the Bunyaviridae Study Group of the International Committee on Taxonomy of Viruses (ICTV) has decided that members of the Anopheles A serogroup be placed into the Bunyavirus genus (3).

Using information obtained from recently completed antigenic studies, SIRACA has placed Mirim and Guaratuba viruses in the Guama serogroup (2). Previously, Mirim and Minatitlan viruses constituted the Mirim serogroup. Recently, Minatitlan virus was found to be more closely related to an unregistered virus known as Palestina virus. These two viruses now form the Minatitlan serogroup (2). Formerly, Guaratuba virus was considered to be a member of the Bunyamwera Supergroup but unassigned to any serogroup (SBU).

In a paper to be published shortly, antigenic data are presented which support the authors' proposal that a Gamboa serogroup be formed (4). A total of 26 virus strains were shown to be related to prototype Gamboa virus. Antigenic comparison of eight strains, including prototype Gamboa virus, indicated the existence of four distinct viruses and three variants in this serogroup. At present, only prototype Gamboa virus is registered.

By means of HI, indirect immunofluorescence, and neutralization tests, Casals and Tignor have demonstrated the existence of antigenic relationships between 5 serogroups composed of tick-borne viruses (5). The relationships described here were analogous to those observed in formulating the Bunyamwera Supergroup. The serogroups antigenically linked together by the authors include CHF-Congo (CHF-CON), Dera Ghazi Khan (DGK), Nairobi Sheep Disease (NSD), Hughes (HUG) and Qalyub (QYB) groups. Prior to these observations, the CHF-CON and NSD serogroups were taxonomically classified as bunyavirus-like while the other serogroups were unclassified.

A new arbovirus isolated from Ornithodoros capensis ticks has been shown to be related to but distinct from Upolu virus (6). The new arbovirus has been named Aransas Bay virus, and it has been proposed that it and Upolu virus form a new serogroup, Upolu.

Recent studies have indicated that Paramushir virus is antigenically identical to Avalon virus, a member of the Sakhalin group (7). Prior to these observations, Paramushir virus was registered as an ungrouped arbovirus.

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

Information published within the last year or so has indicated that a virus previously isolated from ticks is antigenically related to Bhanja virus (8). The two viruses were determined to be related by the HI test but not by other serological tests, including neutralization and agar gel diffusion tests. This information has been noted on the registration card for Bhanja virus.

Several important decisions were made as a result of SIRACA's review of the antigenic relationships among rhabdoviruses. It was decided that there was insufficient antigenic evidence for the incorporation of the former Mossuril serogroup (with Mossuril and Kamese viruses as members) into the Hart Park serogroup. SIRACA has directed that two distinct serogroups be reconstituted. The Hart Park serogroup should contain Hart Park, Flanders and Mosqueiro viruses as members. The Mossuril serogroup should consist of Mossuril and Kamese viruses. Furthermore, it was decided that antigenic data was insufficient for including Gray Lodge virus in the Hart Park serogroup. For the present, Gray Lodge virus will not be listed as a member of the Hart Park serogroup.

Highlands J virus, isolated in 1960, was finally registered during this year. This virus was not registered previously because it was considered to be an antigenic variant of WEE virus (9). Recent testing by plaque-reduction neutralization and short incubation HI reactions have demonstrated that HJ virus is distinct from both Fort Morgan virus and strains of WEE virus (10). Furthermore, serological tests with antibody to the isolated E_1 glycoprotein of HJ indicated that this virus was antigenically distinct from WEE virus by HI, plaque-reduction neutralization and radio-immune precipitation tests (11). Highlands J virus has been antigenically classified as a separate virus type within the WEE complex (10,12).

Taxonomic Status of Registered Viruses: The Bunyaviridae Study Group of the ICTV has proposed that three new genera be designated within the family Bunyaviridae (3). In addition to the previously established *Bunyavirus* genus, the new sets would be known as the *Nairovirus*, *Phlebovirus*, and *Uukuvirus* genera. According to this proposal which has been accepted, the *Phlebovirus* genus would consist of members of the phlebotomus fever group with sandfly fever Sicilian virus designated as the prototype member. The *Nairovirus* genus would consist of members of the CHF-Congo, Nairobi sheep disease, Hughes, Dera Ghazi Khan, and Qalyub serogroups. In addition to the members of these various serogroups, it is proposed that the antigenically ungrouped Dhori virus be designated as a member of the *Nairovirus* genus. Members of the Uukuniemi serogroup would constitute the *Uukuvirus* genus. In addition, the *Bunyavirus* genus has been expanded to include members of the Anopheles A serogroup (3). A report to be submitted for publication will propose that, in addition to members of the Anopheles A serogroup, members of the Anopheles B and Turlock serogroups be included in the *Bunyavirus* genus (13).

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 435 viruses registered in the Catalogue as of December 1980. 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 (14). 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 56 antigenic groups have been established for viruses registered in the Catalogue. The Gamboa and Upolu serogroups have been added and the previously enlarged Hart Park antigenic group has been broken down again to the Hart Park and Mossuril serogroups. 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 now consists of 14 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 Supergroup was expanded by the addition of the Anopheles A and Gamboa serogroups, and the Mirim group was replaced by the Minatitlan serogroup. Mirim was placed into the Guama antigenic group and Minatitlan was found to be closely related to an unregistered virus known as Palestina virus. The Bunyamwera Supergroup was formulated to reflect low-level but reproducible intergroup relationships usually by complement-fixation and/or hemagglutination-inhibition reactions. In a somewhat analogous situation, the nairoviruses consist of 5 distinct serogroups which share low-level intergroup relationships among themselves. Registered viruses belonging in the Bunyamwera Supergroup constitute approximately one-fourth of all registered viruses.

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

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

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

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

Table 5. Group A arboviruses: Alphaviruses clearly are mosquito associated, although a few have been isolated from other arthropods. 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 in the laboratory. All of the latter viruses are rated as Arbovirus (11 viruses) or Probable Arbovirus (1 virus).

The recently registered Highlands J virus has not been implicated in causing disease in man and has been isolated principally in the eastern United States. Highlands J virus is considered to be a distinct virus type in the WEE complex.

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. 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.

Tables 9, 10, 11, 12, 13, 14, and 15. Bunyamwera Supergroup: There are now 14 antigenic sets of viruses plus the unassigned viruses that comprise the Supergroup. The number of individual registered viruses comprising the Bunyamwera Supergroup increased from 98 to 102 during the past year.

Table 9. Anopheles A and Bunyamwera group viruses: Members of the Anopheles A serogroup have been isolated either from Anopheline or Culicine mosquitoes, or both. Of the three members of this serogroup, only Tacaiuma virus has been reported to cause a febrile illness in man. In addition, this virus has been isolated from man and from a sentinel monkey.

In addition to the three registered members of the Anopheles A serogroup, there are an additional 7 unregistered viruses which have been antigenically characterized. Three of these are considered to be

distinct virus types while the other four are considered to be subtypes or varieties. One of the latter seven viruses has been submitted for registration, and has been named Virgin River virus. 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. Three members of this serogroup have been recovered from rodents, one from livestock, and several others from lagomorphs.

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.

Members have been found most frequently in North America (8), South America (5) and Africa (4). Thus far, only one virus has been recovered in Asia, two in Europe and none in Australasia.

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. Bwamba virus has been reported to produce a febrile illness in man as a result of infections acquired in nature. 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. With the exception of Gumbo Limbo and Nepuyo viruses, all other members have been associated with human febrile illness. In addition, Apeu and Oriboca viruses have been reported to infect man as a result of laboratory mishaps. Ten of the group viruses have been classified as Arboviruses and one as Probable Arbovirus.

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. Capim group members have been recovered only in North and South America.

Table 12. Gamboa, Guama and Koongol group viruses: Gamboa virus has been removed from SBU and placed in a serogroup within the Bunyamwera Supergroup. This new serogroup has been named after Gamboa virus and, at present, Gamboa virus is the only registered member of this antigenic group. The serogroup contains six other antigenically well-characterized but unregistered members. Four are considered to be distinct virus types while the other three are considered to be variants. All seven viruses have been isolated only from Aedeomyia squamipennis mosquitoes; and there is some evidence that their natural cycle may involve transmission between the above-named vector and birds (4).

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 disease in man infected in nature.

The number of members comprising the Guama serogroup has increased from eight to ten with the addition of Guaratuba and Mirim viruses. Previously, Guaratuba virus was placed in the Bunyamwera Supergroup but unassigned (SBU) while Mirim and Minatitlan virus formed the Mirim serogroup. These judgements were made by SIRACA after reviewing recently completed antigenic studies involving these two viruses and other Guama group viruses (2).

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

Table 13. Minatitlan, Olifantsvlei and Patois group viruses: The newly formed Minatitlan serogroup contains one registered member (MNT) and one presently unregistered virus designated as Palestina virus. Previously, Mirim and Minatitlan viruses formed the relatively new Mirim serogroup. When subsequent antigenic studies showed that Mirim virus was more closely related to Guama group viruses, it was removed from the Mirim group and placed into the Guama serogroup. Minatitlan virus was isolated from a sentinel hamster exposed near Minatitlan, Mexico. Little is known concerning its role in nature.

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 14. 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 Peaton virus represents a new member of the Simbu serogroup. It was isolated from Culicoides insects and cattle in Australia, and has been classified as a Probable Arbovirus.

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 15. 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.

As a result of the placement of Guaratuba virus in the Guama serogroup and the formation of the Gamboa serogroup, only two viruses remained unassigned. Jurona virus was isolated from mosquitoes while Kaeng Khoi virus was isolated from bats, sentinel mice and rats, and cimicid bugs.

Table 16. 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. Until recently, their precise taxonomic status was considered unresolved and they were simply designated as bunyavirus-like. Now, three new distinct genera of the family Bunyaviridae have been defined including the Phlebovirus genus consisting of members of the PHL serogroup (3). Sicilian sandfly fever virus has been named the type virus for this new genus.

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 newly registered viruses have been added to the PHL serogroup. Both Toscana and Turuna viruses were isolated from phlebotomine insects. The former virus was recovered in Italy and Turuna virus in Brazil.

Rift Valley fever virus 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 17. Tick-borne groups other than group B viruses. Nairoviruses: Members of the five antigenic groups shown in Table 17 now officially constitute the newly defined Nairovirus genus in the Bunyaviridae family (3). CHF-Congo virus was designated the type virus for this genus. Furthermore, reproducible intergroup antigenic relationships have been demonstrated for these five sets of viruses (5). The investigators who described these observations and, in part, SIRACA have decided that these relationships are no greater than those used to establish the Bunyamwera Supergroup. Thus, they recommend that these serogroups be kept as distinct sets. Only members of the CHF-CON and NSD serogroups have been associated with the production of disease in man.

Crimean hemorrhagic fever virus is in the process of being officially registered. It must be reiterated that 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 CHF-Congo virus.

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.

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 Gamjam to be a variety of NSD.

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.

Table 18. Tick-borne groups other than group B viruses: Viruses belonging to the Uukuniemi serogroup now officially constitute the Uukuvirus genus, a new taxonomic set in the Bunyaviridae family (3). Other serogroups listed in that table remain provisionally classified as bunyavirus-like or are unclassified (Quaranfil Group).

Except for Uukuniemi virus, all members of the Uukuniemi serogroup have been isolated only from ticks. Uukuniemi virus has been recovered from both rodents and birds. 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.

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.

Members of the Sakhalin group provisionally have been designated as bunyavirus-like on the basis of electron microscopic observations of Avalon and Sakhalin viruses. Paramushir virus is now being listed with other members of the Sakhalin serogroup. Recent antigenic data would indicate that Paramushir and Avalon viruses are strains of the same virus (7). Pending withdrawal of one of these viruses, both registered viruses will be listed in the Sakhalin group.

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.

The newly formed Upolu serogroup consists of the registered Upolu virus and a presently unregistered antigenic relative (Aransas Bay virus). Upolu virus was recovered in Australia while Aransas Bay virus also was isolated from ticks collected on coastal islands of southern Texas. Antibody to Upolu virus has not been detected in approximately 150 sera collected from man.

Quaranfil virus has been isolated from both man and rodents, and has been associated with causing disease in man infected in nature. Little is known concerning the behavior of Johnston Atoll virus in nature.

Table 19. Tick-borne groups other than group B viruses: While the viruses in Table 19 also are tick-borne agents, they differ taxonomically from those in Tables 17, 18 in that they have been classified as orbiviruses in the family Reoviridae. The orbiviruses are relatively resistant to lipid solvents, are inactivated at acid pH, and possess 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. The arthropod-borne status of Wad Medani virus has been upgraded by SEAS from Possible Arbovirus to Probable Arbovirus.

Tables 20, 21, 22, and 23. 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 20 are characterized taxonomically as bunyavirus-like. All virus members of these minor antigenic serogroups have been primarily associated with mosquito vectors.

Anopheles B viruses have been isolated only from mosquitoes collected in South America. If a proposal which is to be published shortly is officially accepted, the Anopheles A and Turlock serogroups will be designated taxonomically as members of the Bunyavirus genus. Pending the approval of that proposal, they are presently listed as bunyavirus-like.

Bakau group viruses have been recovered only in Asia. Bakau virus has been isolated from both mosquitoes and ticks, and rodents as well. Additional information concerning these viruses is not available.

Thus far, all four viruses of the Mapputta group have been found only in Australia. Maprik virus is rated as a Probable Arbovirus while the other three virus members are classified as Possible Arboviruses.

Members of the Turlock serogroup are found in Africa, Asia, Australasia, and North and South America. Both Turlock and Umbre viruses appear to be associated with birds.

Table 21. 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. The number of viruses comprising the Changuinola serogroup now stands at seven following the registration of five additional antigenic relatives. All five viruses were isolated in Brazil from phlebotomine flies. Of the present seven serogroup members, only Irituia virus has not been isolated from an arthropod. All others, including Changuinola virus, appear to be associated with phlebotomine insects.

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.

Table 22. 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 only three virus members. At present, SIRACA does not believe that the available antigenic data are sufficient to warrant inclusion of Gray Lodge, Kamese and Mossuril viruses in the Hart Park serogroup. Thus, Gray Lodge virus will not be listed with any serogroup, while Kamese and Mossuril viruses again will form the previously discontinued Mossuril serogroup. All of the present Hart Park group members are associated with a mosquito vector and two of the viruses (Hart Park and Flanders) have been isolated from birds. SIRACA has reviewed the antigenic classification of the Hart Park serogroup and has determined that the set contains one complex (Hart Park) consisting of three separate viruses (Hart Park, Flanders, Mosqueiro). The Mossuril group also contains one complex (Mossuril) comprised of two separate viruses (Mossuril, Kamese).

Thus far the antigenic relative of Kwatta virus has not been registered. Kwatta virus appears to be associated with mosquitoes. Connecticut virus was recently registered and added to the Sawgrass serogroup. This virus was isolated from ticks and 4/14 eastern cottontails possessed NT antibodies to Connecticut virus. Thus, all 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. The Kwatta antigentic set has one complex which is named Kwatta and contains one virus (Kwatta). The unregistered member (BeAn 15757) is considered to be a variety of Kwatta virus. The Sawgrass serogroup consists of one complex which is named Sawgrass and contains three distinct viruses (Sawgrass, New Minto, Connecticut). The Timbo antigenic group also contains one complex (Timbo) comprised of three distinct viruses (Timbo, Chaco, An 303197). The latter virus is currently unregistered.

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 have 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. SIRACA has judged the VSV serogroup to consist of 5 complexes. 1) The VS-Indiana complex contains three distinct viruses (VS-Indiana, Cocal, VS-Alagoas). 2) The VS-New Jersey complex contains VS-New Jersey virus as a single virus type. The Hazelhurst and Concan viruses are considered to be varieties of VS-New Jersey. 3) The Piry complex contains Piry virus as a single virus type. 4) The Chandipura complex contains Chandipura virus as a single virus type. 5) The Isfahan complex contains Isfahan virus as a single virus type.

Table 23. Minor antigenic groups of viruses: These antigenic groups consist of members which are taxonomically unclassified.

Both Boteke group 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. Malakal virus was recovered from mosquitoes collected in Africa, while Puchong virus was found in Asia.

Both Marburg and Ebola viruses cause human disease in nature and have been associated with laboratory-acquired infections. Ebola virus recently was found to possess a single-stranded RNA which is noninfectious.

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

Nyando virus has been isolated from a single case of febrile illness in man. It also was isolated from mosquitoes collected in Africa.

The two 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 24. 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. In addition, Pichinde virus has produced subclinical infections in laboratory workers.

The antigenic classification of Tacaribe group viruses was reviewed recently by SIRACA, and it was decided that the antigenic group consists of two complexes. The Tacaribe complex contains nine viruses including Tacaribe, Junin, Machupo, Latino, Parana, Amapari, Pichinde, Tamiami, and Flexal viruses. The latter virus is currently unregistered. The second complex has been designated the LCM complex and contains LCM and Lassa viruses. Two agents designated as West African and South African viruses are considered to be varieties of Lassa virus. The LCM, West African and South African viruses are not registered.

Table 25. 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.

Of the ungrouped orbiviruses associated with mosquito vectors, two viruses have been found in Africa (LEB, ORU), two in Australasia (JAP, PR) and two in North America (LLS, UMA). Llano Seco virus is antigenically related to Umatilla virus but its relationship to other established orbivirus groups has not been resolved. Thus it and Umatilla virus have been placed with the ungrouped viruses pending a clarification of their 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. It also has been shown that it replicates in mosquitoes and is experimentally transmitted by mosquitoes.

Cotia, a poxvirus, has been reported to produce disease in man during infection acquired in nature. Very little is known concerning the rhabdoviruses listed at the bottom of the table. Gray Lodge virus has been added to the list of rhabdoviruses. Although Gray Lodge virus shows low level CF and NT reactions with one Hart Park and one Flanders strain, SIRACA does not believe that those antigenic data warrant inclusion of Gray Lodge virus in the Hart Park serogroup.

Table 26. Ungrouped mosquito-associated viruses: These serologically ungrouped viruses are associated with a mosquito vector but are taxonomically unclassified. Only Gomoka virus has been recovered from a source other than mosquitoes. It was isolated from a bird collected in Africa. Little else is known of these viruses.

Table 27. Ungrouped tick-, Culicoides-, or Phlebotomus-associated viruses: The serologically ungrouped viruses listed in Table 27 appear to be primarily associated with non-mosquito vectors. Approximately two-thirds of the listed viruses are taxonomically unclassified. All of the bunyavirus-like agents are associated with ticks, as are African swine fever virus and one of the rhabdoviruses (BAR). The other rhabdovirus (BEF) plus the taxonomically unclassified Ngaingan are associated with Culicoides insects. Two other taxonomically unclassified viruses are associated with phlebotomine flies, while the rest are associated with ticks. A laboratory-acquired infection with Bhanja virus has been documented. None of the other viruses have been implicated in causing human disease.

Dhori virus recently was isolated from ticks collected in Portugal. Its geographic distribution has now been expanded to include Europe as well as Africa and Asia. The Bunyaviridae study group of the ICTV has classified Dhori virus as a member of the newly defined Nairovirus genus. It is the only member of this genus which is antigenically ungrouped.

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. It is also possible that it is an entirely distinct Coronavirus.

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 28. 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 forty percent of the viruses listed in Table 28 have been taxonomically classified.

Two recently registered viruses were isolated from bats and both have been given provisional taxonomic classifications. Agua Preta virus, isolated in Brazil, resembles a herpesvirus by electron microscopy. Ife virus was isolated in Nigeria, and by electron microscopic examination, it appears to be an orbivirus. Thus far it is antigenically unrelated to any of the other orbiviruses.

Three of the listed rhabdoviruses are associated with bats. One of these, Lagos bat virus, is antigenically related to rabies virus. Three rhabdoviruses have been recovered in Africa and two each in North and South America.

A majority of the unclassified viruses shown in Table 28 appear to be bird viruses. Several have been recovered from rodents, two from bats, and several others from various other vertebrates. Simian hemorrhagic fever virus has produced severe disease in rhesus monkeys recently imported from India. Other monkey species developed disease following contact with recently imported sick rhesus monkeys. Simian hemorrhagic fever virus has been classified by SEAS as Not Arbovirus.

Table 29 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 4.1% have been found on 3 or more continents. The largest number of viruses have been isolated in Africa.

Table 30 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 16% from all other classes. Ninety (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 (317 of 345, 91.9%).

Table 31 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 (176 of 254, 69.3%).

Table 32 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 33, and it shows that 232 (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.

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Table 1

ALPHABETICAL LISTING OF 435 VIRUSES REGISTERED AS OF 31 DEC. 1980
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
AGUA PRETA	AP	
AINO	AINO	SIM
AKABANE	AKA	SIM
ALENQUER	ALE	PHL
ALFUY	ALF	B
ALMPIWAR	ALM	
ALTAMIRA	ALT	CGL
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	

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

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

* Gray Lodge virus shows a low-level CF and NT relationship with Hart Park and Flanders virus strains (non prototypes)

NAME	ABBR.	ANTI-GENIC GROUP
GUAMA	GMA	GMA
GUARATUBA	GTB	GMA
GUAROA	GRO	CAL
GUMBO LIMBO	GL	C
GURUPI	GUR	CGL
HANZALOVA	HAN	B
HART PARK	HP	HP
HAZARA	HAZ	CON
HIGHLANDS J	HJ	A
HUACHO	HUA	KEM
HUGHES	HUG	HUG
HYPR	HYPR	B
IBARAKI	IBA	
ICOARACI	ICO	PHL
IERI	IERI	
IFE	IFE	
ILESHA	ILE	BUN
ILHEUS	ILH	B
INGWAVUMA	ING	SIM
INHANGAPI	INH	
ININI	INI	SIM
INKOO	INK	CAL
IPPY	IPPY	
IRITUIA	IRI	CGL
ISFAHAN	ISF	VSV
ISRAEL TURKEY MEN.	IT	B
ISSYK-KUL	IK	
ITAITUBA	ITA	PHL
ITAPORANGA	ITP	PHL
ITAQUI	ITQ	C
JAMANXI	JAM	CGL
JAMESTOWN CANYON	JC	CAL
JAPANAUT	JAP	
JAPANESE ENC.	JE	B
JERRY SLOUGH	JS	CAL
JOHNSTON ATOLL	JA	QRF
JOINJAKAKA	JOI	
JUAN DIAZ	JD	CAP
JUGRA	JUG	B
JUNIN	JUN	TCR
JURONA	JUR	SBU
JUTIAPA	JUT	B
KADAM	KAD	B
KAENG KHOI	KK	SBU
KAIKALUR	KAI	SIM
KAIRI	KRI	BUN

NAME	ABBR.	ANTI-GENIC GROUP
KAISODI	KSO	KSO
KAMESE	KAM	MOS
KAMMAVANPETTAI	KMP	
KANNAMANGALAM	KAN	
KAO SHUAN	KS	DGK
KARIMABAD	KAR	PHL
KARSHI	KSI	B
KASBA	KAS	PAL
KEMEROVO	KEM	KEM
KERN CANYON	KC	
KETAPANG	KET	BAK
KETERAH	KTR	
KEURALIBA	KEU	
KEYSTONE	KEY	CAL
KHASAN	KHA	
KLAMATH	KLA	
KOKOBERA	KOK	B
KOLONGO	KOL	
KOONGOL	KOO	KOO
KOUTANGO	KOU	B
KOWANYAMA	KOW	
KUMLINGE	KUM	B
KUNJIN	KUN	B
KUNUNURRA	KNA	
KWATTA	KWA	KWA
KYASANUR FOR. DIS.	KFD	B
KYZYLAGACH	KYZ	A
LA CROSSE	LAC	CAL
LAGOS BAT	LB	*
LA JOYA	LJ	
LANDJIA	LJA	
LANGAT	LGT	B
LANJAN	LJN	KSO
LASSA	LAS	TCR
LATINO	LAT	TCR
LEBOMBO	LEB	
LE DANTEC	LD	
LIPOVNIK	LIP	KEM
LLANO SECO	LLS	* *
LOKERN	LOK	BUN
LONE STAR	LS	
LOUPING ILL	LI	B
LUKUNI	LUK	ANA
MACHUPO	MAC	TCR
MADRID	MAD	C
MAGUARI	MAG	BUN

* 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
MAHOGANY HAMMOCK	MH	GMA
MAIN DRAIN	MD	BUN
MALAKAL	MAL	MAL
MANAWA	MWA	UUK
MANZANILLA	MAN	SIM
MAPPUTTA	MAP	MAP
MAPRIK	MPK	MAP
MARBURG	MBG	MBG
MARCO	MCO	
MARITUBA	MTB	C
MATARIYA	MTY	MTY
MATRUH	MTR	TETE
MATUCARE	MAT	
MAYARO	MAY	A
MELAO	MEL	CAL
MERMET	MER	SIM
MIDDELBURG	MID	A
MINATITLAN	MNT	MNT
MINNAL	MIN	
MIRIM	MIR	GMA
MITCHELL RIVER	MR	WAR
MODOC	MOD	B
MOJU	MOJU	GMA
MONO LAKE	ML	KEM
MONT. MYOTIS LEUK.	MML	B
MORICHE	MOR	CAP
MOSQUEIRO	MQO	HP
MOSSURIL	MOS	MOS
MOUNT ELGON BAT	MEB	
M'POKO	MPO	TUR
MUCAMBO	MUC	A
MURRAY VALLEY ENC.	MVE	B
MURUTUCU	MUR	C
NAIROBI SHEEP DIS.	NSD	NSD
NARIVA	NAR	
NAVARRO	NAV	
NDUMU	NDU	A
NEGISHI	NEG	B
NEPUYO	NEP	C
NEW MINTO	NM	SAW
NGAINGAN	NGA	
NIQUE	NIQ	PHL
NKOLBISSON	NKO	
NODAMURA	NOD	
NOLA	NOLA	SIM
NORTHWAY	NOR	BUN

NAME	ABBR.	ANTI-GENIC GROUP
NTAYA	NTA	B
NUGGET	NUG	KEM
NYAMANINI	NYM	
NYANDO	NDO	NDO
OKHOTSKIY	OKH	KEM
OKOLA	OKO	
OLIFANTSVLEI	OLI	OLI
OMSK HEM. FEVER	OMSK	B
O'NYONG NYONG	ONN	A
ORIBOCA	ORI	C
OROPOUCHE	ORO	SIM
ORUNGO	ORU	
OSSA	OSSA	C
OUANGO	OUA	
OUBANGUI	OUB	
OUREM	OUR	CGL
PACORA	PCA	
PACUI	PAC	PHL
PAHAYOKEE	PAH	PAT
PALYAM	PAL	PAL
PARAMUSHIR	PMR	SAK
PARANA	PAR	TCR
PAROO RIVER	PR	
PATA	PATA	EUB
PATHUM THANI	PTH	DGK
PATOIS	PAT	PAT
PEATON	PEA	SIM
PHNOM-PENH BAT	PPB	B
PICHINDE	PIC	TCR
PICOLA	PIA	
PIRY	PIRY	VSV
PIXUNA	PIX	A
PONGOLA	PGA	BWA
PONTEVES	PTV	UUK
POWASSAN	POW	B
PRETORIA	PRE	DGK
PUCHONG	PUC	MAL
PUNTA SALINAS	PS	HUG
PUNTA TORO	PT	PHL
QALYUB	QYB	QYB
QUARANFIL	QRF	QRF
RAZDAN	RAZ	
RESTAN	RES	C
RIFT VALLEY FEVER	RVF	PHL
RIO BRAVO	RB	B
RIO GRANDE	RG	PHL

NAME	ABBR.	ANTI-GENIC GROUP
ROCHAMBEAU	RBU	
ROCIO	ROC	B
ROSS RIVER	RR	A
ROYAL FARM	RF	B
RUSS. SPR. SUM. ENC.	RSSE	B
SABO	SABO	SIM
SABOYA	SAB	B
SAGIYAMA	SAG	A
SAINT-FLORIS	SAF	
SAKHALIN	SAK	SAK
SAKPA	SPA	
SALANGA	SGA	
SALEHABAD	SAL	PHL
SAN ANGELO	SA	CAL
SANDFLY F. (NAPLES)	SFN	PHL
SANDFLY F. (SICILIAN)	SFS	PHL
SANDJIMBA	SJA	
SANGO	SAN	SIM
SANTA ROSA	SAR	BUN
SATHUPERI	SAT	SIM
SAUMAREZ REEF	SRE	B
SAWGRASS	SAW	SAW
SEBOKELE	SEB	
SELETAR	SEL	KEM
SEMBALAM	SEM	
SEMLIKI FOREST	SF	A
SEPIK	SEP	B
SERRA DO NAVIO	SDN	CAL
SHAMONDA	SHA	SIM
SHARK RIVER	SR	PAT
SHUNI	SHU	SIM
SILVERWATER	SIL	KSO
SIMBU	SIM	SIM
SIMIAN HEM. FEV.	SHF	
SINDBIS	SIN	A
SIXGUN CITY	SC	KEM
SLOVAKIA	SLO	
SNOWSHOE HARE	SSH	CAL
SOKOLUK	SOK	B
SOLDADO	SOL	HUG
SORO ROCA	SOR	BUN
SPONDWENI	SPO	B
ST. LOUIS ENC.	SLE	B
STRATFORD	STR	B
SUNDAY CANYON	SCA	
TACAIUMA	TCM	ANA

NAME	ABBR.	ANTI-GENIC GROUP
TACARIBE	TCR	TCR
TAGGERT	TAG	SAK
TAHYNA	TAH	CAL
TAMDY	TDY	
TAMIAMI	TAM	TCR
TANGA	TAN	
TANJONG RABOK	TR	TR
TATAGUINE	TAT	
TELOK FOREST	TF	TR
TEMBE	TME	
TEMBUSU	TMU	B
TENSAW	TEN	BUN
TERMEIL	TER	
TETE	TETE	TETE
TETTNANG	TET	
THIMIRI	THI	SIM
THOGOTO	THO	THO
THOTTAPALAYAM	TPM	
TILLIGERRY	TIL	EUB
TIMBO	TIM	TIM
TIMBOTEUA	TBT	GMA
TLACOTALPAN	TLA	BUN
TONATE	TON	A
TOSCANA	TOS	PHL
TOURE	TOU	
TRIBEC	TRB	KEM
TRINITI	TNT	
TRIVITTATUS	TVT	CAL
TRUBANAMAN	TRU	MAP
TSURUSE	TSU	TETE
TURLOCK	TUR	TUR
TURUNA	TUA	PHL
TYULENIY	TYU	B
UGANDA S	UGS	B
UMATILLA	UMA	
UMBRE	UMB	TUR
UNA	UNA	A
UPOLU	UPO	UPO
URUCURI	URU	PHL
USUTU	USU	B
UTINGA	UTI	SIM
UUKUNIEMI	UUK	UUK
VELLORE	VEL	PAL
VEN. EQUINE ENC.	VEE	A
VENKATAPURAM	VKT	
VS-ALAGOAS	VSA	VSV

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

Table 2. Antigenic Groups of 435 Viruses Registered in Catalogue

Antigenic Group	Abbreviation	No. Registered Viruses in Group		%
A	A		25	5.7
African horsesickness	AHS		1	0.2
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			102	23.4
Bunyamwera Supergroup: Anopheles A	ANA	3		
Bunyamwera Supergroup: Bunyamwera	BUN	18		
Bunyamwera Supergroup: Bwamba	BWA	2		
Bunyamwera Supergroup: C	C	11		
Bunyamwera Supergroup: California	CAL	13		
Bunyamwera Supergroup: Capim	CAP	8		
Bunyamwera Supergroup: Gamboa	GAM	1		
Bunyamwera Supergroup: Guama	GMA	10		
Bunyamwera Supergroup: Koongol	KOO	2		
Bunyamwera Supergroup: Minatitlan	MNT	1		
Bunyamwera Supergroup: Olifantsvlei	OLI	3		
Bunyamwera Supergroup: Patois	PAT	4		
Bunyamwera Supergroup: Simbu	SIM	19		
Bunyamwera Supergroup: Tete	TETE	5		
Bunyamwera Supergroup: Unassigned	SBU	2		
Changuinola	CGL		7	1.6
Colorado tick fever	CTF		2	0.5
Corriparta	COR		2	0.5
Epizootic hemorrhagic disease	EHD		1	0.2
Eubenangee	EUB		3	0.7
Hart Park	HP		3	0.7
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
Mossuril	MOS		2	0.5
Nairoviruses			16	3.7
Nairoviruses: Congo	CON	2		
Nairoviruses: Dera Ghazi Khan	DGK	5		
Nairoviruses: Hughes	HUG	4		
Nairoviruses: Nairobi sheep disease	NSD	3		
Nairoviruses: Qalyub	QYB	2		
Nyando	NDO		1	0.2
Palyam	PAL		4	0.9
Phlebotomus fever	PHL		29	6.7
Quaranfil	QRF		2	0.5
Sakhalin	SAK		5	1.2
Sawgrass	SAW		3	0.7
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
Upolu	UPO		1	0.2
Uukuniemi	UUK		5	1.2
Vesicular stomatitis	VSV		7	1.7
Wallal	WAL		1	0.2
Warrego	WAR		2	0.5
Ungrouped viruses			96	22.1
Total			435	

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	County	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,HJ,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,AP,ARA,BEN,BER,BLM, BOR,BUJ,BVS,CAN,CDU,CHO,COT,GTB,GUR,ICO, INH,IRI,ITP,JUR,MCO,OUR,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
		Nigeria	IFE
		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,PEA,PIA,PR,SRE,TAG,TER, TIL,WAL,WGR,YAC
	Europe	Czechoslovakia	SLO
		Germany	EYA,TET
		Italy	TOS
		Scotland	CM,CW
		U.S.S.R.	BAKU
	N. America	Canada	AVA,BAU*, GI*
		Mexico	SAR*
		Panama	CAC,CAI,NIQ
		U.S.A.	CNT,FM,GLO,LLS,NM,NOR,RG,SCA,YH
	S. America	Brazil	ALT,ITA,JAM,MQO,ROC,TUA
		French Guiana	INI,RBU,TON

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

Table 4. Initial Isolation of 435 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	1	10
	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	26	110
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-	Australia				1	32	8	41
ASIA	Hawaii			1				1
and	Johnston Island					1		1
PACIFIC	New Guinea			1				1
ISLANDS	New Zealand					1		1
	Philippines				2			2
	Totals	0	0	2	3	34	8	47
EUROPE	Czechoslovakia			1	2	4	1	8
	Finland				1	2		3
	France					2		2
	West Germany					1	2	3
	Italy			2			1	3
	Scotland	1					2	3
	U.S.S.R.(West)				1		1	2
	Totals	1	0	3	4	9	7	24
NORTH	Canada				1	1	3	5
AMERICA	Guatemala					1		1
	Mexico					2	1	3
	Panama				3	15	3	21
	U.S.A.	1	3	3	10	27	9	53
	Totals	1	3	3	14	46	16	83
SOUTH	Argentina				1			1
AMERICA	Bolivia					1		1
	Brazil			1	18	37	7	63
	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	49	10	98
	Grand Totals	6	10	19	109	210	81	435

Table 5. Group A Arboviruses

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																						
Aura	+														+						+			22	Alphavirus
Bebaru	+										+				+		+				+			22	=
Cabassou	+										+				+		+				+			21	=
Chikungunya	+							+	+		+	+			+	+	+				+	+		20	=
Eastern equine enc.	+	+				+	+	+		+	+		+		+		+	+	+	+	+	+		20	=
Everglades	+	+					+				+		+							+				20	=
Fort Morgan														+						+				20	=
Getah	+	+															+	+						20	=
Highlands J	+										+				+					+				22	=
Kyzylagach	+																+				+	+		20	=
Mayaro	+						+	+						+		+								20	=
Middelburg	+							+		+	+		+		+		+				+	+		20	=
Mucambo	+							+						+										21	=
Ndumu																								20	=
O'nyong-nyong		+						+		+														22	=
Pixuna	+	+																+						20	=
Ross River	+							+			+		+											21	=
Sagiyama	+																							20	=
Semliki Forest	+	+	+				+	+			+													20	=
Sindbis	+	+																						21	=
Tonate	+	+																						21	=
Una	+	+																						20	=
Venezuelan equine enc.	+	+																						20	=
Western equine enc.	+	+																						20	=
Whataroa																									=

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

Table 6. Group B Arboviruses, Mosquito-Borne

VIRUS	ISOLATED FROM																		ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																				
	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																								
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	+	+						+								+	+									22	=
Jugra	+																	+								21	=
Kokobera	+																	+								20	=
Kunjin	+							+										+				+				20	=
Murray Valley enc.	+							+								+										21	=
Ntaya	+																									22	=
Rocio	+							+							+							+				21	=
Sepik	+																									20	=
St. Louis encephalitis	+	+					+	+								+										20	=
Spondweni	+							+																		22	=
Stratford	+																									21	=
Tembusu	+	+																								20	=
Uganda S	+																									22	=
Usutu	+																									20	=
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															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																					
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: Anopheles A and 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																						
ANOPHELES A GROUP																										
Anopheles A		+																			+			21	Bunyavirus
Lukuni	+	+													+						+			22	"
Tacaiuma	+	+						+	+						+						+			21	"
BUNYAMWERA GROUP																										
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		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																					
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		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																					
CALIFORNIA GROUP																									
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	"
CAPIM GROUP																									
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: Gamboa, Guama and Koongol 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		
GAMBOA GROUP																									
Gamboa	+																			+				22	Bunyavirus
GUAMA GROUP																									
Ananindeua	+									+	+		+		+						+			21	Bunyavirus
Bertioga															+						+			22	"
Bimiti	+									+			+		+						+			20	"
Catu	+	+						+		+		+	+		+						+	+		20	"
Guama	+				+			+		+		+	+		+					+	+	+		20	"
Guaratuba	+										+				+						+			21	"
Mahogany Hammock	+									+										+				22	"
Mirim	+														+						+			20	"
Moju	+									+			+		+						+			20	"
Timboteua										+														21	"
KOONGOL GROUP																									
Koongol	+	?																+						21	Bunyavirus
Wongal	+																	+						21	"

* See footnote Table 5

Table 13. Bunyamwera Supergroup: Minatitlan, Olifantsvlei and Patois 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																					
MINATITLAN GROUP																								
Minatitlan															+					+			22	Bunyavirus
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 14. Bunyamwera Supergroup: Simbu 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																					
Aino	+					+											+	+					22	Bunyavirus
Akabane	+					+								+			+	+					21	=
Buttonwillow						+								+						+			20	=
Ingwavuma	+										+			+		+	+				+		20	=
Inini											+										+		22	=
Kaikalur	+																+						22	=
Manzanilla									+												+		22	=
Mermet											+									+			22	=
Nola	+																						20	=
Oropouche	+					+		+						+		+					+	+	21	=
Peaton						+								+				+					21	=
Sabo						+								+		+							22	=
Sango	+					+								+		+							22	=
Sathuperi	+					+								+		+	+						22	=
Shamonda						+								+									22	=
Shuni	+					+		+						+								+	22	=
Simbu	+															+							21	=
Thimiri											+						+						22	=
Utinga														+							+		22	=

* See footnote Table 5

Table 15. Bunyamwera Supergroup: Tete Group and Unassigned 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		
TETE GROUP																									
Bahig			+								+					+			+					21	Bunyavirus
Batama											+					+								22	"
Matruh			+								+					+			+					22	"
Tete											+					+								22	"
Tsuruse											+						+							22	"
UNASSIGNED - "SBU"																									
Jurona	+														+		+				+			22	Bunyavirus
Kaeng Khoi							+					+					+								22	"

* See footnote Table 5

Table 16. 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	Phlebovirus
Alenquer														+							+			22	=
Anhanga					+					+				+		+					+			22	=
Arumowot	+				+					+										+	+			22	=
Buenaventura					+															+	+			22	=
Bujaru					+															+	+			21	=
Cacao					+															+	+			22	=
Caimito					+															+	+			22	=
Candiru					+			+												+	+	+		21	=
Chagres	+				+			+												+				21	=
Chilibre					+															+				22	=
Frijoles					+					+						+					+			22	=
Gordil	+	+			+					+	+				+						+			21	=
Icoaraci													+	+			+				+			22	=
Itaituba	+				+						+									+	+			20	=
Itaporanga					+												+							21	=
Karimabad					+															+	+			22	=
Nique					+					+											+			21	=
Pacui					+															+	+	+		21	=
Punta Toro	+							+		+				+		+				+			+	20	=
Rift Valley Fever																								22	=
Rio Grande					+												+							22	=
Saint-Floris					+																			22	=
Salehabad					+			+									+					+		20	=
SF-Naples					+			+									+					+		20	=
SF-Sicilian					+																			21	=
Toscana					+																+			22	=
Turuna					+					+											+			22	=
Urucuri																									

* See footnote Table 5

Table 17. Tick-Borne Groups Other Than Group B Viruses: Nairoviruses

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																					
CHF-CONGO GROUP																									
Congo			+			+		+						+		+	+		+			+	+	20	Nairovirus
Hazara			+														+							22	"
DERA GHAZI KHAN GROUP																									
Abu Hammad				+												+	+							22	Nairovirus
Dera Ghazi Khan			+														+							22	"
Kao Shuan				+													+	+						22	"
Pathum Thani				+													+							22	"
Pretoria				+												+								22	"
HUGHES GROUP																									
Hughes				+							+									+	+			21	Nairovirus
Punta Salinas				+																	+			22	"
Soldado				+												+			+		+			20	"
Zirqa				+													+							22	"
NAIROBI SHEEP DISEASE																									
Dugbe	+		+			+		+		+				+		+						+		22	Nairovirus
Ganjam	+		+					+									+					+	+	22	"
Nairobi sheep disease			+					+						+		+						+	+	20	"
QALYUB GROUP																									
Bandia				+						+						+								22	Nairovirus
Qalyub				+												+								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		
UUKUNIEMI GROUP																									
Grand Arbaud				+															+					20	Uukuvirus
Manawa			+	+													+							22	"
Ponteves				+															+					22	"
Uukuniemi			+							+	+								+					21	"
Zaliv Terpeniya			+														+							22	"
KAISODI GROUP																									
Kaisodi			+								+						+							22	Bunyavirus-like
Lanjan			+						+								+							22	"
Silverwater			+											+						+				21	"
SAKHALIN GROUP																									
Avalon			+								+									+				21	Bunyavirus-like
Clo Mor			+																+					22	"
Paramushir			+														+							22	"
Sakhalin			+														+			+				22	"
Taggert			+															+						22	"
THOGOTO GROUP																									
Thogoto			+					+						+		+			+			+		22	Bunyavirus-like
UPOLU GROUP																									
Upolu				+														+						22	Bunyavirus-like
QUARANFIL GROUP																									
Johnston Atoll				+														+						20	Unclassified
Quaranfil				+				+			+					+						+		20	"

* See footnote Table 5

Table 19. 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			+													+	+			+				21	"
Yaquina Head			+																	+				22	"

* See footnote Table 5

Table 20. Minor Antigenic Groups of 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																					
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 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	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
AFRICAN HORSESICKNESS																									
African horsesickness						+								+		+	+		+					20	Orbivirus
BLUETONGUE GROUP																									
Bluetongue						+								+		+	+	+	+	+				20	Orbivirus
CHANGUINOLA GROUP																									
Altamira					+																+	+		22	Orbivirus
Caninde					+																+	+		22	"
Changuinola					+			+												+				21	"
Gurupi					+																+			22	"
Irituia					+					+											+			22	"
Jamanxi					+																+			22	"
Ourem					+																+			22	"
CORRIPARTA GROUP																									
Acado	+										+					+		+						22	Orbivirus
Corriparta	+																							21	"
EHD GROUP																									
Epizootic hem. dis.														+		+				+				21	Orbivirus

* See footnote Table 5

Table 21. Minor Antigenic Groups of Viruses (Continued)

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																					
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 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		
HART PARK GROUP																									
Flanders	+										+									+				22	Rhabdovirus
Hart Park	+										+									+				21	"
Mosqueiro	+																				+			22	"
KWATTA GROUP																									
Kwatta	+																				+			22	Rhabdovirus
MOSSURIL GROUP																									
Kamese	+															+								22	Rhabdovirus
Mossuril	+										+					+								22	"
SAWGRASS GROUP																									
Connecticut			+																	+				22	Rhabdovirus
New Minto			+																	+				22	"
Sawgrass			+																	+				22	"
TIMBO GROUP																									
Chaco														+							+			22	Rhabdovirus
Timbo														+							+			22	"
VESICULAR STOMATITIS GR.																									
Chandipura					+			+						+	+	+	+				+	+		20	Rhabdovirus
Cocal	+				+		+			+				+	+						+			20	"
Isfahan					+								+				+				+		+	22	"
Piry								+						+							+	+		22	"
VS-Alagoas					+			+						+	+					+	+	+	+	20	"
VS-Indiana	+						+	+						+	+					+	+	+	+	22	"
VS-New Jersey																									

* See footnote Table 5

Table 23. Minor Antigenic Groups of 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																					
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 24. 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										+															

* See footnote Table 5

Table 25. Ungrouped Mosquito-Associated 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																					
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
Gray Lodge	+																							22	"
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 26. Ungrouped Mosquito-Associated 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																						
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		+																						21	=
Termeil	+																	+						22	=
Venkatapuram	+																							22	=
Wongorr	+																	+						22	=
Yacaaba	+																							22	=

* See footnote Table 5

Table 27. Ungrouped Tick-, Culcoides-, 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	"
African swine fever				+										+		+			+		+**			20	Iridovirus
Dhori			+													+	+		+					22	Nairovirus
Barur			+							+							+							22	Rhabdovirus
Bovine ephemeral fever						+								+		+	+	+						22	"
Charleville					+									+				+						22	"
Aride			+													+								22	Unclassified
Batken	+		+														+							22	"
Chim			+														+							22	"
Chobar Gorge				+								+					+				+			22	"
Inhangapi					+												+							22	"
Issyk-Kul				+								+					+							21	"
Keterah				+																	+			22	"
Matucare				+		+												+						22	"
Ngaingan											+													21	"
Nyamanini				+												+			+					22	"
Slovakia				+															+					22	"
Tettnang			+																					22	"
Wanowrie	+		+					+								+	+							22	"

* See footnote Table 5

** Cuba

Table 28. Ungrouped Viruses: No Arthropod Vector Known

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																					
Bangui								+								+						+		22	Bunyavirus-like
Bobaya											+					+								22	"
Agua Preta												+									+			22	Herpesvirus-like
Ibaraki														+			+							22	Orbivirus
Ife												+				+								22	"
Nariva										+											+			23	Paramyxovirus
Salanga										+						+								22	Poxvirus
Kern Canyon												+								+				23	Rhabdovirus
Keuraliba										+						+								22	"
Klamath										+										+				22	"
Lagos bat												+				+								24	"
Marco														+							+			22	"
Mount Elgon bat												+				+								23	"
Navarro											+										+			22	"

* See footnote Table 5

Table 28. Ungrouped Viruses: No Arthropod Vector Known (Continued)

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																			Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America				
Almpiwar														+				+			+			21	Unclassified
Araguari													+								+			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 29. Continental Distribution of Grouped and Ungrouped Viruses.

Antigenic Group		Total in Group	Africa	Asia	Australasia	Europe	North America	South America	No. of Continents involved 1	2	3	4	5	6
A		25	6	8	5	2	7	10	17	6	0	1	1	0
AHS		1	1	1	0	1	0	0	0	0	1	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	ANA	3	0	0	0	0	0	3	3	0	0	0	0	0
	BUN	18	4	1	0	2	8	5	16	2	0	0	0	0
	BWA	2	2	0	0	0	0	0	2	0	0	0	0	0
	C	11	0	0	0	0	5	8	9	2	0	0	0	0
	CAL	13	1	1	0	2	9	3	11	1	1	0	0	0
	CAP	8	0	0	0	0	3	7	6	2	0	0	0	0
	GAM	1	0	0	0	0	1	0	1	0	0	0	0	0
	GMA	10	0	0	0	0	2	9	9	1	0	0	0	0
	KOO	2	0	0	2	0	0	0	2	0	0	0	0	0
	MNT	1	0	0	0	0	1	0	1	0	0	0	0	0
	OLI	3	3	0	0	0	0	0	3	0	0	0	0	0
	PAT	4	0	0	0	0	4	0	4	0	0	0	0	0
	SIM	19	9	6	3	0	2	4	14	5	0	0	0	0
	TETE	5	4	1	0	2	0	0	3	2	0	0	0	0
	SBU	2	0	1	0	0	0	1	2	0	0	0	0	0
CGL		7	0	0	0	0	1	6	7	0	0	0	0	0
CTF		2	0	0	0	1	1	0	2	0	0	0	0	0
COR		2	1	0	1	0	0	0	2	0	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		3	0	0	0	0	2	1	3	0	0	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
MOS		2	2	0	0	0	0	0	2	0	0	0	0	0
Nairoviruses	CHF-CON	2	1	2	0	1	0	0	1	0	1	0	0	0
	DGK	5	2	4	1	0	0	0	3	2	0	0	0	0
	HUG	4	1	1	0	1	1	3	2	1	1	0	0	0
	NSD	3	2	1	0	0	0	0	3	0	0	0	0	0
	QYB	2	2	0	0	0	0	0	2	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		29	6	4	0	3	10	12	25	2	2	0	0	0
QRF		2	1	0	1	0	0	0	2	0	0	0	0	0
SAK		5	0	2	1	1	2	0	4	1	0	0	0	0
SAW		3	0	0	0	0	3	0	3	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
UPO		1	0	0	1	0	0	0	1	0	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		96	38	22	14	6	11	16	90	2	4	0	0	0
Totals		435	123	98	55	38	96	114	371	46	14	2	2	0

Table 30. 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		25	24	1	0	1	5	21	2	2
AHS		1	0	0	0	1	0	1	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	ANA	3	3	0	0	0	0	3	0	0
	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
	GAM	1	1	0	0	0	0	1	0	0
	GMA	10	8	0	1	0	0	7	1	0
	KOO	2	2	0	0	0	0	2	0	0
	MNT	1	0	0	0	0	0	0	0	0
	OLI	3	3	0	0	0	0	3	0	0
	PAT	4	3	0	0	0	0	3	0	0
	SIM	19	10	0	0	9	0	9	5	0
	TETE	5	0	2	0	0	0	2	0	0
	SBU	2	1	0	0	0	1	2	0	0
CGL		7	0	0	6	0	0	6	0	0
CTF		2	0	2	0	0	0	2	0	0
COR		2	2	0	0	0	0	2	0	0
EHD		1	0	0	0	0	0	0	0	0
EUB		3	3	0	0	0	0	3	0	0
HP		3	3	0	0	0	0	3	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
MOS		2	2	0	0	0	0	2	0	0
MTY		3	0	0	0	0	0	0	0	0
Nairo-viruses	CHF-CON	2	0	2	0	1	0	1	1	0
	DGK	5	0	5	0	0	0	5	0	0
	HUG	4	0	4	0	0	0	4	0	0
	NSD	3	2	3	0	1	0	1	1	1
	QYB	2	0	2	0	0	0	2	0	0
NDO		1	1	0	0	0	0	1	0	0
PAL		4	3	0	0	1	0	4	0	0
PHL		29	5	0	18	0	0	18	1	1
QRF		2	0	2	0	0	0	2	0	0
SAK		5	0	5	0	0	0	5	0	0
SAW		3	0	3	0	0	0	3	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
UPO		1	0	1	0	0	0	1	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		96	40	20	3	2	1	58	4	0
Totals		435	214	95	31	22	15	317	24	4

Table 31. 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		25	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
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	ANA	3	1	1	0	0	0	0	0	0	0	1	0	0	0	0
	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
	GAM	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	GMA	10	2	0	7	2	2	4	0	0	4	1	1	2	0	0
	KOO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	MNT	1	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	19	2	1	0	4	0	0	7	4	12	3	0	0	0	0
	TETE	5	0	0	0	5	0	0	0	0	5	0	0	0	0	0
	SBU	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0
CGL		7	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
COR		2	0	0	0	1	0	0	0	0	1	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		3	0	0	0	2	0	0	0	0	2	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
MOS		2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
MTY		3	0	0	0	3	0	0	0	0	3	0	0	0	0	0
Nairoviruses	CHF-CON	2	1	0	0	0	0	0	1	1	0	1	0	0	0	0
	DGK	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	HUG	4	0	0	0	1	0	0	0	0	1	0	0	0	0	0
	NSD	3	3	0	1	0	0	0	2	1	1	1	1	0	0	0
	QYB	2	0	0	1	0	0	0	0	0	1	0	0	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		29	7	0	8	2	0	2	1	2	14	4	0	0	0	0
QRF		2	1	0	0	1	0	0	0	0	0	1	0	0	0	0
SAK		5	0	0	0	1	0	0	0	0	1	0	0	0	0	0
SAW		3	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
UPO		1	0	0	0	0	0	0	0	0	0	0	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		96	8	1	12	15	11	1	4	4	51	1	1	0	0	0
Totals		435	96	13	87	69	32	21	36	33	176	48	15	10	3	2

Table 32. 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		25	11	8	12	48.0
Afr. horsesickness		1	0	0	0	
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	Anopheles A	3	1	0	1	33.3
	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	
	Gamboa	1	0	0	0	
	Guama	10	2	0	2	20.0
	Koongol	2	0	0	0	
	Minatitlan	1	0	0	0	
	Olifantsvlei	3	0	0	0	
	Patois	4	0	0	0	
	Simbu	19	2	1	2	10.5
	Tete	5	0	0	0	
	SBU	2	0	0	0	
Changuinola		7	1	0	1	14.3
Colorado tick fever		2	1	1	1	50.0
Corriparta		2	0	0	0	
Epizoot. hem. dis.		1	0	0	0	
Eubenangee		3	0	0	0	
Hart Park		3	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	
Mossuril		2	0	0	0	
Nairo-viruses	CHF-Congo	2	1	1	1	50.0
	Dera Ghazi Khan	5	0	0	0	
	Hughes	4	0	0	0	
	Nairobi sheep dis.	3	3	2	3	100.0
	Qalyub	2	0	0	0	
Nyando		1	1	0	1	100.0
Palyam		4	0	0	0	
Phlebotomus fever		29	6	1	6	20.7
Quaranfil		2	1	0	1	50.0
Sakhalin		5	0	0	0	
Sawgrass		3	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	
Upolu		1	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		96	6	1	7	6.3
Totals		435	95	55	104	23.9

Table 33. Evaluation of Arthropod-Borne Status of 435 Registered Viruses (SEAS)

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		25	16	5	4	0	0	21	84.0	0	
AHS		1	1	0	0	0	0	1	100.0	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	ANA	3	0	2	1	0	0	2	66.7	0	
	BUN	18	8	5	5	0	0	13	72.2	0	
	BWA	2	1	1	0	0	0	2	100.0	0	
	C	11	10	1	0	0	0	11	100.0	0	
	CAL	13	9	1	3	0	0	10	76.9	0	
	CAP	8	4	2	2	0	0	6	75.0	0	
	GAM	1	0	0	1	0	0	0		0	
	GMA	10	5	3	2	0	0	8	80.0	0	
	KOO	2	0	2	0	0	0	2	100.0	0	
	MNT	1	0	0	1	0	0	0		0	
	OLI	3	0	0	3	0	0	0		0	
	PAT	4	1	1	2	0	0	2	50.0	0	
	SIM	19	3	4	12	0	0	7	36.8	0	
	TETE	5	0	1	4	0	0	1	20.0	0	
	SBU	2	0	0	2	0	0	0		0	
CGL		7	0	1	6	0	0	1	14.2	0	
CTF		2	1	0	1	0	0	1	50.0	0	
COR		2	0	1	1	0	0	1	50.0	0	
EHD		1	0	1	0	0	0	1	100.0	0	
EUB		3	0	0	3	0	0	0		0	
HP		3	0	1	2	0	0	1	33.3	0	
KSO		3	0	1	2	0	0	1	33.3	0	
KEM		16	0	3	13	0	0	3	18.8	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
MOS		2	0	0	2	0	0	0		0	
MTY		3	0	0	3	0	0	0		0	
Nairoviruses	CHF-CON	2	1	0	1	0	0	1	50.0	0	
	DGK	5	0	0	5	0	0	0		0	
	HUG	4	1	1	2	0	0	2	50.0	0	
	NSD	3	1	0	2	0	0	1	33.3	0	
	QYB	2	0	0	2	0	0	0		0	
NDO		1	0	1	0	0	0	1	100.0	0	
PAL		4	0	0	4	0	0	0		0	
PHL		29	4	9	16	0	0	13	44.8	0	
QRF		2	2	0	0	0	0	2	100.0	0	
SAK		5	0	1	4	0	0	1	20.0	0	
SAW		3	0	0	3	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	
UPO		1	0	0	1	0	0	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	
Ungrouped		96	3	11	74	5	3	14	14.6	8	8.3
Totals		435	106	71	232	9	17	177	40.7	26	6.0

ABSTRACTS OF ARBOVIRUS PAPERS PRESENTED AT ANNUAL MEETING, AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE, ATLANTA, GEORGIA, NOVEMBER 5-7, 1980

Genetic Diagnosis of the Geographic Origin of *Aedes aegypti*

Jeffrey R. Powell and Walter J. Tabachnick

Allelic variation at 19 to 22 enzyme-coding gene loci has been studied in more than 40 samples of *Aedes aegypti* from throughout its geographic range. Regional and subspecific differentiation is great enough to allow diagnosis of the geographic origin of a population with a 90% or greater probability. The importance of such diagnosis and regional genetic differentiation is related to the epidemiology of some arboviral diseases, especially yellow fever and dengue fever. In particular, the genetic distinctness of Asian *A. aegypti* populations and southeast U.S. populations may be related to the absence of yellow fever and dengue fever, respectively, in these two regions.

The location of San Angelo virus in developing ovaries of transovarially infected mosquitoes as revealed by fluorescent antibody technique

Robert B. Tesh[1] and Michel Cornet[2]

A line of Aedes albopictus mosquitoes, chronically infected with San Angelo virus (genus Bunyavirus, California encephalitis group), has recently been established. Females in this line transmit the virus transovarially from generation to generation to a high percentage of their progeny. In order to determine how the virus enters the developing oocytes, whole mounts and frozen sections of hereditarily infected mosquitoes were examined by fluorescent antibody technique during various stages of ovarian development.

Upon emergence from the pupal stage, viral antigen was observed only in the oviduct and ovariole sheath. During the next 3 to 4 days, viral antigen appeared in the follicular epithelium and then in the nurse cells and oocytes of the primary follicles. Subsequently, in the 72 hour period between ingestion of blood and oviposition, there was a marked increase in the amount of viral antigen in the oocyte, indicating rapid virus replication. After oviposition, viral antigen was also present in the secondary follicles. Results of this study suggest that San Angelo virus gains entry into the developing oocyte by direct extension from the surrounding tissues through the follicular epithelium, in a manner analogous to that described with certain endosymbionts of insects. A series of color slides demonstrating the development of viral antigen in the oocyte will be shown.

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Incidence and Prevalence of Chagres and Punta Toro Viruses in Panama

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A project is in progress to quantify the incidence and prevalence of Punta Toro (PT) and Chagres (CHG) viruses in Panama. Both viruses are endemic/enzootic in Panama and are known to cause human disease. To determine incidence rates we are monitoring US military personnel assigned to Central Panama for febrile disease due to infection with these agents and for development of antibody against them. To date, we have recovered 3 arboviruses (CHG, PT and VEE) from 56 samples taken from acutely febrile soldiers. Serological studies indicate that PT virus incidence is about twice that of CHG virus and that both sides of the isthmus support similar infection rates.

Sera from residents of various areas of Panama have been tested for neutralizing antibody to CHG and PT viruses to determine antibody prevalence rates. Antibody to CHG virus was generally restricted to residents of Central and Eastern Panama at a rate of from less than 10% to about 17%. Antibody to PT virus was found throughout Panama at a rate of from 10% to nearly 35%.

OCCURRENCE OF OVARIOLAR DILATATIONS IN NULLIPAROUS MOSQUITOES

By

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A B S T R A C T

Determination of physiological age of mosquitoes, based on the number of ovariolar dilatations, has been extensively used as a technique to study the potential for pathogen transmission and survival rates, which can be valuable in assessing the impact of control measures. This technique has been extensively used by researchers working on Anopheline mosquitoes, however, validity of this technique has been questioned in some Aedes and Culex mosquitoes. Recently, autogenous nulliparous females of Culex tarsalis have been shown to have dilatations and we have found a similar phenomenon occurring in essentially an anautogenous mosquito, Culex nigripalpus. The implication of these findings and an explanation of occurrence of this phenomenon will be discussed.

For presentation at the American Society of Tropical Medicine and Hygiene on November 5-7, 1980 in Atlanta, GA

Abstract

Selection of lines of Culex tritaeniorhynchus for reduced and increased susceptibility to infection with West Nile virus and preliminary genetic studies.

C. G. Hayes and R. H. Baker

Pakistan Medical Research Center, University of Maryland, Lahore, Pakistan

Initially 24 genetic marker and geographic colonies of Cx. tritaeniorhynchus were screened for susceptibility to infection with West Nile (WN) virus using a membrane-feeding technique. Only the e ma, ebony body-maroon eye, colony was significantly less susceptible than a wild-type colony used as a control.

Using the same membrane-feeding method, 9 stocks of Cx. tritaeniorhynchus have been selectively inbred from 2-15 generations for reduced susceptibility, and 9 stocks also have been selected from 4-15 generations for enhanced susceptibility. These include genetic marker as well as long established and newly colonized wild-type stocks. None of the selected lines have shown a consistent decrease in susceptibility; however, two stocks, Changa Manga and Taipei, have shown increased susceptibility.

The selected Changa Manga stock (designated as line No.0046) has been crossed with the e ma colony, and the susceptibility of the resulting F_1 female progeny tested. In a representative experiment in which 95% and 40% of the parental 0046 and e ma females become infected, respectively, the infection rates for the F_1 females were similar to the parental 0046 line, suggesting that the trait of increased susceptibility is probably dominant. When individual females from this same experiment were plaque assayed in primary chick-embryo cells, the 0046 line females also were shown to replicate WN virus to higher titer than the e ma females. The parental 0046 females had an average titer of $10^{2.8}$ plaque forming units (PFU)/female which was significantly higher than average for e ma of $10^{1.8}$ PFU/female. The F_1, female progeny resulting from the crosses had intermediate titers between the two parental stocks, but the factor(s) responsible for enhanced ability to replicate virus were more strongly associated with the 0046 parental female than with the 0046 male. These results, suggest that different genetic factors may be responsible for controlling the initial susceptibility of females to infection with WN virus and the subsequent ability of infected females to modulate the replication of virus.

F_1 progeny from the above crosses were backcrossed to the parental types and the resulting female progeny tested for virus susceptibility and the ability to replicate virus. Initial results from these experiments indicate that the genetic factor (s) controlling both of these characteristics are associated with linkage group II. Further experiments are in progress to verify this relationship.

APPLICATION OF A COMPUTERIZED INFORMATION MANAGEMENT SYSTEM (SELGEM) TO MEDICALLY IMPORTANT ARTHROPODS (NATIONAL MUSEUM MOSQUITO COLLECTION)

by

Michael E. Faran, Charlotte B. Klarman and Charles L. Bailey

In 1979, a definitive study (Mosquito SELGEM Project) was begun that will result in the development of a computer based, systematic and ecological master file (data bank) of the National Museum Mosquito Collection housed in the Smithsonian Institution. This study is directed at providing easily accessible, coordinated ecological and vector data to public health organizations, the military, and other scientific and environmental agencies concerning vector species of mosquitoes. The specimens and associated data regarding the approximately 700,000 specimens constitute the best mosquito collection in the world. The collection data and specimens have come from primarily 3 sources: "Mosquitoes of Middle America", University of California, Los Angeles and; the "South East Asia Mosquito Project" and the Medical Entomology Project", Smithsonian Institution. Included in the data is detailed information on the medical importance, ecology, distribution, systematics, etc., of each species. To test SELGEM's capability, 61 collections containing about 3600 specimens from Ecuador were submitted to the computer via ENTREX, a hardware-software data entry system. Several subprograms were developed to address specialized questions of epidemiological importance (see below). Distribution maps of the potential vector species Culex ocossa and Anopheles rangeli were generated by the computerized plotter. A computer program, MILREF, was obtained from the Defense Mapping Agency that permits computer plotting of extremely accurate distribution maps using either geographic coordinates or military grid reference coordinates. By early 1980 it is anticipated that collection records on approximately 70,000 specimens (125 species) from Central and South America, and the Caribbean will have been added to the master file. These records concern primarily species of the subgenus Nyssorhynchus of Anopheles (i.e. darlingi, albimanus, aquasalis) and species of the subgenus Melanoconion of Culex (i.e. taeniopus, ocossa, panocossa). A separate file is being compiled that will consist of a narrative on the medical importance, bionomics, distribution, and important references of every species of known or suspected medical importance. Ultimately, this narrative will become part of an information packet that could be rapidly provided to individuals or organizaations on request responding to a crisis such as an outbreak of Venezuelan encephalitis or malaria in some part of the world.

Sample of the Types of Requests SELGEM is Capable of Answering:

1. What is (are) the malaria vector(s) in Guatemala? 2. In what habitats are the latter species collected? 3. Plot distribution to the nearest 100 meters of all Anopheles albimanus collected in Panama. 4. Has A. albimanus ever been collected in Nicaragua above 500 meters? 5. Where has the vector of jungle yellow fever, Haemagogus janthinomys, been collected in Trinidad? 6. What other species have been collected in association with Ha. janthinomys? 7. What months of the year have the immatures of Ha. janthinomys been collected?

Correlation between cool season environmental temperatures and dengue hemorrhagic fever (DHF) case rates in Bangkok, Thailand

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ABSTRACT

Reported case rates of DHF in Bangkok follow an annual cycle with peak case rates in the mid-rainy season (June to October), falling case rates in the late rainy season and cool dry season (November to March) and rising case rates in the hot season (April to May). Although the cycle pattern is relatively constant the magnitude may vary by a factor of ten-fold between mild and severe years. Linear regression analysis of monthly case rates versus total yearly case rates for the years 1962 through 1978 showed that case rates in May of a given year, just before the onset of the rainy season are strongly correlated with the total number of cases in that year (N=17, $r=+.858$ $p < .001$). Case rates in March of a given year (typically the nadir of DHF activity) are also significantly correlated with the total number of cases in that year (N=16, $r=+.696$ $p < .01$). These observations suggest that factor(s) which govern DHF case rates are operative well before the onset of the rainy season.

Linear regression analysis of the relationship between monthly DHF case rates and environmental temperatures during the month of January is presented in Table 1. A strong positive correlation was observed between the mean monthly temperatures of December and January and the DHF case rates in December, January and February; in years with relatively warm cool seasons the DHF case rates tended to remain higher than years with lower temperatures.

In this analysis hot and rainy season temperatures were found not to be correlated with DHF cases rates. Other meteorologic variables evaluated included total rainfall per month, number of days with rain per month, mean relative humidity per month, days with minimum relative humidity less than 70% per month, mean wind velocity per month, and days with mean wind velocity per month greater than 10 km/hr. None of these variables were found to correlate with DHF case rates at anytime throughout the year.

Although high DHF case rates can be correlated with high cool season environmental temperatures, the mechanism is unknown; one good explanation is that temperature may exert an effect on the extrinsic incubation period of dengue virus in *Aedes aegypti*.

Table 1. Correlation of temperatures recorded during the month of January with DHF case rates in preceding and subsequent months for the period 1962-1978. (Number in the table represent p values of correlation; a plus (+) sign signifies a positive correlation and a negative (-) sign a negative correlation).

Meteorologic condition in January	P value of correlation of meteorologic condition with number of DHF cases in:							
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Aver daily mean T	NS	NS	+.02	+.005	+.005	+.02	+.02	NS
Days mean T $< 27^{o}$	NS	NS	-.01	-.001	-.001	-.01	-.01	NS
Absolute minimum T	NS	NS	NS	+.02	+.02	NS	+.02	NS

Effects of Temperature on the Extrinsic Incubation Period of Dengue Virus in Aedes aegypti.

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ABSTRACT

Experimental studies were conducted to determine the ability of a local strain of Aedes aegypti to transmit a local strain of dengue virus type 2 to Rhesus monkeys at temperatures comparable to those of the different seasons in Bangkok, Thailand. After feeding on a dengue 2 infected monkey with an undetectable viremia, mosquitoes maintained at 20°C, 24°C, 26°C, 28°C and 30°C became infected but only the mosquitoes maintained for 25 days at 30°C transmitted virus to monkeys. Infection rates ranged from 25% for mosquitoes at 20°C to 58% for mosquitoes at 30°C. In the second experiment, Ae. aegypti were fed on a viremic monkey with $2 \times 10^{3.0}$ plaque forming units per 0.3 ml of dengue virus type 2. Virus transmission was not demonstrated using mosquitoes maintained for 3, 7, 12, 18 and 25 days at 26 C while mosquitoes maintained at higher temperatures transmitted virus to monkeys. Mosquitoes maintained at 32°C and at 35°C were capable of transmitting virus 4 to 7 days after feeding on infected monkeys, while 8 to 12 days were required for mosquitoes maintained at 30°C to transmit virus. These experimental findings indicate that the efficiency of Ae. aegypti as a vector of dengue virus type 2 was influenced markedly by the amount of virus ingested and by minor changes in temperature. The significance of the findings under field conditions is unknown; however, these data may account for the marked decrease in human dengue virus infections during the cool season (mean daily temperature of 24-25°C) and the rapid and sustained rise in the incidence of human infections during the hot and rainy seasons (a mean daily temperature of 31°C and 27°C, respectively) in Bangkok, Thailand.

Genetic Analysis of *Culex pipiens* Populations in the Central Valley of California

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We have used electrophoresis to study genetic variation at 10 enzymatic loci among 9 different populations of *Culex pipiens* from the central valley of California. The populations were collected along a north-south transect representing the length of the valley and include populations of *C. p. pipiens*, *C. p. quinquefasciatus*, and populations which were primarily hybrids between the two subspecies. In each population individual males were identified to subspecies or as a hybrid using the DV/D ratio of the terminalia, and then subjected to electrophoresis. Individuals could be assayed for all 10 loci using our techniques. None of the loci we have surveyed is totally diagnostic for distinguishing the two subspecies. However, several of the loci show gene frequency clinical variation which significantly correlates with the observed variation in the morphological character, DV/D ratio. These loci when used together enable one to determine subspecies identity with high probability in both males and females.

Epidemic Dengue 3 in Central Java, Associated with Low Viremia in Man

D. J. Gubler, W. Suharyono, I. Lubis, S. Eram, and S. Gunarso

An outbreak of dengue type 3 was studied in Central Java, Indonesia, in 1978. In contrast to previous dengue 3 epidemics in Central and East Java, this outbreak was less explosive, associated with mild illness, and low viremia. The dengue virus isolation rate from serologically confirmed patients was only 32% compared to 65% for an epidemic in Bantul a year earlier. Neither dengue hemagglutination-inhibition (HI) antibody titers nor day of illness on which specimens were collected accounted for this difference. Data suggest that some strains of dengue virus (epidemic strains) which are associated with low viremia and generally cause only mild illness in man may occur naturally.

Ecological studies of St. Louis encephalitis in Memphis, Tennessee

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St. Louis encephalitis epidemics occurred in Memphis in 1964 and again in 1974-75. Entomological studies were initiated in 1974 as part of the epidemic investigation and have continued on an annual basis since. In 1979, field studies were expanded to include sampling of both wild vertebrate and mosquito populations in suburban and sylvan habitats peripheral to urban Memphis. The intent was to compare SLE virus transmission in these habitats as compared with virus activity detected in the peridomestic urban house sparrows, and mosquitoes collected from urban resting sites.

During the early years of the investigations when human cases occurred (1974-76) numerous SLE virus strains were recovered from Culex mosquitoes and the isolations started early in the summer. Following 1976, when 276 SLE strains were isolated from mosquitoes, the frequency of virus recovery from mosquitoes dropped sharply to 7, 11, and 21 in 1977-79, respectively. Virus recoveries in these latter years also occurred later in the season as compared with those years in which human cases occurred.

The overall SLE antibody prevalence from the 2957 avian, 149 mammalian, and 14 reptilian sera collected in 1979 was 4.1%, 10.1%, and 0. Antibody prevalence for house sparrows (2.9%) collected from urban sites was lower than the seasonal mean prevalence for the 89 wild bird species (4.9%) collected at the sylan sites. Bobwhite quail, Cardinals, and Mockingbirds had the highest antibody prevalences among bird species collected in the sylvan sites with 45.8%, 13.8%, and 8.6% positive, respectively for the season. Of mammals, rodents had the highest N antibody prevalences (18.9%); whereas opposums (6.3%) and raccoons (1.9%) were much lower.

Virus activity and seasonal distribution in epidemic and non-epidemic years, and virus activity in urban areas versus suburban-sylvan sites are evaluated and related to the detection of SLE virus transmission and risk of human disease.

A Survey for Arboviral Antibodies in Sera of Humans and Animals in Lombok, Republic of Indonesia.

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Sera were collected from humans, cattle, horses, goats, ducks, chickens, wild birds, bats and rats in Lombok, Indonesia. Sera were tested by hemagglutination inhibition (HI) for antibodies to Japanese encephalitis (JE), Zika (ZIKA), Chikungunya (CHIK) and Ross River (RR) viruses. Selected sera were tested by microneutralization test for antibodies to the following viruses: JE, ZIKA, Murray Valley encephalitis (MVE), Tembusu (TMU), Langat (LGT), Kunjin (KUN), Sepik (SEP), dengue-2 (DEN-2), CHIK, RR, Getah (GET), Sindbis (SIN), Bunyamwera (BUN), Batai (BAT) and Bakau (BAK).

Human sera had JE HI antibody in 135 (30%) of 446 tested. Neutralization tests indicated that DEN-2, ZIKA, TMU, KUN and SEP may have caused flavivirus infections. No other infections were documented in humans.

Hemagglutination inhibition and neutralization tests on animal sera indicated that flavivirus infections with JE, MVE, KUN, SEP and that infections with BAT and BUN occurred among domestic animals. There was no evidence in the form of neutralizing antibodies that any animal had been infected with alphaviruses or other viruses used in the test.

Transovarial transmission of arboviruses: Virus or vector function?

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The precise mechanism which determines whether an arbovirus will be transovarially transmitted in its mosquito host is unknown. There are at least 3 hypothetical mechanisms which are obvious:

1) Vertical transmission is a function of a particular mosquito species.

2) Vertical transmission is a function of a specific virus or group of viruses.

3) Vertical transmission is a unique event which only occurs in a specific vector-virus interaction.

We attempted to answer these questions using 2 virus-vector models which have been well studied. The La Crosse (LAC) virus - Aedes triseriatus system was used as a model of efficient transovarial transmission; filial infection rates (FIR's) have been demonstrated to be 70%. Our model of inefficient vertical transmission was the yellow fever (YF) virus - Aedes aegypti system; FIR's for this interaction are in the neighborhood of 0.2%. Aedes triseriatus and Aedes aegypti were infected with either LAC or YF virus or a mixture of both viruses. FIR's were calculated for individual progeny as well as for pooled individuals.

FIR's were remarkably similar for A. aegypti and A. triseriatus when parent mosquitoes were infected with LAC virus (see table), although there seemed to be a trend for lower FIR's with each ovarian cycle in A. aegypti. The 12% FIR of LAC virus in our colony of A. triseriatus was suprisingly low when compared to midwestern strains; whether or not this low FIR is characteristic of eastern populations of A. triseriatus is currently under investigation.

Our rationale for infecting mosquitoes with a mixture of LAC and YF viruses was to ascertain if the efficient virus (LAC) might somehow allow the inefficient virus (YF) to be "carried" across the so-called ovarian barrier during the initial stages of infection. The data indicate that only LAC virus was vertically passed in both mosquito species at rates similar to those found in mosquitoes infected solely with LAC virus.

These preliminary data indicate that in the virus-vector interactions of LAC and YF viruses in A. triseriatus and A. aegypti, the virus is a major determinant of transovarial transmission.

Transovarial Transmission of yellow fever and La Crosse viruses in *Aedes aegypti* and *Aedes triseriatus* mosquitoes

MOSQUITO SPECIES	Virus								
	La Crosse			yellow fever			La Crosse - yellow fever		
	# POOLS POSITIVE	# PROGENY	MIR	# POOLS POSITIVE	# PROGENY	MIR	# POOLS POSITIVE	# PROGENY	MIR
Aedes aegypti	21	975	2.2%	0	339	0	LAC - 12 YF - 0	390 366	3.1%
Aedes triseriatus	11	296	3.7%	1	390	.3%	LAC - 3 YF - 0	129 129	2.3% 0

A Survey for Antibodies to Arthropod-Borne Viruses in the Human Population of Sudan

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Since Sudan lies in a transitional climatic zone between equatorial and northern Africa, it is likely the many arthropod-borne viruses which circulate in both areas are disease problems in Sudan. This investigation was the first phase of a study to determine the impact of arthropod-borne viruses on human beings and animals in Sudan. Over 800 sera were collected from male and female outpatients of all ages seen at the Khartoum Hospital, and from mostly male military recruits. The hospital patients were from the greater Khartoum area and from the heavily populated agricultural areas south of the city. The soldiers were recruited from all regions, but most had never travelled from the limited areas near their home villages. Four hundred sera have been screened for antibodies using the indirect fluorescent antibody and the complement-fixation tests. Antigens were those arthropod-borne viruses which have been implicated as human pathogens in equatorial and northern Africa. The percentages of antibodies obtained to date are: West Nile 32.1%, polyvalent alphavirus antigen (Sindbis, Chikungunya, and O'nyong-Nyong) 3.5%, Rift Valley fever 3.2%, polyvalent Bunyamwera (Bunyamwera, Germiston, Ilesha) 5.7%, Tataguine 18.8%, Quaranfil 1.0%, Bwamba 3.4%, Tahyna 1.0%, polyvalent Bangui and Zinga 1.5% and Sandfly Sicilian 22.3%. Other antibodies detected at less than 1.0% were Gordil, Gabek Forest, Sandfly Naples, Arumowot, Shuni, St. Floris, Nyando, and Dugbe. Tests for antibodies to Thogoto, Wad Medani, and Malakal viruses were negative.

RECOVERY OF MULTIPLE SEROTYPES OF BLUETONGUE VIRUS FROM INFECTED SHEEP AND CATTLE

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The mechanism by which the bluetongue virus (BTV) group evolved to include 20 distinct serotypes is not understood. This report describes the recovery of multiple serotypes of BTV from infected sheep and cattle and points out a mechanism by which the evolution of serotypes might proceed. The evolutionary and epidemiological significance of multiple BTV serotype infections in sheep and cattle will be discussed.

Multiple serotypes of BTV were identified from field-collected samples of sheep and cattle blood. Two sheep, each infected with BTV serotypes 10 and 17, were found in a flock with bluetongue disease. A sheep infected with BTV serotypes 11 and 17 was found in a 2nd flock; it was the only viremic sheep and was not clinically ill. Multiple infections of individual beef and dairy cattle were found in 3 geographically separate herds; mixtures recovered included a) BTV serotypes 10 and 17 and b) serotypes 11 and 17. Clinical signs of illness were absent in the cattle in 2 herds (B-1 and B-2) but severe conjunctivitis was seen in several calves in a 3rd herd (B-3), including one with a multiple serotype infections (BTV 11 and 17). The cattle herds (B-2 and B-3) with multiple infections had no serological evidence of BTV, as determined by the agar gel precipitin test; serum was not available from the other cow (herd B-1) with a multiple serotype infection.

Histopathologic Observations in the Central Nervous System of Indicator Rabbits Exposed to LaCrosse Virus.

by

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Abstract

Clark and coworkers have established that Peoria County, Illinois is a major focus of endemic transmission of LaCrosse(LAC) virus of the California encephalitis group. The Illinois Department of Public Health has studied LAC activity in this area since 1976. During the summer of 1979, ten seronegative domestic rabbits were placed in wooded areas around the county on June 1 and bled weekly through September 29. At the conclusion of their exposure in the field to naturally-occurring Aedes triseriatus mosquitoes, the authors used these animals to perform a post-mortem examination and histopathologic study.

The study was motivated by dual considerations. First, because of its low case-fatality rate, no comprehensive study of the histopathologic changes of LAC in man was available. Second, neither clinical illness nor histopathologic lesions have been recorded in any lower animal species, except intracerebrally inoculated sucklin mice.

Nine rabbits were available for examination and eight of these developed neutralizing antibodies to LAC virus. Two of the nine exhibited clinical illness consistent with an insult to the central nervous system. One animal had posterior ataxia while a more seriously ill rabbit presented many signs referable to disease of the basal ganglia.

At necropsy, three of the nine exhibited gross meningeal changes; two had vascular congestion; and the most severely ill animal showed definite clouding of the meninges. This animal also showed a poorly defined area of yellowish discoloration in the cerebrum.

Histopathologic studies revealed focal lymphocytic and plasmacytic infiltrate in the meninges of eight of nine animals and areas of perivascular cuffing, ependymal granulations, and glial nodules were seen in seven. The rabbit with extensive left-sided spastic paresis and a macroscopic lesion had an extensive necrotizing lesion of the basal ganglion. Stains specifically for Encephalitozoon cuniculi were uniformly negative for this protozoan parasite.

In summary, we have unequivocal evidence of encephalitis in indicator rabbits-all but one of which were naturally infected with LAC virus. Our data do not allow us to conclude that LAC induced all of the lesions that we observed but does provide us with the incentive to initiate a controlled investigation to assess the potential of the domestic rabbit as an animal model of LaCrosse viral encephalitis in man.

An Indirect Fluorescent Antibody Technique for the Rapid Diagnosis of La Crosse Encephalitis

By

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An indirect fluorescent antibody technique (IFA) was developed for the rapid serologic diagnosis of La Crosse (LAC) encephalitis. The IFA was as sensitive as the HI and NT test for the early detection of antibodies; 65% of the confirmed LAX cases were positive by IFA in the acute phase serum as compared to 58 and 59% for the HI and NT tests respectively. Convalescent phase serum antibody titers ranged from 1:64 to greater than 1:2048. Antibody titers were remarkably specific considering the known serologic cross reactivity of the California group viruses. Representative sample titrations are shown in the Table.

IFA Antibody titers to California Group virus antigens

Subject	La Crosse	Trivittatus	Jamestown Canyon	Snowshoe Hare
A	1:128	1:16	1:128	1:128
B	1:256	1:16	1:16	1:128
C	1:256	1:8	1:64	1:64
D	>1:2048	1:4	1:4	1:64
E	1:256	0	0	1:32
F	1:256	1:4	0	1:64
G	1:256	0	0	1:8

Antibodies were also detected by IFA in cerebral spinal fluid samples. Studies are currently underway to determine the immunoglobulin class of antibodies detected in serum and CSF.

GENETIC INFLUENCES ON THE SUSCEPTIBILITY OF MICE TO SINDBIS VIRUS

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The outcome of central nervous system (CNS) infections produced in 6-8-week-old inbred mice intracerebrally (i.c.) inoculated with a standardized dose ($10^{3.8}$ PFU) of a neuroadapted strain (AR339) of Sindbis virus (SV) varies greatly between different mouse strains. Based on SV-induced mortality in 30 or more animals of each strain tested, the influence of the major histocompatibility gene complex (H-2) and/or non-H-2 background on susceptibility was assessed. It was found that mouse strains could be ranked in the following descending order of susceptibility: C57BL/6J ($H\text{-}2^b$) > C3H/HeJ ($H\text{-}2^k$) > BALB/cJ ($H\text{-}2^d$) > CBA/J ($H\text{-}2^k$). All C57BL/6J mice died within 8-10 days while all CBA/J mice survived. Any major role of H-2 in determining susceptibility to SV was excluded by the mortality patterns observed in a variety of infected H-2 congenic mouse strains. These included B10.K ($H\text{-}2^k$) and B10.D2 ($H\text{-}2^d$) all of which died, and C3H.SW ($H\text{-}2^b$) which, like infected C3H/He ($H\text{-}2^k$), exhibited a mortality rate of 70%.

Comparisons of SV replication in brains of susceptibile C57BL/6J and resistant CBA/J mice at 3,5 and 7 days post-infection revealed no striking differences. On day-3, brain virus titers in mice of both strains were $10^{7.0}$ PFU/gm; less than $10^{1.6}$ PFU/gm were detected on day-7, at which time mice of neither strain had developed significant levels of circulating SV neutralizing antibody. Resistance of CBA/J mice to SV was abolished by immunosuppression with lethal irradiation (800R). Infected irradiated mice all developed fatal CNS disease well before the onset of radiation illness in uninfected controls. However, even in brains of infected irradiated mice, virus levels, per gm of brain, fell from $10^{7.0}$ PFU, on day-3, to $10^{3.8}$ PFU, on day-7, in the presumed absence of antiviral antibody production. On the other hand, SV neutralizing antibody given passively to susceptible C57BL/6J mice 2 days post-infection, when brain virus titers are nearly maximal, completely protected then from fatal CNS disease.

Collectively, these observations suggest that, for a given mouse strain, the outcome of SV infections of the CNS is influenced largely by genes which are not H-2-linked. Strains of mice having a black (B6/B10) background are susceptible and all die when inoculated i.c. with SV even though the kinetics and magnitude of virus replication in their brains, and in those of resistant CBA/J mice, seem quite similar. Furthermore, neither virus clearance nor survival necessarily correlate with the appearance of a measurable antiviral immune response, although radiation-induced immunosuppression can abrogate resistance, and the presence of antiviral antibody can override susceptibility. (Supported by Contract DAMD 17-78-C-8042 from the U.S. Army R & D Command, and USPHS Grant NS-11286)

RECONSIDERATION OF THE TAXONOMY OF THE WESTERN EQUINE ENCEPHALITIS VIRUS COMPLEX

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For more than 40 years, western equine encephalitis (WEE) complex virus strains have been isolated in North and South America. It has been recognized for many years that WEE virus ecology differs in western and eastern North America. In the West, the virus is associated with epidemic disease in horses and humans and is transmitted by Culex tarsalis mosquitoes; whereas in the East, the agent exists as an enzootic infection of birds, transmitted by Culiseta melanura mosquitoes and rarely associated with disease. Antigenic differences have been described between strains from the East (e.g., Highlands J virus) and strains from the western part of the continent, but no extensive antigenic comparisons had been made. This has led to problems of diagnosis, epidemiology, and nomenclature.

Thirty-three WEE complex strains isolated from mammals, birds, and mosquitoes collected in North and South America were compared in short-incubation, hemagglutination-inhibition, and serum dilution plaque reduction neutralization tests. WEE complex virus strains from Louisiana, Georgia, North Carolina, Maryland, New Jersey, and Massachusetts were, in all tests, indistinguishable from each other and from prototype Highlands J (HJ) virus from Florida. Virus strains from Brazil, Mexico, Texas, New Mexico, California, Oregon, Montana, Utah, Iowa, Illinois, Ohio, and Canada were indistinguishable from each other and from standard laboratory strains (e.g., McMillan and Fleming) of WEE virus from the western U.S. and Canada. The virus strains similar to HJ virus were distinct from viruses identified as WEE virus and from other alphaviruses belonging to the WEE complex (Sindbis, Whataroa, Aura, and Y62-33).

A newly recognized member of the WEE complex (Fort Morgan virus) with unusual ecological relationships (transmission by swallow nest bugs, Oeciacus vicarius) has been characterized and found to be a distinct member of the WEE virus complex. It shares closer antigenic relationships with HJ than with WEE virus.

Epidemiologic, epizoologic, ecologic, biochemical, and pathogenetic differences between WEE, HJ, and Fort Morgan viruses are discussed and a new classification scheme proposed for alphaviruses of the WEE complex.

DISTRIBUTION AND PREVALENCE OF MERMET VIRUS INFECTIONS IN THE CENTRAL UNITED STATES

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Mermet virus, a Simbu serogroup bunyavirus (family Bunyaviridae), has been isolated from birds captured in Illinois, Ohio, and Texas and from Culex sp. mosquitoes collected in Tennessee.

A serosurvey of 3,085 birds, primarily house sparrows (Passer domesticus) captured for St. Louis encephalitis virus surveillance, revealed generally low prevalence rates of Mermet neutralizing antibody throughout the central U.S. Antibody prevalences by state were: Wisconsin 6.1%; Illinois 1.1%; Ohio 6.9%; Kentucky 0%; Tennessee 0.9%; Missouri 0%; and Mississippi 3.8%. In Indiana, 11.6% of birds had neutralizing antibody in 1977 and 19.1% in 1978; prevalence rates varied from as low as 5.0% in 1977 to as high as 17.5% in 1978 in the same area of the state.

The geographical and temporal distributions of neutralizing antibody in birds suggest that low-level transmission is the rule but that periodic, focal amplification of the virus occurs.

For the first time, evidence was obtained for the presence of Mermet virus in Wisconsin, Indiana, and Mississippi.

ABSTRACT

COMPARATIVE STUDY OF DENGUE VIRUS REPLICATION IN THE CELL LINES DERIVED FROM HEMATOPHAGOUS AND NONHEMATOPHAGOUS MOSQUITOES

Goro Kuno, Ph.D.

Although several cell lines from hematophagous mosquitoes are known to support replication of dengue viruses, little is known about their replication in cell lines derived from nonhematophagous mosquitoes. Recently, a continuous cell line (TRA-171) was obtained from a nonhematophagous mosquito, Toxorhynchites amboinensis. Therefore, I studied dengue virus replication in the cell line and compared the sensitivity to virus infection of this cell line with that of two cell lines derived from hematophagous mosquitoes, Aedes albopictus and A. pseudoscutellaris.

All four serotypes of laboratory-adapted dengue viruses replicated in TRA-171 cells and in the other two cell lines. With the adapted viruses, in general, extracellular virus titers in the supernatants rose earlier and higher in A. albopictus cells. When 16 unadapted dengue virus strains (types 1, 2 and 3) were inoculated, again virus titers, often but not always, rose earlier and higher in A. albopictus cells. However, in terms of virus isolation rate, the TRA-171 cell line was slightly more sensitive than the other two, since 16 viruses were isolated in that cell line, whereas 14 and 15 were isolated in A. albopictus and A. pseudoscutellaris cells, respectively. When an additional 12 human sera, from which dengue viruses had been previously isolated by intrathoracic inoculation of adult mosquitoes, were inoculated only into TRA-171 cell cultures, 11 viruses (10 DEN 1 and 1 DEN 2) were isolated. The results revealed further that dengue viruses could be isolated in the three cell lines even though the inocula contained no detectable plaque forming agent and that slower rises in virus titers in the supernatants did not necessarily reflect lower dosages of virus in the inocula.

Cytopathic effects, syncytia in particular, developed extensively in both TRA-171 and A. pseudoscutellaris cells maintained at 28°C. However, the syncytial development in TRA-171 cells was judged unreliable as an indicator of dengue virus infection, since small syncytia and vacuolar structures developing in normal cell controls after 7-10 days in culture could be confused with early signs of virus-induced cytopathic effects.

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ABSTRACT

THE POTENTIAL FOR SPREAD OF RIFT VALLEY FEVER (RVF) IN THE MIDDLE EAST

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Since 1977, RVF has been epidemic and epizootic in Egypt and West Africa. In 1979, an explosive epizootic occurred south of Egypt stretching from the Sudan into Kenya. This widespread outbreak raised concerns that RVF would penetrate into the Middle East. A sudden outbreak of febrile deaths in Saudi Arabia during July and August appeared to confirm these fears. These human deaths were eventually attributed to heat stroke, but a study of the potential for spread of RVF into Saudi Arabia was also undertaken due to its proximity to countries with enzootic and epizootic RVF.

A study of importation practices revealed that nearly 2.7 million sheep, goats, cattle and camels are imported annually into Saudi Arabia from Egypt, Sudan, Somalia, and Australia. One million of these animals are imported for ritual slaughter at Mecca during the Haj and begin to enter the country four months before the Haj. These animals are frequently held in close proximity to native herds. Strict quarantine measures are employed only for imports from Egypt and Sudan. An estimated 250,000 animals were imported for slaughter during 1978 but never slaughtered under government supervision. Illegal importation of animals was observed but not quantified. All of these practices tend to put potentially infected imported animals in close proximity to indigenous herds.

A non-random serosurvey at the government slaughterhouse in Jeddah during one week produced 143 specimens from sheep, goats, camels, and cattle from Somalia, Sudan and Australia. One hundred and thirty six of these specimens were analyzed and six (4.4%) had positive complement fixing titers of at least 1:8 to RVF. The country of origin and species of animal were: Sudan (1 sheep) and Somalia (2 camels, 2 sheep, and 1 goat). Plaque reduction neutralization tests revealed titers of 1:2560 in each of the Somalia camels, 1:40 in the Sudanese sheep and 1:10 in the remaining animals. These results suggest that RVF may also be present in Somalia and that, due to a lack of quarantine measures for Somalian animals, RVF may enter Saudi Arabia by that route.

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Mouse macrophage cell line for assay of human dengue-enhancing antibodies

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Peiris and Porterfield (1) recently demonstrated antibody-enhanced growth of West Nile virus (WNV) in P388D_1 cells, a continuous line of mouse macrophages. These cells show a number of macrophage characteristics. Among them are: immune phagocytosis, Fc and C3 receptors, absence of surface immunoglobulin and effector functions in ADCC assays. We have found that P388D_1 cells demonstrate antibody-enhanced infection with dengue 2 virus, 16681 strain (D2V). The test antibody preparation was added together with D2V at a multiplicity of infection of 0.5. Cells were infected as suspension cultures (1 x 10^6 per ml) in MEM-Alpha medium supplemented with 10% newborn calf serum. Virus-antibody complexes were washed from P388D_1 cells after 1 hour incubation at 37°C. Cultures were assayed for virus content on days 1-4 after infection. Direct plaques with D2V could not be observed in adherent P388D_1 monolayers as was the case with WNV. Dengue infection-enhancing antibody was measured by published methods (2). Here we report results of tests on 11 human cord blood sera obtained from infants born to dengue-immune natives of the Philippines, India and Vietnam. These sera were titered for D2V infection-enhancing antibody in human monocytes and P388D_1 cells:

		Reciprocal Dengue 2 Titers			
Serum	Geographic Origin	HI	PRNT	EnhT (human monocytes)	EnhT (P388D_1)
A	Philippines	40	115	<20	100
B	"	10	<10	20,480	25,600
C	"	40	430	81,920	102,400
D	"	20	33	640	400
E	"	40	125	8,320	6,400
F	"	10	24	320	400
G	"	40	130	130	400
H	"	20	140	>655,360	>409,600
I	Vietnam	40	130	133,000	102,400
J	"	40	220	81,920	102,400
K	India	20	62	20,480	25,600

This preliminary study shows a good correlation in D2V infection-enhancing antibody titers measured by the two assay systems. The marked heterogeneity of D2V enhancing antibody in this small sample in notable. We are measuring enhancing activity in a larger collection of cord blood sera from Thailand. These results will be included in the presentation at the ASTM&H meeting.

P388D_1 cells provide a simple and inexpensive enhancing antibody assay system for studies on the pathogenesis of dengue infection. Because of the relative homogeneity of P388D_1 cells, it is expected that this assay system will provide enhancing antibody titers with improved reproducibility compared with those developed from human blood monocytes.

1) Peiris, J.S.M., Porterfield, J.S. Antibody-mediated enhancement of flavivirus replication in macrophage-like cell lines. Nature 282: 509-511, 1979.
2) Halstead, S.B., O'Rourke, E.J. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J. Exp. Med. 146: 201-217, 1977.

Abstract

Horse Virulence of Small and Minute Plaque from P-676 and MF-8 Epizootic VEE Strains

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During the course of studies to investigate whether epizootic VEE virus persisted in horses, we found that explant cultures from fetuses infected with strain P-676 (which produces about 30% fatality in horses), yielded two plaque types; one a minute plaque (MP), 0.2 - 0.5 mm in diameter, and the other a small plaque (SP), 1.5 to 2.0 mm in diameter. the SP was the predominant plaque type of the parental pool.

The finding of a P-676 MP variant prompted us to search for MP variants in the MF-8 VEE strain (which produces about 60% fatality in horses). A similar MF-8 MP variant was found. In order to characterize the P-676 and MF-8 plaque variants, 4 horses each were inoculated subcutaneously with the P-676 and MF-8 SP and MP plaque cloned virus.

All 4 horses ~~in~~fected with either P-676 SP or MP developed fever and viremia, but no other clinical signs. None of the animals died and all developed neutralizing antibodies. Of the 4 equines infected with MF-8 SP, all developed severe signs of encephalitis and 3 died. None of the horses inoculated with MF-8 MP died and only two showed viremia, with no fever or any other symptoms; all the horses developed neutralizing antidies.

Neutralization tests carried out with cloned plaque variants and horse serum obtained early after infection detected differences among the plaque variants. These results will be presented and discussed.

The Re-establishment of Aedes aegypti and Its Invasion of Natural Breeding Sites in New Orleans. By Robert S. Fritchey and Harold Trapido.

ABSTRACT

Efforts to eradicate Aedes aegypti in the New World largely have been premised on its breeding in artifactual containers in close proximity to human habitations. Its utilization of natural water holding containers, as in Africa, would greatly complicate any attempt at eradication.

Prior to the introduction of the general use of DDT in 1946, there exist for the Americas only 2 reports of larvae in rock holes, 2 of leaf-axil breeding and 8, possibly 9, of tree hole breeding. In subsequent years, frequent breeding in natural containers, particularly tree holes, was reported in the West Indies and South Florida. This suggests that a genetic selection resulting in the invasion of natural containers followed the widespread use of residual insecticide in domestic situations.

Aedes aegypti had spontaneously disappeared from New Orleans before the national eradication effort in the sixties. It reappeared in artifactual containers in heavily populated areas of the city in 1972. In 1974 it was found breeding in a tree hole for the first time. This prompted a survey to establish the extent of tree hole breeding within the city, and an attempt to differentiate morphologically between populations from tree holes and those from artifactual breeding sites. Of approximately 3,000 trees examined, 13 were found to contain tree holes capable of breeding mosquito larvae. A. aegypti were recovered from 11 (84.6%) of these 13 trees. The majority of breeding sites was located at distances greater than 133 meters from the nearest human dwelling. On the basis of variation in the abdominal pale scale pattern, the two populations could not be distinguished.

ABSTRACT

"Correlation Between Aedes aegypti Infestations and Dengue Infection in in Two Cities in Mexico" — D. A. Eliason*, M. Moore*, L. Cabrera-C.**, E. Hernandez-C.**, and G. E. Sather *. *Center for Disease Control, Atlanta, Georgia 30333. **Mexican Ministry of Health, Mexico D. F., Mexico.

A field study in 1980 was aimed at describing and quantitating dengue activity in two cities in Mexico and correlating the incidence of disease with abundance of the vector mosquito, Aedes aegypti. The study involved sero-surveys and mosquito sampling in randomly selected households in two areas each in Tampico and Merida during February and August 1980. In February, in Tampico, 2 of 111 (2%) single serum specimens from one area had serologic evidence of recent dengue infection (reciprocal hemagglutination inhibition titers of > 640 to one or more dengue virus types), Aedes aegypti larvae were found on 12% of the premises in that areas. In the other area sampled in Tampico, there was serologic evidence of recent dengue infection in 11 of 81 (14%) single serum specimens and in this area 27% of the premises inspected had Aedes aegypti larvae. In one area of Merida, 6 of 46 (13%) single serum specimens had evidence of recent dengue infections compared to 35 or 78 (45%) specimens in the other area. Aedes aegypti larvae were found on 18% and 19% respectively of the premises sampled in the two areas. A second visit will be made to each area in August to obtain a second serum sample from individuals who were bled during the first visit in February. Premises which were inspected for Aedes aegypti in February will be reinspected in August. Comparisons are made of changes in vector populations and these are correlated with dengue incidence, i.e. seroconversions during the period from February to August.

Manipulation of Immune Resistance to Ticks in Guinea Pigs with Transfer of Sensitized Cells or Serum and with the Administration of an Anti-Basophil Serum,

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Immune resistance of guinea pigs to feeding of ectoparasitic ticks has been demonstrated with several species of ticks. In all cases, this immune resistance is associated with large infiltrates of basophils to the site of tick rejection. The current study examined whether sensitized cells or immune serum were responsible for this basophil associated cutaneous resistance to ticks, and whether the basophils were necessary for expression of the resistance mechanism.

Larval Ambyloma americanium ("Lone star tick") were employed. Hartley guinea pigs acquire resistance to these ticks after a single infestation. Immune resistance is expressed in subsequent infestations and results in decreased tick repletion (engorgement and drop off) and decreased tick weight. Sensitization feeding resulted in 80% tick repletion and an average tick weight of 0.93 mg. Subsequent challenge feeding 3-4 weeks later resulted in 10% repletion (an 80% rejection compared to ticks placed on non-immune hosts), with an average tick weight of 0.69 mg (a 25% decline).

Sites of tick feeding in naive hosts contained just neutrophils and mononuclear cells. The cellular response in the dermis of resistant hosts consisted principally of basophils (69% of the infiltrate) until 72-96 hours after commencement of feeding when eosinophils were prominent (54%).

Immune resistance could be passively transferred to normal guinea pigs with either cells or serum from sensitized hosts. Tick challenged recipients of peritoneal exudate cells had only 11% tick repletion (an 80% rejection) with an average tick weight of 0.65 mg, while immune serum recipients had less of a decrease in tick repletion (67%) and tick weight (0.81%), but these transfers were reproduceable and this represented statistically significant resistance.

Administration of a rabbit anti-guinea pig basophil serum (J. Immunol., 121: 1157, 1978) to tick sensitized guinea pigs eliminated basophils from the bone marrow, blood and tick feeding sites, and abolished acquired resistance to tick feeding (73% repletion, 1.05 mg average tick weight), compared to controls treated with normal rabbit serum or with saline.

These studies demonstrate that sensitized cells and immune serum factors (presumed to be respectively T cells and antibody) can mediate cutaneous basophil associated immune resistance to ticks, and that the basophils are necessary for the expression of the tick resistance mechanism.

Synthesis of Viral Proteins in Cells Infected with Sandfly Fever Viruses

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Virus-directed protein synthesis has been studied in BHK or vero cell cultures infected with nine serologically distinct sandfly fever viruses (Arumowot, Candiru, Gordil, Itaporanga, Karimabad, Naples, Punta Toro, Rio Grande, or Sicilian). The inhibition of host cell protein synthesis following infection by these viruses was found to be both delayed and incomplete. Consequently, infected and isotopically labelled cells were lysed in detergent-containing buffers and viral proteins were selected by immunoprecipitation or immuno-affinity chromatography using hyperimmune ascitic fluids. These samples were then analyzed by discontinuous polyacrylamide gel electrophoresis and fluorography. The results have indicated that although overall similarities in the profiles of virus-specific polypeptides exist, each of the viruses studied directs the synthesis of a set of polypeptides which is sufficiently distinct such that these viruses could be identified on this basis. In addition to virion structural polypeptides, immunoprecipitates from Karimabad, Sicilian, Gordil and Punta Toro virus-infected cells contain a 31,000 dalton protein which may be termed non-structural insofar as it does not become an integral component of the mature virion. Other presumed nonstructural proteins have been detected and are currently under study. Although it is probable, it remains to be shown that these putative non-structural proteins are unique gene products. By one-dimensional electrophoresis only the glycoproteins of Arumowot, Naples, and Punta Toro clearly resolve into two distinct species.

The proteins specified by Karimabad virus have been studied in greater detail to monitor the kinetics of their synthesis, post-translational processing, and function in virion morphogenesis. Pulse-chase procedures which have been carried out in the presence or absence of amino acid analogues have failed to reveal precursor-product relationships among the proteins detected by immunoprecipitation. Pulse labelling at various times after infection has, however, shown that the onset of synthesis of viral proteins is asynchronous. The synthesis of the nucleocapsid and 31K protein is detected concurrently at three hours after infection whereas the glycoproteins are not detected until seven hours after infection. By electron microscopy the assembly (budding) of Karimabad virus occurs at Golgi membranes in which the spike glycoproteins and viral RNP condense only at contralateral sides of the same membrane area, suggesting some form of transmembranal recognition. Analysis of the viral proteins in membrane vesicles prepared from cytoplasmic membranes has shown that all structural proteins but not all nonstructural proteins become membrane-bound. The resistance of viral proteins in these vesicles to protease digestion has suggested that at least one, and perhaps both glycoproteins are transmembranal during virus budding. The significance of these observations with respect to virus morphogenesis will be discussed.

IMMUNE ENHANCEMENT OF DENGUE-2 VIRUS REPLICATION IN ADHERENT HUMAN MONOCYTES AND IN A HUMAN MONOCYTE CELL LINE

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The current hypothesis on the mechanism of immune enhancement of dengue virus replication (described by Halstead) is that the virus, complexed with dilute non-neutralizing or cross-reactive antibody, is internalized by more cells via their Fc receptors. We tested this hypothesis in adherent human monocytes obtained by Ficoll-Hypaque centrifugation of whole blood. First, we found that gentle trypsinization of the adherent monocytes (0.075% for 30 minutes at 35C) essentially eliminated their ability to support dengue virus replication when the cells were exposed to virus in normal medium. However, the addition of dilute antibody to the culture system resulted in yields of up to 5 $\log_{10}$/ml from the trypsin-treated monocytes. These results indicated that dilute trypsin removed the viral receptors but not the Fc receptors.

We used this system of immune enhancement of dengue-2 virus replication in trypsin-treated monocytes to determine if heat-aggregated normal IgG, which binds to Fc receptors, would inhibit replication of the virus. Gamma globulins in Cohn Fraction II were heated at 63C for 10 minutes and used at 1, 10, 100, and 1000 ug/ml in the culture system. Viral yields were reduced 40 to 50 fold at the 1000 ug/ml level, and approached the normal yields (in control cultures) at 10 ug/ml of aggregated IgG. These results indicate that infection of monocytes by virus complexed with non-neutralizing antibody is through the Fc receptor.

Experience with a human monocyte cell line (U-937) has shown that trypsinization of the cell line is not required to demonstrate reproducibly the phenomenon of immune enhancement of virus replication. Dengue virus will only replicate in these cells when non-neutralizing antibody is mixed with the inoculum and the culture medium. We conclude that the U-937 cell line does not possess the trypsin sensitive viral receptors found on freshly harvested human adherent cells, but does possess Fc receptors by which they can be infected with virus complexed with antibody. Quantification of immune enhancement activity can nowbe accomplished without the necessity of human blood donors or having to remove viral receptors with trypsin. A human antiserum with a dengue-2 neutralization titer of 1:400 and a titer of 1:100 against the serologically related yellow fever vaccine virus exhibited an immune enhancement titer of $\geq$ 1:200,000. This serum will be tested for its ability to enhance the effectiveness of a live attenuated dengue-2 virus vaccine in vivo.

EARLY INTERACTIONS OF ATTENUATED DENGUE VIRUSES WITH MAMMALIAN AND MOSQUITO CELLS

K. H. Eckels, P. L. Summers, and P. K. Russell

Early infection events of attenuated dengue-2 and dengue-3 virus clones and their parent viruses were studied in mammalian and mosquito cells. The attenuated S-1 clone of dengue-2 virus has been used in clinical trials as a live virus vaccine for immunization of humans, and the C-5 clone of dengue-3 (found to be attenuated for mice and monkeys) is currently a candidate human vaccine virus. The attenuated viruses were less efficiently adsorbed than the parental, non-attenuated viruses. Virus was adsorbed by incubation for 2 hours on fetal rhesus lung or Aedes Albopictus mosquito cell monolayers. The cells were washed with medium containing homologous antibody, trypsinized, and then assayed for infectious centers in a standard plaquing system. Adsorption carried out at various temperatures indicated that the attenuated viruses produced 4-fold to 10-fold fewer infectious centers than the parent viruses at temperatures of 35C to 38.5C. Little difference was seen between the virus pairs at 4C or 23C.

Pretreatment of cells with polyethylene glycol increased the efficiency of adsorption of the attenuated S-1 clone up to the level of the parent virus while pretreatment with trypsin increased the efficiency of adsorption to within 2-fold of the parent virus. Both of these agents can cause rearrangement and concentration of viral receptors on a cell membrane. Concentration or "patching" of receptors in a given area on the membrane may be important for multivalent binding and successful adsorption of the attenuated viruses. Temperature studies indicate that initial adsorption is similar for the parent and S-1 viruses and that other events occurring later in the adsorption period including receptor concentration are important for a cell to be successfully infected by the S-1 virus.

CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST DENGUE VIRUSES

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Diagnosis of dengue virus infection and specific identification of isolated dengue virus serotypes have presented certain problems for the arbovirus laboratory, primarily because of extensive serological cross-reactions frequently observed between the four dengue serotypes and other flavivirus antigens. A recently established technology for the production of monoclonal antibodies using lymphocyte hybridoma cell cultures shows great promise for the production of type-specific serological reagents as well as valuable research reagents fo assist in the investigation of the molecular basis for the antigenic complexity of these viruses. Our primary objectives is this study were to demonstrate that monoclonal antibodies can be produced to dengue-2 virus, that type-specific determinants are recognized by certain of these antibodies, and that these immune reagents can be used in traditional serological tests.

Lymphocyte hybridoma cultures were prepared using spleen cells from BALB/c mice immunized with dengue type 2 (New Guinea C) virus fused with the P3x63Ag8 myeloma cell line. Antibody-secreting cell clones were selected by solid phase radioimmune assay (SPRIA), and a representative collection of these cell lines were examined in some detail. Mouse ascitic fluids were prepared using these hybridomas to provide the highest-titered reagents possible.

Ten of 16 hybridoma clones examined were positive in a plaque reduction neutralization test. Of these 10, only one appeared truly type specific, in that it neutralized homologous dengue-2 virus to a titer of 1:3200 and was negative at a 1:10 dilution with the other three dengue serotypes. The 16 antibody preparations were chosen because they inhibited dengue virus hemagglutination (HAI). Four of these antibody preparations specifically inhibited dengue-2 virus and included the hybridoma clone with the type-specific neutralization reaction described above. The HAI cross-reactive clones were approximately equal in titer to each of the other three serotypes, as well as yellow fever and Japanese encephalitis virus, suggesting an antigenic component common to each of the flaviviruses tested. Complement fixation (CF) results were less precise, and certain antibody preparations were anticomplementary; however, many of those selected were CF positive.

A variety of experiments were performed using antibody from these hybridoma cultures, including immune fluorescence, immune precipitation of extracts from radiolabeled infected cells, and an examination of their ability to immunologically enhance virus replication in monocytes. These studies support the concept that monoclonal antibodies produced by lymphocyte hybridomas have enormous research potential as well as direct application as diagnostic immune reagents.

An attenuated mutant of Venezuelan encephalitis virus: Biochemical alterations and their genetic association with attenuation. EMILIO A. EMINI and MICHAEL E. WIEBE, Cornell University Medical College, New York, N.Y.

A temperature-sensitive mutant, ts 126, derived from the hamster-virulent 68U201 wild-type (wt) strain of Venezuelan encephalitis virus, an alphavirus, has been shown to be attenuated. Although it is temperature-sensitive, the mutant replicates in infected hamsters and elicits production of protective antibodies (Krieger et al., 1979, Infect. Immun. 25: 873-879).

We have characterized additional phenotypic differences between ts 126 and 68U201 wt in an attempt to localize the genetic basis of the mutant's attenuated virulence. ts 126 was shown to differ from the parent virus with respect to virion structure-dependent, particularly surface structure-dependent, characteristics: temperature-lability, plaque sizes in Vero cells, and binding properties to hydroxylapatite. A surface difference was identified by isoelectric focusing of the virion envelope glycoproteins as an alteration in the E_1 glycoprotein. The common genetic basis of all these phenotypic differences was demonstrated by the isolation of independently-arising, stable genetic revertants of ts 126. These revertants exhibit characteristics identical in every respect to 68U201 wt.

It appears from these studies that the mutation which gave rise to the ts 126 mutant virus occurred in the structural gene coding for the E_1 envelope glycoprotein and that the resultant phenotypic alteration in this glycoprotein is genetically associated with the mutant's lack of virulence.

Response of Different Aged Chicks to Infection with Temperature Sensitive Mutants of Western Equine Encephalomyelitis Virus

by

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The mechanism(s) which allows western equine encephalomyelitis virus (WEEv) to overwinter in temperate climates is still unknown. Evidence for overwintering of virus in known mosquito vectors is lacking. However, there is experimental evidence that WEEv can persist in some avian hosts for nearly a year. As a follow up of this latter observation, we are attempting to determine if temperature sensitive (ts) mutants of WEEv facilitate the establishment of persistent infections in birds since ts mutants have been recovered from animals persistently infected with other viruses.

The initial studies have concentrated on the *in vivo* and *in vitro* characterization of 5 ts mutants produced by mutagenesis and belonging to different complementation groups. For *in vivo* studies 10^4 pfu of wildtype (WT) or ts virus were inoculated subcutaneously into wet (< 1 day) and 21-day old chicks whose body temperatures were in the permissive ($\leq 40^{\circ}C$) and nonpermissive ($\geq 41.5^{\circ}C$) temperature ranges, respectively, for viral replication. Responses measured were mortality, viremia and antibody development.

Infection of 21-day old chicks with WT virus resulted in high titered viremias within 24 hrs after infection but virus was cleared from the blood by 72 hrs after infection. In contrast, the replication of one stable RNA$^-$ ts mutant (ts 158) was apparently restricted in these older chicks with high body temperatures since neither viremia nor antibody development was detected. The multiplication of the other 4 ts mutants was not restricted in the 21-day old chicks; however, viremia titers in chicks infected with these mutants were significantly lower than those observed in chicks infected with WT virus. None of the viruses, including WT, caused mortality in these older chicks.

All viruses produced viremias when inoculated into wet chicks but viremia titers observed with 4 of the 5 ts mutants were 100- to 1000-fold lower than they were with WT virus. The primary difference between these viruses in wet chicks, however, was in their expression of virulence as evidenced by mortality. WT and ts 149 viruses were highly virulent causing 100% mortality within 48 hrs after inoculation. Two ts mutants, including ts 158, were avirulent (i.e., no mortality) whereas the other two were moderately attenuated in that only 20% of the chicks died and death was delayed until 5 to 6 days after infection. Interestingly, death of these latter chicks occurred 1 to 2 days after viremias disappeared, whereas death of chicks infected with WT and ts 149 viruses occurred when viremia titers were at maximal levels. The percent cumulative mortality correlated well with the viremia titer at 24 hrs postinfection. It is unknown whether attenuation of WEEv in wet chicks was related to partial restriction of viral replication at $\leq 40^{\circ}C$.

Studies on the *in vitro* characterization of the ts mutants have been done and will be summarized during the presentation.

American Society of Tropical Medicine and Hygiene
Abstract of paper for presentation at 1980 Atlanta meeting.

Timing of pupation in the Western Treehole Mosquito, Aedes sierrensis.

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Aedes sierrensis is a probable vector of canine heartworm in California. Larvae occur in winter rain-filled treeholes following hatch of estivated eggs. The time of appearance of 4th instar larvae is highly variable and depends on the date of the first heavy fall rain and the subsequent regime of temperature which affects the rate of larval growth. Nevertheless, the duration of the 4th instar varies in such a way that pupation begins at about the same time each year. Previous workers have measured development times in larvae raised at the unrealistic tmperature of 17°C and continuously exposed to either a long or a short photoperiod. They have concluded that long photoperiod shortens the time from hatch to pupation.

We now report the results of experiments conducted at 8°, 11° and 14° where larvae were exposed to different proportions of long versus short photoperiods during their larval development. The results show that the 4th instar is sensitive to small differences in photoperiod (corresponding to midwinter versus early March, at about 38° latitude) and that pupation is delayed by the shorter photoperiod although larval growth rate remains a function of temperature.

These results will be discussed in relation to the basic population model for the tropical treehole breeding Aedes aegypti.

ABSTRACT

ISOZYME CHARACTERIZATION OF LIVE Haemagogus equinus, A VECTOR OF SYLVAN YELLOW FEVER. Petersen, J. L. (Gorgas Memorial Laboratory, Panama)

Isozymes are genetically determined variants of an enzyme that can be detected by differential migration in an electric field. Zymograms of three enzymes, phosphoglucomutase (PGM, E.C. 2.7.5.1), phosphoglucose isomerase (PGI, E.C. 5.3.1.9) and malate dehydrogenase (decarboxylating) (ME, E.C. 1.1.1.40) were obtained by cellulose acetate electrophoresis of live, virgin Haemagogus equinus males and females (Table 1). The technique is based upon the removal of a single hind leg of an anesthetized mosquito. A single leg triturated in 5 µl of buffer provides sufficient tissue homogenate for electrophoresis for the enzymes mentioned above. This permits genotype determination of a mosquito while it is still alive so that it is possible to set planned genetic crosses. Formerly, mass crosses were set then progeny were screened to isolate desired traits from fortuitous matings. To obtain homozygous lines of the isozymes we crossed selected individuals as follows: First, virgin mosquitoes carrying uncommon alleles in the heterozygous state were identified electrophoretically and then crossed among themselves. Then, the progeny of this cross were reared to adults and then characterized electrophoretically while still virgin. Homozygotes were selected and crossed to establish pure breeding lines for each allele (Table 1).

We are using isozyme characterization to find genetic markers useful in identifying geographic variation in vector competence among populations of mosquitoes that transmit yellow fever. At the same time we are studying the formal genetics of Hg. equinus. Our goal is to locate genetic markers on all three linkage groups and summarize these in a genetic map.

Table 1. Enzyme characteristics of Haemagogus equinus.

Enzyme	Locus name	No. of alleles	Subunit structure
Phosphoglucomutase	Pgm-1	4	Monomer
Phosphoglucose isomerase	Pgi-1	2	Dimer
Malate dehydrogenase (decarboxylating)	Me-1	1	Unknown
	Me-2	3	Tetramer

Different intestinal thresholds among strains of Venezuelan encephalitis virus in an enzootic vector mosquito, Culex (Melanoconion) opisthopus

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In 1977, Culex (Melanoconion) opisthopus was found to be a natural vector species of Venezuelan encephalitis (VE) virus at a marsh focus of virus on the Pacific coast of Guatemala. This species was only the third mosquito species proven to be an enzootic vector of VE virus; the other two species are Cu. (Mel.) portesi in Trinidad, West Indies and aikenii in Panama.

During 1979, determinations of the intestinal threshold of VE virus (minimal intestinal dose of virus) in Cu. (Mel.) opisthopus began by examining a virus strain (68U201) from the Pacific coastal marsh focus in Guatemala. These experiments revealed that intestinal thresholds of this strain of VE virus were low (<5 pfu in primary chicken embryonic cell cultures) for both infection and transmission of virus by wild-caught Cu. (Mel.) opisthopus from the Guatemalan focus.

Subsequently a second strain of VE virus was tested and found to have remarkably higher intestinal thresholds for infection and transmission of virus by Cu. (Mel.) opisthopus. This strain (69U332) was isolated from a sentinel hamster at the Pacific focus in Guatemala in October 1969, about two months after the VE epidemic-epizootic had terminated in that region. It was identified as VE virus by plaque-reduction neutralization and by complement-fixation tests, and had equine-virulence,hemagglutination-inhibition, hemagglutination-pH and Vero plaque properties like epizootic VE virus. Cu. (Mel.) opisthopus were allowed to bite hamsters at selected times after inoculation to obtain different input quantities of virus and mosquitoes were kept at 27°C, 70% relative humidity with 15 hours of light, including artifical dusk and dawn. The following numbers of mosquitoes and average input levels of virus strain 69U332 were testedin7experiments after 20-23 days of extrinsic incubation: 12 and 6 at <5 pfu, 42 at 25 pfu, 9 at 320 pfu, 21 at 800 pfu, 20 at 10,000 pfu and 11 at 16,000 pfu. The generation levels of these mosquitoes were F_0, F_2, F_2, F_0, F_2, F_4 and F_4 respectively. The only mosquito that contained detectable virus was 1 of the 21 at 800 pfu. This mosquito did not engorge blood on day 23 and the hamster remained well. Thus the 50% intestinal infection threshold of the 69U332 strain of VE virus in Cu. (Mel.) opisthopus exceeded at least 800 pfu and probably 16,000 pfu.

That the Cu. (Mel.) opisthopus propagated at the insectary in Ithaca, New York had not lost their susceptibility to VE virus was verified in control experiments. Using virus strain 68U201 and F_2 generation mosquitoes, 13 of 20 mosquitoes became infected with an input of 12 pfu/mosquito and 5 of 10 infected mosquitoes that bit normal hamsters transmitted virus; with 15 pfu/mosquito, 8 of 12 mosquitoes became infected, and 4 of 5 infected mosquitoes transmitted virus. Using another virus strain (70U80) from an enzootic focus on the Atlantic coast of Guatemala, 10 pfu infected 15 of 15 F_4 generation mosquitoes and 4 of 6 mosquitoes transmitted virus, and 630 pfu infected 7 of 7 F_2 mosquitoes, and 7 of 7 transmitted virus.

Thus a striking difference was found among strains of VE virus with respect to intestinal thresholds for infection and transmission of virus by the enzootic vector mosquito, Cu. (Mel.) opisthopus. Obviously further studies are required to learn whether these results reflect fundamental differences between enzootic and epizootic VE strains.

Detection of LaCrosse Antigen with ELISA

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The use of immunoglobulins prepared by column chromatography as coating antibody has resulted in an ELISA test for the detection of LaCrosse antigen. A high titered mouse immune ascitic fluid was precipitated with ammonium sulfate and subjected to chromatography on G-200 Sephadex. The ELISA was performed by a multilevel sandwich technique. After the coated plates had been exposed to specimens with or without antigen and washed, a rabbit antiLaCrosse serum was used as a detector. The rabbit was immunized with a single dose of gradient purified virus grown in Vero cells and not mouse brain material to prevent undesirable reactions with the mouse derived coating antibody. Alkaline phosphatase labeled goat antirabbit conjugate and p-nitrophenyl phosphate were used to complete the procedure. The plates were evaluated visually and in some instances spectrophotometrically for comparison. The detector step was added to the standard procedure to allow one conjugate to be used with a number of viruses and to make use of a commercially available conjugate.

Initial experiments showed that antigen could be detected in infected mouse brain (20% in 10% FCS), sucrose acetone extracted baby mouse brain (5%) (SABMB), gradient purified material derived from BHK cells, and supernatant from Vero cells. A weak reaction was noted with gradient purified material from BHK cells infected with either snowshoe hare virus or Tahinia virus. Negative reactions were found with normal mouse brain (20% in 10% FCS), Dengue SABMB, gradient purified material derived from Dengue infected LLCMK2 cells, the supernatant from Modoc infected Vero cells, and gradient purified material from BHK cells infected with Trivitatus virus. The test was sufficiently sensitive to detect 10^5 PFU of virus.

To determine if deterimental background would occur with other potential sources of antigen, LaCrosse virus was added to one aliquote of the following materials while another remained untreated: mouse liver (10%). mouse serum (undiluted), human CSF (undiluted), and both large (50) and small (10) pools of Aedes aegypti, Aedes triseriatus, Culex fatigans and Culiseta melanura. Antigen was detected in all samples to which virus had been added and no background was noted in untreated material as long as PBS-Tween (0.5%) was added. A number of human serum samples were included in the test, and one in twenty five showed a nonspecific background reaction at both the 1/10 and 1/20 dilutions. These sera were nonspecific in several ELISA tests.

Experiments are currently being planned to determine if antigen can be detected in mosquitoes infected in the laboratory.

ABSTRACT

AEDES AEGYPTI SURVEILLANCE AND CONTROL MEASURES IN SANTO DOMINGO, DOMINICAN REPUBLIC

C. G. Moore,[1] N. Gañan-C.[2]

Dengue virus activity has been widespread throughout the Caribbean since the mid 1970's. In 1977, the government of the Dominican Republic, under sponsorship of the Pan American Health Organization and the Center for Disease Control, began a study of dengue epidemiology in that country, with vector surveillance and control carried out by the National Malaria Eradication Service (SNEM). The objectives were to gather baseline data on Aedes aegypti abundance and to achieve some level of protection of the population through vector control. We report the results of the first 23 months of that program.

From September, 1977, through January, 1978, adult A. aegypti control was attempted by ultra-low volume (ULV) treatment of the city of Santo Domingo with malathion. In May, 1978, a program of larval surveillance and control was begun. A total of 115,000 premises within Santo Domingo were targeted for inspection and treatment. Aedes aegypti control measures consisted of public education and application of Abate 1-SG(R)[3] larvicide to non potable water. Aedes aegypti abundance was monitored on a monthly basis in nine representative zones within the target area. An additional zone, comprising the International Airport and surrounding area, was located approximately 20 km from the city. There were no untreated areas. All but 4 of the original 10 zones were changed during the study for administrative reasons. Seventeen different zones were inspected and treated during the study. Two hundred houses, 20 in each zone, were inspected each month, a different set of houses within a given zone being selected each time. Inspections were conducted both inside and outside houses and other buildings, and data were collected on total numbers of containers and numbers containing larvae.

Seasonal changes in the 4 zones studied since the beginning of the project reflected local rainfall patterns. In 1978, there was a peak in larval abundance in June and another in December. In 1979, an unusually wet year, Breteau indices remained at or above 30 through August. In September and October the index fell below 10, probably because of the effects of Hurricane "David," which did extensive damage to the city. By November, the Breteau index had again risen to 45. Of the 17 zones studied, the lowest average Breteau index was found at the International Airport (14.5), an area consisting mostly of commercial and industrial premises. Within Santo Domingo, average Breteau indices ranged from a low of 15.0 to a high of 48.6, with center-city areas most heavily infested. Although Breteau indices in several zones declined during the study, there was no clear evidence of reduction in larval abundance. Since new groups of houses are visited each month, we expected little decline due to control. However, if public education efforts had been highly effective, communication among local residents should have resulted in fewer positive breeding sites. We found no evidence of this.

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ABSTRACT

THE LABORATORY DIAGNOSIS OF DENGUE-1 VIRUS OUTBREAKS IN MEXICO

J. P. Woodall,[1] M. L. Zárate,[2] C. E. Sather,[1]
L. Cabrera-C.,[4] C. G. Moore,[1] G. Kuno,[1] M. Moore,[3]
I. B. Fabrikant[1] and A. Moya-C.[2]

In early 1978 the Caribbean pandemic of dengue-1 virus reached the American mainland at Honduras. From there it spread through Central America, reaching the northern borders of Guatemala and Belize by the end of the year. No outbreak of dengue had been reported from Mexico since 1941. Sera were collected in January 1979 from 55 Mexicans with histories of recent febrile illness; they lived in the area of Chetumal, Quintana Roo state, at the Belize border on the Caribbean coast of Mexico. Nine were found to contain flavivirus hemagglutination-inhibition (HI) antibodies, and one had complement fixing (CF) antibodies at a titer compatible with recent dengue-1 infection. At the same time, there was an outbreak of fever, with muscle and joint pains, at Tapachula, Chiapas state, at the Guatemala border on the Pacific coast of Mexico. Serum samples were taken from 92 patients, and 21 had HI and CF antibodies, suggesting current cases of dengue. Several had HI titers which indicated that this was not their first exposure to flavivirus infection, and some of these people were born after the 1941 epidemic.

Subsequently, outbreaks of dengue-like disease were reported from the southern states of Oaxaca and Veracruz, and towards the end of 1979, in the state of Yucatán.

In February 1980 a joint Mexico-U.S. effort in Mérida, Yucatán, resulted in the collection of 122 sera from a household survey (findings reported elsewhere), five convalescent sera from patients who had previously been bled in the acute phase of a dengue-like illness, and one blood specimen from an acutely ill person. Dengue-1 virus was isolated from the acute specimen by inoculating the serum into Toxorhynchites amboinensis mosquitoes and into Aedes albopictus, Ae. pseudoscutellaris, and T. amboinensis tissue cultures. The same virus type was isolated from the clot. Identification was by the CF and plaque reduction neutralization tests. This represents the first isolation of a dengue virus from Mexico. Four of the five pairs of sera from suspect cases had dengue-1 CF antibody, including two with diagnostic rises in titer. These findings confirmed the cause of the Mérida outbreak was dengue-1 virus and suggested that other chronologically related outbreaks producing flavivirus antibodies were due to the same agent. We have developed a rationale for interpreting complex flavivirus antibody patterns to diagnose current dengue infection.

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Genetic Resistance to Lethal Flavivirus Encephalitis: Effect of Host Age and Immune Responsiveness on Production of Interfering Virus *in vivo*.

Abigail Smith and Robert Connell

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Resistance of nonimmune mice to lethal flavivirus encephalitis is inherited as a simple autosomal dominant trait. We have previously reported the importance of the immune system and interfering virus (IV) production to survival of genetically resistant mice infected with Banzi virus. We have extended our studies to young mice (at an age before phenotypic expression of resistance is developed) and to immunologically compromised mice in order to understand further the role of Banzi IV production in resistance to disease.

Three week-old and 8 to 12 week-old C3H/He (He, susceptible) and C3H/RV (RV, resistant) mice were given 500,000 $TCID_{50}$ of Banzi virus by intraperitoneal inoculation; half of the 8 to 12 week-old mice were given cyclophosphamide. Brains were harvested at daily intervals beginning on day 2 post-inoculation; the brain suspensions were titrated for quantitation of virus and interference assays were performed in CER cells using either infected or uninfected brain suspensions or diluent as pre-absorption inocula before low multiplicity challenge with stock Banzi virus. The results are presented qualitatively below (+ denotes detection of IV; ++ denotes $\geq$ 2 $\log_{10}$ reduction in interference assay progeny titers compared to control progeny titers; numbers in parentheses are days post-inoculation of IV detection):

	Susceptible C3H/He	Resistant C3H/RV
Untreated 3 week-old mice	-	+ (3,4)
Untreated 8-12 week-old mice	-	+ (5,6,7,8)
Cyclophosphamide-treated 8-12 week-old mice	+ (4,5)	++ (4,5,6,7)

Both 3 week-old and immunosuppressed 8 to 12 week-old RV mice die, indicating that interfering virus alone, in the absence of a fully functional immune system, does not mediate survival. One question raised by these results is the relation of immunosuppression to IV production, since cyclophosphamide treatment amplified IV production in RV brains and contributed to the detection in He brains. One hypothesis is that, since the primary target of Banzi virus is the lymphoid organ system, IV is first produced in a sub-population of lymphoid cells which continue to proliferate in the presence of cyclophosphamide treatment (possibly T cells). The reduced immunocompetence of these mice prevents clearance and results in rapid viral proliferation and IV amplication in the ultimate target organ, the brain. Alternatively, since the effect of cyclophosphamide on the central nervous system is unknown, it is possible that the drug in some way alters the secondary target organ (brain) in favor of increased IV production. Experiments using other immunosuppressive agents which preferentially act on different cell subsets and studies directed toward direct IV detection in lymphoid organs should help to resolve this question.

Abstract for the 29th Annual Meeting of The American Society of Tropical Medicine and Hygiene, November 4-7, 1980, Atlanta, Georgia.

Genome Characterization of Various Dengue Types 1 and 2 Virus Strains

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The virion RNA species of the four dengue (Den) serotypes have previously been shown to be distinct from one another by oligonucleotide fingerprint analyses. We now report that different strains of the same serotype have unique fingerprints, which may become an extremely useful tool if viral origins can be traced to a specific geographical region of the world. Similarly, the dengue-2 virus vaccine candidate can be distinguished from the parent virus by oligonucleotide mapping, thus making the technique extremely valuable for identifying and characterizing viruses isolated from human volunteers.

The genomes of various strains of dengue-1 viruses as well as the dengue-2 vaccine strain and its parent were compared. Virion RNA fingerprints of each of four Den-1 strains - prototype Hawaiian (1943), African (1962), Jamaican (1977), and a 1977 isolate from the Bahamas - appeared unique. The two Carribbean strains were identical with the exception of two oligonucleotide spots, and exhibited virtually no similarity to the Hawaiian strain. However, a striking resemblance was observed between the cytosine/adenine-rich oligonucleotides of the Carribbean strains and the African strain. Although the similarities were insufficient to establish a close relationship between Carribbean and African strains, it should be emphasized that these viruses were separated by a 15-year interval in isolation.

Separate geographic isolates of dengue-2 viruses also exhibited significantly different fingerprint patterns. The prototype New Guinea C (1945) strain of Den-2 displayed a completely different fingerprint from a Puerto Rican (1969) GMK6 strain. The PR-GMK6 strain was the parent virus used in the selection of the temperature-sensitive vaccine strain PR-159(s1), and although the fingerprints of both strains were essentially identical, seven differences in their oligonucleotide spots could be detected. Such differences should allow characterization of virus isolates from human volunteer vaccine recipients. Fingerprints are similarly being compared from a variety of revertants (i.e. large-plaque, temperature-resistant viruses resulting from propagation of vaccine virus).

PROPERTIES OF LIVE ATTENUATED JAPANESE ENCEPHALITIS VACCINE BEFORE AND AFTER ADAPTATION TO THE C6/36 CLONE OF *AEDES ALBOPICTUS* CELLS

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The 2-8 vaccine strain of JEV was developed in Peking by Chen (Acta Microbiologica Sinica 14(2): 176-184, 1974). The virus was attenuated after 100 passages in primary hamster kidney cells, followed by U-V treatment, 5 passages in baby mice, and 3 plaque clonings in primary chick embryo cells. It is of reduced virulence for mice, pigs, horses, and monkeys. It was administered as a live vaccine to 8,000 children in China with approximately 50% seroconversion, and to 500,000 horses with a high rate of seroconversion.

The virus was adapted to C6/36 cells. It initially titered 10^8 $TCID_{50}$/ml and had CF antigen titer of 1:12 on the fifth day of infection. The cultures remained persistently infected. Titers of 10^8 $TCID_{50}$/ml were maintained during a 100 day period provided the culture medium was changed at weekly intervals. The C6/36-passaged virus remained attenuated for mice, but after 100 days in culture, induced higher antibody titers than the vaccine strain which had not been passaged in mosquito cells.

The basis for attenuation was sought. The vaccine is not temperature-sensitive, but attenuation in mice was reversed by treatment of the mice with cyclophosphamide.

Transovarial transmission of St. Louis encephalitis virus by *Culex pipiens* complex mosquitoes

D. B. Francy, W. A. Rush, M. Montoya, D. Inglish, R. A. Bolin

A study was conducted to determine whether transovarial transmission (TOT) could be experimentally demonstrated with SLE virus in *Cx. pipiens* complex mosquitoes, the principal vector in the central-eastern United States. During 1978 the TOT studies were conducted in conjunction with evaluation of SLE virus fector efficiency of field collected mosquitoes from McLeansboro, IL, Memphis, TN, and material from a colony started with mosquitoes collected in the vicinity of Chicago in 1975. The specimens from McLeansboro were catagorized as *Cx. p. pipiens* by examination of the male genitalia as were the colony mosquitoes from Chicago. The Memphis mosquitoes were both subspecies plus intermediate forms and thus are referred to as *Cx. pipiens* complex.

Female mosquitoes were fed on viremic chicks previously inoculated with a mosquito strain of SLE virus from Memphis, TN. Stock virus for the experiments was prepared by intrathorasic inoculation of *Cx. p. pipiens* with original mosquito suspension.

Most of the progeny mosquitoes tested for TOT from the 1978 study resulted from the first ovarian cycle of mosquitoes following feeding on an infected host. A smaller number of progeny were from a second

ovarian cycle after refeeding of the infected female mosquitoes on a normal host. Elapsed time between infection of the parent and oviposition was 8-26 days for the first blood meal and 19-33 days for the 2nd.

The procedures were modified in 1979 in several respects. Only mosquitoes from the Chicago colony, and from a Memphis colony started in 1976 were used. Mosquitoes were again infected by feeding on viremic chicks. Mosquitoes were allowed to oviposit, fed on a non-infected host, and a second lot of eggs obtained. Eggs were hatched and larvae divided into 2 groups held at 18 $\pm$ 0.5C or 25 $\pm$ 0.5C, respectively.

Progeny mosquitoes were sorted into pools of 50 each after varing periods of incubation and held at -60° for testing. A portion of the pooled females were allowed to feed overnight on a normal chick, held ~ 3 days for blood digestion and frozen. Recipient chicks were bled at ~60 hrs. for virus testing and at 21 days for serological testing for evidence of virus transmission.

In 1978 a single instance of TOT was documented in Memphis mosquitoes reared from 1st ovarian cycle eggs oviposited 26 days post-infectious feeding. In the 1979 experiments a single TOT occurred among progeny from the 1st ovarian cycle. From 2nd cycle mosquitoes virus was recovered from 7 pools. Virus isolations were mostly from Memphis mosquitoes held at the lower temperature. Among 2nd cycle progeny TOT rates were 6/5424 for Memphis mosquitoes compared with 1/3510 from Chicago mosquitoes. Recovery rates were also higher at the lower temperature (6/6160) compared with higher temperature (1/3074).

Of the 4 positive female pools, 3 were from the Memphis colony and 1 from Chicago. The positive Chicago mosquito pool had an opportunity to transmit as did 2 of the Memphis pools. The Chicago mosquitoes failed to transmit virus, but both of the positive Memphis pools did.

The results of the study demonstrate unequivocally that TOT can occur in a major vector species of SLE virus, and more importantly that progeny females infected transovarially can transmit virus efficiently.

The ecological ramifications of these observations are discussed.

Michael J. Turell
James L. Hardy
William C. Reeves

Carbon Dioxide Sensitivity in Mosquitoes Induced by Infection with California Encephalitis Virus

It has long been recognized that fruit flies, Drosophila melanogaster, infected with rhabdoviruses such as sigma and the various serotypes of vesicular stomatitis virus are sensitive to carbon dioxide (CO_2). This sensitivity is expressed as a paralysis following anesthesia with CO_2. Vesicular stomatitis virus and other rhabdoviruses known to produce CO_2 sensitivity in Drosophila have recently been shown to produce this effect in mosquitoes, but attempts to induce CO_2 sensitivity in mosquitoes infected with several togaviruses have failed. We have now demonstrated and partially characterized this same phenomenon in 6 species of mosquitoes infected with a bunyavirus, California encephalitis (CE).

Mosquitoes were infected with recent isolates of CE virus by intrathoracic (i.t.) inoculation, ingestion of virus from gauze pledgets soaked with a defibrinated rabbit blood/virus suspension, or transovarial transmission. Mosquitoes were held in an insectary maintained at 27°C and a relative humidity of 80% until tested for CO_2 sensitivity at room temperature (22°C). This was accomplished by introducing CO_2 into the 0.5 ℓ cardboard holding containers for approximately 20 seconds. Sensitive mosquitoes continued to lie on their backs and were unable to right themselves. Some mosquitoes were able to get back on their feet and walk or hop, but could not fly normally. These were designated questionably sensitive (+/-). The infection status of each mosquito was determined by plaque assay in cells.

CO_2 sensitivity was observed in all 6 mosquito species that had been infected with CE virus by i.t. inoculation 4-10 days previously. These included: Aedes dorsalis, Aedes melanimon, Aedes nigromaculis, Aedes triseriatus, Culiseta incidens, and Culex tarsalis. CO_2 sensitivity occurred in both field collected and laboratory colonized mosquitoes. CO_2 sensitivity was also demonstrated following oral infection from pledgets; however, both +/- and misclassified mosquitoes were more common than they had been in mosquitoes infected by i.t. inoculation. While mosquitoes infected either orally or by i.t. inoculation became sensitive, this phenomenon was not observed in transovarially infected progeny of Ae. dorsalis or Ae. melanimon.

CO_2 sensitivity and infection were inhibited by incubating the CE virus with antiserum to the BFS 283 strain of CE virus for 1 hour prior to i.t. inoculation. When chloroform was used to anesthetize both infected and uninfected Ae. dorsalis and Cx. tarsalis, all mosquitoes recovered. Thus, this phenomenon appears to be related specifically to infection with CE virus and anesthesia with CO_2.

Holding Ae. dorsalis and Cx. tarsalis for various periods of time after inoculation before first exposing them to CO_2 showed that they first became sensitive at approximately 3 days post inoculation, remained sensitive for nearly 1 week, and then gradually lost their sensitivity.

The finding that CO_2 sensitivity occurs in mosquitoes following infection with CE virus is significant because it is the first demonstration of CO_2 sensitivity caused by an arbovirus in its natural vector; it extends the phenomenon of CO_2 sensitivity to another family of viruses, Bunyaviridae; and it allows, under certain circumstances, for a rapid and inexpensive method of determining the infection status of mosquitoes with CE virus.

SLE VIRUS ACTIVITY BEFORE, DURING, AND AFTER ECOLOGICAL UPHEAVAL IN A TROPICAL ECOSYSTEM

Abdiel J. Adames, Pauline H. Peralta and Pedro Galindo

A study was designed to detect the effects of changes of arboviral cycles in a drastically changing tropical environment due to the construction of a hydroelectric dam and the formation of a large man-made lake in Eastern Panama. Studies were conducted on a forested island formed within the lake after impoundment. Project was designed in three periods, namely: Pre-impoundment, Impoundment and after impoundment and was carried from 1973 to 1978. Viral activity was detected by systematically sampling mosquito population for virus isolation attempts and also by serological studies from sentinel and wild vertebrates, birds and mammals.

Changes in SLE virus activity were detected and correlated with environmental changes caused by the formation of the lake.

SLE virus was isolated only once during the pre-impoundment period from a diurnal arboreal mosquito (Haemagogus lucifer). During the impoundment period, virus was isolated 9 times from a nocturnal mosquito (Mansonia dyari), which lives associated with aquatic weeds that proliferate in a newly formed lake. No isolations from blood-sucking insects were obtained during the post-impoundment period.

Results from serological studies from birds during the pre-impoundment period demonstrated that, 1 out of 38 sentinel chickens and 1 out of 39 passerine birds had antibodies against SLE virus. During the impoundment period, SLE antibodies were found in 12 out of 165 passerine birds, 3 out of 19 Cormorants, 9 out of 54 non-passerine birds and 3 out of 19 sentinel chickens. During post-impoundment period, SLE antibodies were found in 1/113 passerine birds, 2 out of 52 non-passerine birds, and 1/25 Cormorants.

Pattern of SLE antibodies in sentinel hamsters and wild mammals was as follows: Pre-impoundment period: 5 out of 113 sloths and 2 out of 13 sentinel monkeys were seropositives and all sentinel hamsters, rodents and marsupials were negative. Impoundment period: 19 seropositives out of 357 sentinel hamsters, 19 out of 78 sloths, 4 out of 11 sentinel monkeys and no seropositives from rodents. Post-impoundment period: only one seropositive was found out of 540 sentinel hamsters.

Upsurge of SLE virus activity occurred within the impoundment period which is the destabilization period of the different ecosystems. The observed changes were predicted in the general hypothesis postulated for the project.

Venereal, transovarial, and oral infection rates with La Crosse virus in Aedes triseriatus following engorgement on chipmunks with antibody.

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Ten-fold higher rates of venereal infection (VI) with La Crosse (LAC) virus in Aedes triseriatus mated by infected males following engorgement, and one-third lower rates in females previously engorging on chipmunks with than without antibody to LAC have been previously observed in our laboratory.

Additional trials have since been conducted using F_1 generation field collections of Aedes triseriatus and males transovarially infected with field strains of LAC, and with females infected transovarially or orally on viremic chipmunks.

Venereal infections: VI rates in females mated by TO infected males 5-9 days following engorgement on chipmunks with antibody were again reduced as in previous trials. Thirty-one percent of 106 females pre-fed on chipmunks without antibody to LAC, and only 16% of 111 pre-fed on those with antibody, later transmitted LAC to suckling mice. Similar reductions were not observed in females mated 10 or more days following engorgement of antibody.

Transovarial infections: Rates of transovarial transmission in the trials with VI females were similarly reduced. Most VI infected females later transmit LAC by TO as well as oral route. Filial infection rates (FIR) in progeny reared from second or later ovarian cycles of 20 of the venereally infected females were 50-70%, including both male and female progeny. No differences in filial infection rates were noted between progeny of females previously engorged on chipmunks with or without antibody. In several trials with females origionally infected by transovarial transmission, LAC virus was later transmitted to mice by all of 56 females re-fed 10 days after engorgement on chipmunks with antibody as well as by all of 59 previously engorged on chipmunks without antibody.

Oral infections: In three trials with females infected orally on viremic chipmunks 10-15 days following original engorgement on a chipmunk either with or without antibody, there was no apparent reduction in later transmissions of LAC virus to suckling mice.

Reductions of venereal infection rates with LAC virus have been repeatedly observed only in Aedes triseriatus mated by infected males 5-9 days after engorgement of antibody in chipmunks. This could however be important as a natural control mechanism in the maintainance cyle of La Crosse encephalitis virus since: (1)-antibodies neutralizing LAC are present in about two-thirds of chipmunks in endemic areas; (2)-about half of female Aedes triseriatus are believed to take a blood meal before mating; and (3)-male as well as female progeny are infected during transovarial transmission.

Abstract: submitted for presentation at the 29th. Annual Meeting, American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, November 5-7th., 1980.

EFFECT OF TEMPERATURE ON DISSEMINATION BARRIERS TO WEE VIRUSES IN MOSQUITOES

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The effect of temperature on western equine encephalomyelitis (WEE) viral infections in Culex tarsalis was examined to more clearly elucidate the mechanism(s) of barriers in mosquitoes that affect the transmission of WEE virus by bite. The barriers examined were the mesenteronal infection (MI) barrier where infection is not initiated in the mesenteron, the mesenteronal escape (ME) barrier where the infection does not disseminate beyond the mesenteron, and the salivary gland infection (SGI) barrier where the virus spreads from the mesenteron to the hemocoel but the salivary glands fail to become infected.

A colonized strain and a genetic hybrid line of Cx. tarsalis were used for these studies. The Yuma colony originated from specimens collected in Yuma, Arizona, and is highly susceptible to viral infection with WEE virus. The genetic line, designated WB, consisted of the F_1 progeny of females selected for resistance to viral infection with WEE virus (WR-1) and mated with males from the WS-3 strain which had been selected for WEE viral susceptibility. All WB females are susceptible to infection with WEE virus since susceptibility is dominant. Mosquitoes were allowed to ingest intermediate doses of virus from a viremic chick or virus-soaked pledget and then divided into three groups for incubation at 18°C, 25°C or 32°C. Viral transmission rates at various times after infection were determined by allowing mosquitoes to feed on normal chicks.

The results obtained with the Yuma strain were as expected. Both the percent mosquitoes infected and the percent infected mosquitoes transmitting were increased as either the temperature or length of extrinsic incubation period increased. The percent of infected females exhibiting the SGI barrier decreased as the temperature or length of extrinsic incubation was increased whereas the percent infected females exhibiting the ME barrier was not significantly affected by either of these variables. Similar results were obtained with WB females incubated 6 or 12 days at 18°C and 25°C. However, only 30% of 20 infected WB females in the 32°C group transmitted virus at 6 days after infection. Fifty percent of the infected but nontransmitting females had ME barriers. At 12 days after feeding only 22% of 23 females held at 32°C even contained infective virus in their mesenterons as compared to 71% of 28 on day 6. In contrast, the infection rates in the two groups held at 18°C and 25°C remained 89 - 100% through day 12.

We do not understand at this time why WEE viral infections were eliminated from WB females when they were incubated at 32°C. Perhaps an enzyme is activated at 32°C in this genetic line of Cx. tarsalis that enhances the development of dissemination barriers. Other studies have suggested that insect rearing at 32°C increases refractoriness to oral infection with WEE virus. Alternately, these results may be due to interference of WEE viral replication by an indigenous mosquito virus present in WB but not in Yuma females. Further research is in progress to examine these two alternatives.

INTERACTIONS BETWEEN ARCTIC BUNYAVIRUSES AND MOSQUITOES

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Although two bunyavirus isolates from Aedes communis mosquitoes in the western Canadian Arctic during 1978, snowshoe hare (SSH) and Northway (NOR) have multiplied readily in salivary glands of domestic A. aegypti mosquitoes after intrathoracic infection, multiplication of SSH virus has been observed relatively infrequently after feeding 0.1 - 100 plaque-forming units (PFU) to A. aegypti, and multiplication of NOR virus after feeding comparable virus doses is rare. Current experiments involved feeding of A. aegypti with 10,000 PFU SSH virus or 1000 PFU NOR virus from semipurified preparations after ultracentrifugal banding on a 20-60% sucrose density gradient. Mosquitoes were injected intrathoracically with similar high virus doses. Multiplication of SSH virus was first detected after 20 days incubation at 13°C, 6-34 days at 23°C and 6-13 days at 32°C after virus feeding, and in all mosquitoes incubated at each temperature for 6 or more days after injection. Demonstration of SSH antigen in salivary glands and in head squashes of mosquitoes correlated closely with demonstration of infectious virus by plaque assays in baby hamster kidney tissue culture monolayers. Multiplication of NOR virus after feeding was detected after 27 days incubation at 23°C and 6 days at 32°C, but not after incubation at 13°C. However NOR virus multiplied readily after intrathoracic injection and incubation at each temperature, and detection of virus antigen in salivary glands and head squashes paralleled the presence of infectious virus. Although virus titers of SSH and NOR viruses in salivary glands remained relatively unchanged at about 3.0 log PFU during the initial month of incubation at 13 and 23°C after intrathoracic injection, more than a tenfold decline of titer was noted after 20 days incubation at 32°C. This paralleled a similar decline of Semliki Forest virus infectivity during one month's incubation at 23°C and 32°C following virus feeding or intrathoracic injection of 50,000 PFU virus.

Electron Microscope Study of Transovarial Transmission of San Angelo Virus in *Aedes albopictus*

Fran Schwier and A.R. Barr, University of California, Los Angeles

ABSTRACT

Transovarial transmission of San Angelo virus was studied by conventional electron microscopy in *Aedes albopictus*. F_{16} generation mosquitoes were reared from a "transovarial transmission efficient" line of *Aedes albopictus* (the virus has been passed consecutively 15 times by vertical transmission). Ovaries from F_{16} generation females were embedded and serial sectioned for examination. Virus particles were discovered in paracrystalline arrays in the follicular sheath and follicular epithelium. This study presents the first electron micrographs of vertical transmission of a California group virus.

Molecular Studies of Nairoviruses

John P.M. Clerx and David H.L. Bishop

Nine viruses belonging to at least 5 different serogroups of arboviruses with an ungrouped or bunyavirus-like status, have been found to have certain biochemical characteristics in common with those of accepted members of the Bunyaviridae family, thereby fulfilling the requirements for their placement into this family of arthropod borne viruses. The viruses have a tripartite, single stranded, RNA genome, distinct in their individual sizes by comparison with other members of the family Bunyaviridae. The viruses also have three major structural proteins: one unglycosylated, 48,000-54,000 dalton polypeptide (which is associated with the viral RNA), and two glycosylated proteins which can be removed from virions by protease treatment. The larger glycoprotein has a mol. wt. of around 75,000; the smaller glycoprotein has a mol. wt. of around 40,000. Recently, it has been demonstrated that viruses from these serogroups are distantly related to each other in serological tests, and have been proposed as a new supergroup of viruses in the family Bunyaviridae (Dr. J. Casals, personal communication). Our biochemical analyses substantiate the formation of a new supergroup of viruses (nairoviruses) with generic status (*Nairovirus* genus) in the family Bunyaviridae.

REPORT FROM THE

ARBOVIRUS PROGRAM, PACIFIC BIOMEDICAL RESEARCH CENTER

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Epidemic polyarthritis (Ross River) virus infection in the Cook Islands

An outbreak of epidemic polyarthritis due to Ross River virus was recognized in February, 1980 on the island of Rarotonga in the Cook Islands - the easternmost extension of the infection in the Pacific to date. An HI antibody survey of humans, cattle, dogs, and pigs carried out several months later indicated that about 70 percent of the humans and between 30 and 40 percent of each of the lower animal species had been infected by that time.

The clinical manifestations did not differ in general from those described previously. However, in contrast to previous studies, it was possible to isolate Ross River virus from the serum of about one-half of approximately 100 patients who had no Ross River antibody in their acute serum specimens. Viral isolates were obtained by inoculating the C6/36 clone of Aedes albopictus cells and examining the latter several days later by an indirect fluorescent antibody procedure. However, the C6/36 cells were adversely affected by undiluted human sera and hence such specimens had to be diluted at least ten-fold before testing. Viral isolates also were made by inoculating Toxorhynchites amboinensis mosquitoes and later assaying the mosquitoes in C6/36 cells. Assay in Tx. amboinensis was about ten times more sensitive than direct assay in C6/36 cells. However, because of the larger amount of inoculum employed, direct assay of sera in C6/36 cells yielded as many viral isolates as tests in Tx. amboinensis. The geometric mean amount of Ross River virus in positive human sera varied from 10^5 to 10^6 ID_{50} per ml depending on time after onset of illness.

Two observations were made on the minimum incubation period of the disease. In one instance, a patient developed arthritis not more than 78 hours after arriving in Rarotonga from a virus-free area (New Zealand). Virus was recovered from this patient's serum and a rise in Ross River antibody titer was observed. In another similar case, the incubation period was shown to be not longer than five days.

Only three species of mosquitoes occur on Rarotonga, Aedes polynesiensis, Culex quinquefasciatus, and Culex annulirostris. A total of 323 mosquitoes were collected from the vicinity of patients' houses and killed for viral assay after being held for several days to assure the digestion of any blood that they might contain. Six viral isolates were made from the total of 267 Ae. polynesiensis tested and none from the 38 Cx. quinquefasciatus or 18 Cx. annulirostris.

Ross River infection also occurred on other islands of the Cook group. Viral isolates were obtained from patients infected on the island of Aitutaki and HI antibody was demonstrated in the sera of persons who had not left the island of Atiu. Illnesses clinically compatible with Ross River infection were reported from Mangaia, Manihiki, Mauke Penrhyn, and Rakahanga, but no specimens were available for laboratory tests.

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REPORT FROM THE MINISTRY OF HEALTH, THAILAND, AND THE DEPARTMENT

OF TROPICAL MEDICINE, UNIVERSITY OF HAWAII, HONOLULU

A Prospective Study of Dengue Hemorrhagic Fever in Thailand: The Rayong Study. First Annual Report

The Southeast Asia Regional Office of the World Health Organization is sponsoring a five-year longitudinal intercountry study on the epidemiology of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The purpose is to measure transmission rates of dengue viruses in a defined population in DHF/DSS endemic and "silent" areas and to relate these to the occurrence in this population of carefully characterized clinical dengue cases. In 1980, a study was begun in Rayong, Thailand (DHF/DSS endemic) and in 1981, in Jogjakarta (DHF/DSS endemic) and Medan (no prior DHF/DSS), Indonesia.

Rayong Study: First Year report

Serosurveys:

Age-stratified sample

In January-February 1980, blood was obtained from 2750 children ages 4-14 years resident in Rayong municipality and 4 adjacent villages (population 52,935, area - 73 sq. km). These children attended 12 primary and one secondary schools. In April, an additional 421 infants and pre-school children were bled. Two Nobuto's filter paper strips were taken from each subject. Dried filter papers were stored at -20°C in plastic bags. Of the 3171 blood specimens, 1086 were subselected for test as an age-stratified serological survey. Of children ages <1-4, 60-70% had dengue 2 HI antibodies; antibody prevalence rose progressively from 70-100% in children ages 5-13 years.

Cohort sample

A total of 965 blood specimens were collected from first grade children with a modal age of 7 and a range of 4-10 years.

Serosurvey: Results

From the age-stratified and cohort studies, 86 children (partial sample) without dengue HI or neutralizing antibodies in January-April 1980 were bled in December 1980. Dengue 1-4 plaque-reduction neutralizing antibodies were measured in microvolumes in LLC-MK2 cells at an initial dilution of 1:30. The 86 post-epidemic sera had the following antibody patterns:

Dengue Neutralizing Antibodies (PRNT) in December 1980 Sera from Children without Dengue Antibody in January 1980.

	PRNT vs Dengue Type				Two infections in 1980	No antibody
	1	2	3	4		
%	29	9	1	1	31	28

Hospital study

Sixty-seven children with an illness satisfying the WHO criteria for DHF/DSS were admitted to Rayong Hospital from the study area. Of these, 50 had satisfactory diagnostic specimens and serological results suggesting primary (2) or secondary (48) dengue infections. Of 50 DHF cases with serological evidence of recent dengue infection, 41 had an acute phase serum permitting isolation attempt. To date, two strains of dengue 2 and one dengue 1 have been isolated.

DHF cases occurring in cohort or age-stratified populations

Fifteen children bled in January-April 1980 were subsequently hospitalized with a diagnosis of DHF. Eight of these were hypotensive and are classified as DSS. Available results indicate that three children were monotypically immune to dengue 1 and two children were without any dengue neutralizing antibody in January 1980. Coincident with DSS all children experienced a second dengue infection. A dengue 2 virus was recovered from one of these children:

		PRNT Pre-Epidemic*				PRNT Post-Epidemic				HI Titers	
Case	Age/ Sex	D1	D2	D3	D4	D1	D2	D3	D4	Acute	Conval.
52	7/F	0	0	0	0	850	380	110	30	160**	≥10,240
84	8/F	+	0	0	0	>9000	720	1400	200	320	≥10,240
86	10/M	+	0	0	0	>9000	>1920	2000	450	320	≥10,240
94	2/F	+	0	0	0	>9000	>1920	920	80	5120	≥10,240
120	7/M	0	0	0	0	1000	>1920	2000	110	≥10240	≥10,240

* + = PRNT ≥1:30; 0 + PRNT <1:30. ** Dengue 2 isolated.

Comment: This study provides, for the first time, type-specific dengue infection rates developed from population-based data. Assuming that the present trends will continue when the sample is enlarged, in 1980, Rayong experienced infections with all four dengue viruses. The outbreak was due predominantly to dengue 1. Notable is the observation that each of 5 DSS cases were susceptible to dengue 2 virus. Each experienced a secondary dengue infection coincident with DSS. In two children, the first and second infection occurred within a 7-9 month period; furthermore, 31% of susceptible children experienced two dengue infections in 1980. In three instances children with DSS were infected in a sequence beginning with dengue 1. Based upon virus isolation results and community infection rates, the most likely infection sequence which resulted in DSS is 1-2.

These preliminary first year results provide the first evidence that closely spaced dengue infections may be pathogenic. In 1980, more than 40,000 DHF hospitalizations were reported country-wide in Thailand. Fortuitously, the Rayong study was begun just prior to the largest outbreak in Thai history. This first application of microneutralization techniques and finger-tip blood to dengue epidemiology will encourage a continuation of this important attempt to solve the enigma of DHF/DSS.

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REPORT FROM THE DEPARTMENT OF VIROLOGY AND RICKETTSIOLOGY,
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Survey for human overt Japanese encephalitis(JE) in Japan in 1980

The national committee for JE surveillance could confirm 39 cases including 15 deaths. Case fatality rate was 38.5%. Four out of 15 deaths could not be confirmed by virological methods and was defined as presumptive deaths considering typical clinical findings. Out of 24 survivals, 10 cases were completely cured, but some sequellae were observed in 14 cases. Therefore, complete recovery rate was calculated as 10/39 = 25.6%.

Geographical distribution of cases is shown on Table 1.

Table 1. Geographical (Prefectural) distribution of human overt JE in 1980 in Japan.

Prefectures	Cases(Deaths)	Prefectures	Cases(Deaths)
Kyushu district		Other district	
Kumamoto	14 (6)	Wakayama	4 (4)
Fukuoka	2 (1)	Yamaguchi	3 (0)
Nagasaki	2 (1)	Ehime	3 (1)
		Tokushima	3 (2)
Subtotal	18 (8)	Osaka	2 (0)
		Kanagawa	1 (0)
		Hyogo	1 (0)
GRAND TOTAL : 39 (15)		Hiroshima	1 (0)
		Okayama	1 (0)
		Kochi	1 (0)
		Okinawa	1 (0)

All cases except one in Kanagawa were from the western half part of Japan. It was remarkable as the previous year to see that 14 cases (35.9%) including 6 deaths occured in a single prefecture, Kumamoto. The reason of the peculier incidence is not known yet.

Sex ratio of cases was 15 including 9 deaths in male and 24 including 6 deaths in female. Age distribution is on Table 2.

Table 2. Age distribution of human overt JE in 1980

Ages	Cases	Deaths
More than 69	17	8
60 - 69	8	4
50 - 59	5	2
40 - 49	1	1
30 - 39	3	0
20 - 29	0	0
10 - 19	0	0
5 - 9	3	0
0 - 4	2	0
Total	39	15

Twenty five cases were found in the age group of more than 59 which consist 64.1% of the total. Further, case fatality rate of the same group was extremely high being 48%. On the other hand, no fatal case was found in cases under 40 in age. No case was found to have JE vaccination history.

(Akira Oya)

REPORTS FROM DEPARTMENT OF MICROBIOLOGY, DEPARTMENT OF MEDICAL ZOOLOGY, AND INTERNATIONAL CENTER FOR MEDICAL RESEARCH, KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Inactivation mechanism of Chikungunya virus by tannic acid

The mechanism of inactivation of Chikungunya virus(CHIKV) by tannic acid(TA) was investigated. The purified CHIKV and purified TA, nonagalloyl glucose having a molecular weight of 1,549, were used. When the virus was mixed with TA, the infectivity and hemagglutination(HA) activity were reduced in parallel. Aggregates of the TA-treated virus particles were observed under an electron microscope and stained bluish-black by treatment with $FeCl_3$. The reaction was reversible depending on pH of the mixture: the aggregates which were once formed at pH 6.2 dispersed again at pH 9.0-10.0 with recovery of infectivity. However, mere dilution of TA-virus mixture, addition of bovine serum albumin(BSA), or treatment with tannase(TA-decomposing enzyme), did not recover the reduced infectivity.

Quantitative relationships between TA and CHIKV in "equivalent points" of the binding were expressed by the following formula:

$$T = 1.1 \times 10^4 \times \sqrt{V}$$

where T is the equivalent amount of TA(nmole) in the reaction mixture and V is viral infectivity(PFU) in the initial reaction mixture.

TA also inhibited the infectivity of RNA extracted from CHIKV by SDS-phenol method, and the HA activity of CHIKV-membrane isolated by treatment with Triton X-100 was also suppressed. Electron microscopic observations revealed aggregates of TA-treated CHIKV-membrane which stained bluish-black by $FeCl_3$.

The affinity between TA and CHIKV structural proteins was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in discontinuous buffer system. The CHIKV without TA treatment revealed three bands by staining with Coomassie blue; two of them(E_1 and E_2) were shown to be associated with the membrane, and one(C) with the core. The grades of the affinity of TA to each of the proteins were in order $C > E_1 > E_2$.

The binding rates(at pH 3.0-9.0) of TA and CHIKV, as well as four kinds of representative proteins(protamine, gelatin, BSA, and globulin) were studied. The proteins had different pH ranges for their precipitation by TA. Within the particular pH ranges, the binding rate of TA to each protein was rather fixed, except for CHIKV which showed three different rates of binding. (Investigations conducted by E. Konishi, T. Matsumura, and S. Hotta) (Reported by S. Hotta)

REPORT FROM THE DEPARTMENT OF VIROLOGY, INSTITUTE FOR TROPICAL MEDICINE, NAGASAKI UNIVERSITY

1. Virus Isolation from *Culex tritaeniorhynchus* by *Aedes albopictus* Clone C6/36 Cell Cultures

In collaboration with Drs. Matsuo, Higashi, Tamoto, and Kuwatsuka of Nagasaki Prefectural Institute of Public Health and Environmental Sciences, (NPI), virus was isolated from *C. tritaeniorhynchus* captured from July 2 to September 1 at pigpens and cowsheds in Aino Town. Altogether 18,000 females in 180 pools were processed, of which 3,800 in 38 pools were engorged and others were unengorged. Each pool was homogenized and centrifuged, and the resulting supernatant was inoculated intracerebrally into a litter of suckling mice (SMB) using 0.02 ml/brain. These procedures were performed at NPI. The remainder of the supernatant was transported to Nagasaki University and passed through Millipore HA filter with 25 mm diameter. One-tenth ml of the filtrate was inoculated into a tube culture and two-tenth ml into a Petri dish (60 mm) culture of C6/36 cells, respectively. Adsorption was carried out at 28°C for 2 hours, then the cells in the tube culture were covered by the maintenance medium and the cells in the Petri dish with agar overlay, respectively. The cells were then incubated at 28°C. Infected fluid was collected from the tube culture 7 days after the inoculation, and the presence of the virus was screened by the hemagglutination (HA) test and by the secondary inoculation into C6/36 cells on 8-chamber slides, in order to detect intracellular viral antigens by immunoperoxidase (IP) method. Specimens with positive HA or IP were considered as positive virus isolates, and their infectivity was tested on BHK21 cells. Final identification was performed by the neutralization test. Cells in Petri dish cultures were stained by neutral red introduced as the second overlay 5 days after the inoculation.

Japanese encephalitis virus (JEV) was first detected from a pool collected on July 28, both by SMB and by C6/36 cells. The last JEV isolation by C6/36 cells was on September 1, in contrast to the last JEV by SMB on August 21. Number of JEV-positive pools by SMB method was 8, of which 7 pools were also positive by C6/36 cells. However, one of the 8 pools did not yield JEV by C6/36 cells, possibly because of bacterial contamination of the cell culture. There were 10 pools from which JEV was detected by C6/36 cells but not by SMB.

Attempts were made to isolate virus from postmortem specimens of a 61 years old male dying on Auguat 30 with acute neurological symptoms of 4 days duration. Pieces of cerebrum, cerebellum, and pons, as well as cerebrospinal fluid (CSF), were taken and inoculated into SMB, followed by the inoculation into C6/36 cells. JEV was isolated by C6/36 cells from the brain materials, but not from CSF, however, the virus was not detected by SMB inoculation.

Getah virus (GET) was isolated by SMB from 2 pools collected on July 2 and August 13. These 2 pools yielded GET also by C6/36 cells. There were 3 pools (collected on August 1, 13, and 21), from which GET was detected by C6/36, but not by SMB. From the pool collected on August 21, JEV was also isolated by C6/36 cells and by SMB.

These results agree with previous observations in Osaka Prefecture showing that C6/36 cells have similar or even better efficiency to isolate JEV and GET from field-caught *C. tritaeniorhynchus*. Moreover, this method appears to be applicable to isolate JEV from postmortem brain materials as well. In previous observations in Osaka Prefecture, many unidentified filtrable agents could be detected from *C. tritaeniorhynchus* by plaque formation on C6/36 cells. Our recent study in Nagasaki, however, revealed that the prevalence of such unknown viruses appears to be much less, and that the distribution of such viruses could be different from place to place in Japan.

2. Application of Enzyme-linked Immunosorbent Assay (ELISA) to assay anti-JEV human immunoglobulin level.

Plastic microplates (Immulon, rigid 96 well U-plate) were sensitized with purified and formalin-inactivated JEV vaccine obtained from Research Foundation for Microbial Diseases of Osaka University. The vaccine was diluted to contain 25 ug protein/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6, and 0.1 ml amount was distributed in each well. The plates were incubated at 4°C overnight. Antigen solution was removed and the plates were washed 3 times with PBS-Tween for 3 min. each. One-tenth ml of human serum specimens diluted in PBS-Tween was added in the well and reacted for 1 hour at 37°C. Sera used in the test were 460 specimens collected from patients clinically suspected of JE during the year of 1965-1978 and kept frozen at -30°C. As negative control, serum was taken from an African coming from Nairobi, Kenya. Plates were emptied and washed 3 times with PBS-Tween as before. One-tenth ml of 1:400 diluted peroxidase-conjugated anti-human immunoglobulin goat globulin (Cappel) in PBS-Tween was distributed in each well and reacted for 1 hour at 37°C. Plates were emptied and washed as before. Peroxidase reaction was performed at room temperature for 1 hour using 0.1 ml/well of the reaction mixture containing 0.5 mg/ml of o-phenylenediamine, 0.02 % H_2O_2 in 0.05 M citrate-phosphate buffer, pH 5.0. Color density was recorded by CORONA double-beam spectrophotometer at 500 nm, as well as by visual colorimetry.

One of the patient sera having the HI titer of 1280 was chosen as the positive standard serum. ELISA test with serial 2-fold dilution of the positive and the negative sera gave dose-response curves and the positive standard serum had the endpoint titer of 10240, when the end point was taken as the reciprocal of the highest dilution of the serum giving the O. D. of more than twice of the negative serum at the same dilution. Comparison of the O. D. of the color reaction made by a series of the standard serum dilution with that by the test specimen at a given dilution, we could estimate the endpoint ELISA titer of the test specimen. The ELISA titer estimated from the O. D. at a given dilution of 64 serum specimens agreed quite well with the endpoint ELISA titer obtained by serial 2-fold dilution of the same specimen within the error of 2-fold. The ELISA titer estimated by visual colorimetry agreed very well with that obtained by spectrophotometry. The reproducibility of this assay method for several test sera revealed the titer fluctuation was within 2-fold from the average.

Based on these fundamental observations, criteria of serodiagnosis on JE by ELISA were worked out.

(A. Igarashi, S. Matsuo, Y. Makino, and K. Bundo)

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

Japanese encephalitis outbreak in Assam, India in 1978 and 1980 - Virological investigations.

An epidemic of encephalitis was recorded in the Dibrugarh district of Assam, India between May and September 1978 in which 120 cases clinically diagnosed as Japanese encephalitis(JE) were admitted in hospitals with 67 (55.8%) deaths.

Virological investigation was carried out by this department with 44 hospitalized cases at various stages of illness. The result of serological examinations showed rise of JE antibodies (HI and/or CF) in 14 out of 16 paired sera and evidence of JE infection in 9 out of 28 single serum samples.

Another epidemic of encephalitis broke out in the above state on a wider scale involving a number of districts during the month of August 1980 and continued till December. A total of 276 cases with 137 (46.6%) deaths was recorded upto 31.1.81. Virological investigations were carried out in two districts, viz: North Lakhimpur and Dibrugarh.

In North Lakhimpur, JE specific antibodies (HI and CF) were detected in two cases. Out of 94 contact cases, 13 (13.8%) showed specific JE antibody.

From Dibrugarh district, one post-mortem brain material could be collected, which however, did not yield any virus. HI and/or CF antibodies were detected in 16 (69.5%) out of 23 single serum samples collected at varying stages of illness. Besides this, out of 54 contact blodd specimens, JE antibodies were detected in 13 (24%) samples.

The most striking feature of this investigation appears to be the isolation of JE virus from a mosquito pool of Mansonia annulifera. This isolation could be effected out of a limited number of mosquito pools (numbering 73) comprising chiefly of Anopheline, Culicine and Mansonia species. Although JE virus has been isolated from Mansonia uniformis and Mansonia bonneae dives from Malayasia (Simpson et al, Annals of Trop. Med. and Parasit, 68, 4, 1974), this is no doubt the first isolation report of JE virus from M. annulifera from India and probably elsewhere.

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REPORT FROM THE DEPARTMENT OF ENTOMOLOGY, KIMRON VETERINARY INSTITUTE, P. O. Box 12, BEIT DAGAN 50200, ISRAEL.

Pathogens of Veterinary Importance Isolated from Mosquitoes and Biting Midges in Israel

SUMMARY

Mosquitoes and Culicoides were examined for the presence of pathogens known to occur in Israel, e.g. bluetongue (B.T.) virus, Turkey Meningo-Encephalitis (T.M.E.) virus and the spirochaete Borrelia anserina. The appearance of Rift Valley Fever (R.V.F.) in Egypt in 1977 prompted a similar survey in the Sinai peninsula despite the fact that there has never been even a suspicion of a clinical case in Israel. In a B.T. disease project light trappings of Culicoides were conducted in 19 localities in which 39000 biting midges were examined. B.T. virus type 16 was isolated in 1968 at Devora from a pool consisting mainly of C. schultzei gp and C. imicola; B.T. virus type 4 was isolated in 1969 at Yesodot from a pool of 183 C. imicola; B.T. virus type 2 was isolated in 1979 at Talmé El'azar from pools consisting of 2462 C. imicola and B.T. virus type 6 was isolated three times in 1980 at Bet Dagan from pools of 204, 258 and from 143 C. imicola respectively. All virus isolations and typing were carried out by the Department of Virology. In the Akabane project (1976 and 1977) 4632 Culicoides and 1867 mosquitoes from 4 localities were trapped and examined. An arbovirus which was not Akabane, B.T. or T.M.E. which are checked routinely in the institute, was isolated from a pool of 44 engorged Culex pipiens complex trapped in Gan Shemuel. The isolation of virus was made by the Department of Vaccine Control. In the R.V.F. project which was conducted in 1978 and 1979 mainly in the northern part of Sinai, 351 mosquitoes of four species and 7098 Culicoides of fourteen species were tested and found to be negative. All the tests were performed in the Department of Virology of the Israel Institute for Biological Research, Ness Ziona. Within the T.M.E. project (1972 to 1977) more than 12000 Culicoides and 9000 mosquitoes from 14 localities were tested in the Department of Avian Diseases. Positive isolations of T.M.E. were made from an unsorted pool of mosquitoes from Gesher haZiw, an unsorted pool of 13 Culicoides from Nahalal, a pool of 223 unidentified Culicoides from Zetan and a pool of 12 engorged Culex pipiens complex from the same locality. From a pool of 77 Culicoides comprising mainly C. imicola

and trapped in Bizzaron an unidentified arbovirus which was not T.M.E., was isolated. From a pool of 22 Culicoides comprising mainly C. agathensis and C. newsteadi and caught in Giv'at Hayyim an arbovirus gp A was isolated. From one specimen of C. agathensis from the same locality Leucocytozoon sp was isolated in the Deapartment of Parasitology. From two specimens of Aedes caspius caught at Qidron an arbovirus which was not T.M.E. was isolated. Within the project of spirochaetosis (1972, 1973 amd 1974) 592 mosquitoes of 4 species and 2386 Culicoides of 9 species from 4 localities were tested for Borrelia anserina in the Department of Avian Diseases and all were found to be negative.
It appears that proportionally the number of pathogens isolated from the number of insects tested, was relatively high.

(Y. Braverman, M. Rubina and K. Frish)

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

I. IDENTIFICATION OF TICK-BORNE ARBOVIRUSES ASSOCIATED WITH SEABIRD COLONIES ON CAPE SIZUN, FRANCE, AND BELONGING TO THREE DIFFERENT SEROGROUPS.

The ornithological reserve "Michel-Henri Julien" is located near Douarnenez on the northern bord of Cape Sizun, Finistere, North western, France (48°04 N - 4°35 W).

It is a sanctuary for many seabirds including kittiwakes, alcidae, shags, fulmars and different species of gulls.

During July 1979, two types of ticks were collected from ground and nestling birds :
- *Ornithodoros (A.) maritimus* (116 L, 7 N and 3 ♀) from ground, the actual host of these ticks beeing probably a herring gull (*Larus argentatus*);
- *Ixodes uriae* (44 N and 7 ♀) from kittiwakes (*Rissa tridactyla*).

This material was processed for virus isolation. Three isolates were obtained by IC inoculation of suckling mice and tentatively identified as follows :

1. A STRAIN OF SOLDADO-LIKE VIRUS (Hughes group) from a pool of 3 ♀ *O. (A.) maritimus*. This virus (Brest/Ar/T247) was found antigenically related to Soldado and quite distant from Hughes, Farallon, Punta Salinas, Zirqa and Sapphire-2 viruses. By cross CF tests with Soldado reference strain (TR 52 214), our virus T247 appeared as an antigenic subtype of Soldado virus.

2. A STRAIN OF ZALIV TERPENIYA VIRUS (Uukuniemi group) or of a virus very close to it, from a pool of 5 N *I. uriae*.

This virus (Brest/Ar/T260) was highly sensitive to ethyl ether, pH 3.0 and heating. No hemagglutinating activity was demonstrated from sucrose aceton extracted brains of infected suckling mice. Antigenically, it was a member of UUK group of arboviruses.

By CF test, T260 was found different from Grand Arbaud, Manawa, Uukuniemi (S-23), Sumakh and RML 105 355, but closely related to Zaliv Terpeniya and Oceanside viruses. These close relationships with ZT and Oceanside were confirmed by cross CF (Table 1) and ID tests.

3. A STRAIN OF A VIRUS APPARENTLY RELATED TO UPOLU GROUP, from a pool of 7 ♀ *I. uriae*.

This agent (Brest/Ar/T261) was pathogenic only for suckling mice by IC route. It was heat-labile and sensitive to ethyl ether and pH 3.0 exposures. No hemagglutinin was detected.

By CF tests, this agent is not related to viruses of groups A, B, Bunyamwera, California, Quaranfil, Nyamanini, Hughes, Uukuniemi, Kemerovo, Sakhalin and some others tick-borne arboviruses. A weak but reproductible serological relationship was evidenced by CF tests for two seabird-associated viruses, Upolu and Aransas Bay. However, these two viruses were previously isolated from ticks of the genus *Ornithodoros* and of the *capensis* complex (Doherty *et al*, 1969 - Yunker *et al*, 1979), and not from *Ixodes uriae*.

Preliminary survey by electron microscope of suckling mice brains infected by T261 showed very pleomorphic particles, not certainly viral in origin, with a mean diameter of 47.5 nm.

The simultaneous occurence of two specialized ticks, namely *O. (A.) maritimus* and *I. uriae*, at Cape Sizun (as at Cape Frehel, another ornithological reserve of Brittany) infected by three different viruses, may be of great significance. In this area, these two species of ticks occupy two very close ecological niches, *Ornithodoros* beeing associated with gulls and shags, and *I. uriae* with kittiwakes.

II. SEROLOGICAL EVIDENCE OF ARBOVIRUS INFECTIONS IN SMALL MAMMALS IN BRITTANY.

In 1979-1980, 480 sera were collected from small mammals (rodents, insectivora) trapped in Finistere, Cotes-du-Nord and Ille et Vilaine, France. This material, coming from 12 different places, was studied for antibody against arboviruses by IH and CF tests using micromethods and the following antigens: Sindbis, West Nile, Wesselsbron, tick-borne encephalitis (european type), dengue type 2, Calovo, Tahyna, Uukuniemi (S-23), Bhanja, Tribec and Soldado. In addition, an antigen obtained from a serological variant of Soldado virus, Brest/Ar/T101, previously isolated from *O. (A.) maritimus* at Cape Frehel, was included in CF tests. Non specific inhibitors were removed by aceton.

A total of 59 sera was found positive, 49 in IH tests (10.21%) and 10 in CF tests (2.08%). Positive reactions were found with one or more antigens including flaviviruses (WN, DEN 2, WSL), Tahyna, Uukuniemi, in IH tests, and Tribec, Soldado and T101 viruses in CF tests.

Antibodies against Soldado virus were found in 3 *Apodemus sylvaticus* at St Renan and Lanildut ; antibodies against T101 were found in 3 *Clethrionomys glareolus* (St Briac) and one *Rattus norvegicus* (Chausey islands).

From these unexpected results, it appears that antibodies against Hughes group viruses may occur in sera of rodents, in despite the fact that these viruses are normally borne by ticks paraziting seabirds. However, the places where rodents were trapped are not very distant from the coasts of Brittany and from seabirds colonies; this is particularly obvious for Chausey islands (see map). Some unknown mechanism(s) of shifting the viruses may exist in these areas.

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ANTIGENS	IMMUNE SERA OR ASCITES:			
	Brest/Ar/T260	ZT	Oceanside	RML 105 355
Brest/Ar/T260	512/128⁂	64/64	128/128	16/128
ZT	128/64	128/32	128/64	16/64
Oceanside	256/16	128/8	64/16	16/64
RML 105 355	<8/<4	<8/<4	<8/<4	512/64

⁂ *Reciprocal of immune fluid titer / Reciprocal of antigen titer.*

Table 1. Comparizon by cross CF tests of 4 UUK group viruses isolated from Ixodes uriae.

Map of Brittany. Places of collection of rodent sera (*).

REPORT FROM THE LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE,
ARBOVIRUS RESEARCH UNIT, WINCHES FARM FIELD RESEARCH STATION,
ST. ALBANS, HERTS, ENGLAND

Isolation and Characterisation of Monoclonal Antibodies against Sindbis Virus

Five Sindbis-specific monoclonal antibody producing hybridoma cultures were obtained by fusion of mouse myeloma cells (X63-Ag8-653) with immunised BALB/c mouse spleen cells. Each antibody was positive when tested by indirect immunofluorescence using acetone-fixed Vero cells infected with Sindbis Virus. Clones 2,14 and 22 were negative when tested with non-fixed cells whereas clones 11 and 12 were positive. Clone 11 specifically inhibited Sindbis virus haemagglutination, clone 12 specifically inhibited Sindbis virus haemolytic activity and clone 22 precipitated only the ribonucleoprotein antigen of Sindbis virus. Clones 2 and 14 have not yet been identified but they are not reactive with Sindbis virus surface antigens. These and further monoclonal antibodies, currently in preparation, are being used to develop rapid diagnostic procedures and also for detailed studies of virus pathogenesis.

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REPORT FROM THE ARBOVIRUS RESEARCH UNIT
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ANTIBODIES AGAINST CERTAIN TICKBORNE VIRUSES IN RODENT SERA FROM EGYPT

Serological surveys for antibodies against arboviruses in Egypt have dealt chiefly with human and domestic animal samples. Few Egyptian rodents have been investigated serologically for antibodies against arboviruses. However, rodents are often important reservoirs of arboviruses, especially those transmitted by ticks. Here we report serological results from 361 rodent sera collected by the NAMRU-3 Medical Zoology and Veterinary Medicine Departments in three Governorates (Cairo, Alexandria, and Suez) and tested against 11 tickborne viruses previously reported from Egypt. Certain of these viruses present a known or potential threat to human health.

Table 1 shows the distribution of CF antibodies against the 11 viruses in Rattus rattus, R. norvegicus, and Arvicanthis niloticus. Table 2 shows the distribution by Governorate of the positive sera against these viruses. The CF titers are shown in Table 3. The results are discussed in order of prevalence of antibodies in the tested sera.

Matariya (MTY) virus exhibited the highest prevalence rate (19.9%), mainly in R. norvegicus (30%) from Alexandria. In a previous study of 145 R. rattus sera from the Cairo area, antibodies against this virus were not detected (Darwish et al., 1975), which is in accordance with the low rate of MTY infection in the Cairo R. rattus in the present study. More than 50% of the positive sera had a CF titer of 1:8 or more (Table 3). MTY virus was originally isolated in Egypt from the blood of a southward-migrating bird collected in 1961 at Matariya (near Port Said) on the Mediterranean coast. Rodents from Alexandria and Suez show a higher prevalence rate than those from Cairo (30.0%, 22.6%, and 6.3%, respectively; Table 2). There is little bioepidemiological information on MTY virus and the related Burg el Arab (BEA) virus (MTY serogroup) and their vectors, human and animal hosts, and disease relationships. MTY virus may have originated in Eastern Europe or Western Asia but appears now to be established in the Egyptian fauna.

Wad Medani (WM) virus showed the second highest prevalence rate (12.5%). The positive reactions were only in R. norvegicus (21.6%)

and R. rattus (9.7%) from Alexandria and Suez (Tables, 1, 2). About half of the reactive sera had a CF titer of 1:8 or more (Table 3). In a previous serological study on 94 rodent sera (mainly R. rattus) from the greater Cairo area, only one serum showed antibodies against WM virus (Darwish et al., 1975). WM virus was originally isolated from ticks collected in 1952 in the Sudan and subsequently from other African, Asian, and Caribbean countries. There is little data on the epidemiology, hosts, and pathogenesis of WM virus. Sera from rodents and other animals in different Governorates, particularly in southern Egypt, should be investigated for antibodies against WM virus to help understand the spread of this virus into Egypt from the Sudan or elsewhere. In Egypt, at least 3 tick species from which WM virus has been isolated in Africa are common (Rhipicephalus turanicus) or are introduced with imported livestock (R. e. evertsi, and Amblyomma variegatum).

Dhori (DHO) and Wanowrie (WAN) viruses follow in prevalence, with rates of 7.75% and 6.6%, respectively. Antibodies against both viruses, with CF titers up to 1:16, were detected in the 3 rodent species from Cairo, Alexandria, and Suez (Tables 1-3). In a previous study of domestic animal and rodent sera, CF antibodies against DHO and WAN viruses were detected in sera from the camel, buffalo, cow, dog, and R. rattus (Darwish et al., 1974). In both studies, the prevalence of DHO virus antibodies was higher than that of WAN virus. These viruses were isolated in Egypt by Williams et al. (1973) from female Hyalomma ticks; DHO virus from H. dromedarii from camels in the Imbaba market near Cairo, WAN virus from H. impeltatum from camels at the El Hammam camel market west of Alexandria. Imbaba market camels usually originate in the Sudan, those of El Hammam market come from the Western Desert and Libya. DHO and WAN viruses were originally isolated in India from Hyalomma from sheep and camels, respectively; both appear to be established in Egypt as indicated by isolations and antibody prevalence in domestic animals and rodents. Both viruses are widely distributed in Africa, southern Europe, USSR, and India, probably owing largely to the age-old, long-distance trekking of tick-infested domestic animals over caravan and marketing routes. Hyalomma adults infest many domestic animals; immatures parasitize smaller-sized mammals, especially rodents and hares, and sometimes birds and other vertebrates.

The epidemiology of these two viruses has not been studied, but clinical data indicate their hazard to man. WAN virus had been isolated from a human corpse in Sri Lanka (Pavri et al., 1976).

Thogoto (THO) virus exhibited an overall prevalence rate of 6.1%; the rate in R. norvegicus (10.8%) was higher than in R. rattus (4.2%); Arvicanthis niloticus was free of antibodies (Table 1). Rodents from Cairo were negative for antibodies to THO virus but those from Alexandria and Suez had infection rates of 11.4% and 5.6%, respectively (Table 2). The CF titers of more than 40% of the positive sera were 1:8 or higher. A previous serological study in Egypt showed that THO virus infects humans and cattle, camels, sheep, goats, (which were also reported to be serologically and/or virologically positive in Africa south of the Sahara), as well as the donkey, buffalo and R. rattus, which were not previously recorded in THO virus epidemiology (Darwish et al., 1979). In the same study, it was observed that when the HI and CF tests were employed simultaneously, the prevalence rate was more than six-fold higher in the first than in the second test; the R. rattus infection rate by the HI test was 6.3% (Darwish et al., 1979). Extrapolating these data to the present study indicates that the rodent infection rate is probably higher than the 6.1% reported herein. The THO virus isolate from camel-infesting Hyalomma anatolicum from the Imbaba camel market near Cairo in 1970 (Williams et al., 1973) provided the first evidence to associate THO virus with Hyalomma ticks. THO virus was originally isolated in 1960 from Boophilus decoloratus and Rhipicephalus spp. from cattle in Thogoto forest, near Nairobi, Kenya (Haig et al., 1965) and has also been isolated in Africa from Amblyomma, Hyalomma and Rhipicephalus ticks. The THO isolates from human cases presenting in Nigeria with serious optic neuritis and fatal meningoencephalitis (Moore et al., 1975) point to this agent as a distinct threat to human health. THO virus has been widely reported from tropical Africa north of the equator and is probably also active in Egypt as indicated serologically and by isolation from Hyalomma ticks. At least 2 tick species (B. annulatus and R. simus) from which THO virus was isolated in tropical Africa occur in Egypt. THO virus epidemiology and its potential threat to the human and animal health of Egypt should be investigated.

Dugbe (DUG) virus exhibited a prevalence rate similar to that of THO virus (6.1%). Antibodies to both viruses were detected in R. rattus and R. norvegicus from Alexandria and Suez (Tables 1, 2). CF titers were 1:4 to 1:16 (Table 3). Antibodies against DUG virus were first detected in Egypt in sera from buffaloes, camels, sheep, and cattle, thus extending the known distribution of the virus from western and eastern Africa to northeastern Africa (Darwish et al., 1976). The camel and buffalo had not previously been implicated in DUG virus circulation.

Rodent sera were not tested in Egypt for antibodies to DUG virus but the virus has been isolated from rodents in Nigeria and Ethiopia (Kemp et al., 1974; Wood et al., 1977). Results of the serological studies point to the need for an epidemiological investigation in Egypt of DUG virus, which causes febrile illness in humans and has been reported to infect cattle, sheep, goats, rodents, and a bird in Senegal, Central African Republic, Cameroun, Uganda, and Ethiopia. Of the 10 tick species associated with DUG virus in Africa, Hyalomma marginatum rufipes, H. impeltatum, and Boophilus annulatus are common in Egypt, H. truncatum occurs in the southeastern coastal area, and Amblyomma variegatum and A. lepidum are carried on imported cattle and camels and by migrating birds. Rhipicephalus turanicus, which is common on domestic animals in Egypt, is closely related to R. sulcatus from which the virus was isolated in Senegal. DUG virus should be considered among the potential threats to human and animal health in Egypt.

Crimean-Congo hemorrhagic fever (CCHF) virus showed the same overall prevalence rate (6.1%) as THO and DUG viruses. CCHF virus antibodies were detected in the 3 species of rodents tested in the 3 Governorates (Tables 1, 2). CF antibody titers were 1:4 to 1:16 (Table 3). In a previous serological study of CCHF virus in Egypt, antibodies were detected in sera from man, camels, cattle, buffalo, and sheep; rodent sera were not tested (Darwish et al., 1977). Those findings provided the first evidence of CCHF virus circulation in Egypt or anywhere in northern Africa (as also true of our results for DUG and THO viruses; Darwish et al., 1976, 1979). Egypt is geographically situated between many Eurasian and African CCHF foci. Six of the 27 tick species reported to be vectors elsewhere are common members of the Egyptian tick fauna and numerous northward and southward migrating birds transport ticks from within the African and Eurasian distribution zones of CCHF virus during their passages through Egypt. The 6 potential vectors in Egypt are Hyalomma anatolicum anatolicum, H. marginatum rufipes, H. impeltatum, Rhipicephalus sanguineus, R. turanicus, and Boophilus annulatus. These data point to the risk of human infection by CCHF virus in Egypt, where one serologically positive person has already been observed, and also to the urgent need to determine the nature of the epidemiology of this virus in Egypt.

Quaranfil (QRF) virus was next in prevalence (4.4%) to THO, DUG, and CCHF viruses (Table 1). Antibodies to QRF virus were detected in the rodent sera from Cairo and Alexandria; about 70% of the positive sera had a

CF titer of 1 :8 or more (Tables 2, 3). In a previous serological study of humans, domestic animals, and rodents (Darwish et al., 1975), the overall prevalence was 8.45% but no antibodies were detected in the 94 rodent sera tested. CF antibodies to QRF virus were detected in rodent sera from Suez area (Abdel Wahab and Imam, 1970) and positive reactions in R. rattus, R. norvegicus, and Acomys cahirinus, were 2.3-3.2%. QRF, the first arbovirus isolated in Egypt, was originally obtained in 1953 from a febrile child in Quaranfil, Qalyubiya (Taylor et al., 1966). Additional QRF isolates from Egypt in the 1950s were from blood of 2 febrile children, from egrets, a domestic pigeon squab, and Argas ticks [A. (P.) arboreus and A. (A.) hermanni] (Taylor et al., 1966). Subsequent serological studies in Egypt did not show antibodies to QRF virus in human sera, except in a 1975 study (Darwish et al., 1975), in which 2.6% of 191 sera from northern Egypt were positive. The natural history of QRF virus should be investigated to determine the dynamics which may cause cyclic outbreaks. An important question in this respect is how humans and other mammals become infected by QRF virus, as they are seldom bitten by bird-parasitizing Argas ticks, the only vectors of QRF virus so far known.

Abu Hammad (AH) and Abu Mina (AM) viruses exhibited overall prevalence rates of 3% and 1%, respectively. Antibodies to AH virus were detected in R. rattus and R. norvegicus, but antibodies to AM virus only in Arvicanthis niloticus (Table 1). Rodents from Cairo were positive for both viruses; all those from Suez were negative (Table 2). CF titers of 1 :16 were encountered with both viruses (Table 3). In the previous serological study of these viruses, only AM antibodies were detected in rodents; few domestic animal sera reacted with CF antigens (Darwish et al., 1976). AH and AM viruses, of the Dera Ghazi Khan (DGK) serogroup of arboviruses, were first isolated in Egypt; AH virus in 1971 from Argas (A.) hermanni ticks from pigeon houses in Abu Hammad, Sharqiya; AM virus in 1963 from a northward migrating turtle dove trapped in Abu Mina near Bahig, Matruh, and in 1969 from Argas (Persicargas) streptopelia from crevices in a date palm in which doves and herons roosted in Dakhla Oasis, El Wadi El Gedeed.

The results of these two serological studies, with 0.5% to 3.1% positive reactions to both viruses in sera from buffaloes, pigs, sheep, dogs, and rodents, suggests that the epidemiological process of these bird- and bird-tick associated viruses is probably more complex and the range of virus reservoirs and vectors is probably wider than has been recognized. These two viruses - as with QRF - raise the question of how mammals become infected by agents whose only known vectors are bird-parasitizing Argas ticks.

The Bhanja (BHA) virus prevalence rate was the lowest (1.1%) in this study; only 4 rodent sera were positive with CF titers not exceeding 1 : 8 (Tables 1-3). In a previous survey for BHA virus antibodies in human and domestic animal and rodent sera from Egypt, an overall prevalence rate of 4.2% was found by the HI test but CF antibody prevalence (1.0%) was lower (Darwish et al., 1978). The broader nature of HI antibodies to BHA virus, as shown in surveys and also experimentally, may be responsible for the low prevalence by the CF test in rodent sera shown in this study. R. rattus, Arvicanthis niloticus, and the camel, buffalo, and donkey were not previously implicated in BHA virus epidemiology. Of the 9 tick species from which BHA virus has been isolated elsewhere, Hyalomma marginatum rufipes, H. detritum, H. truncatum, Amblyomma variegatum, Boophilus annulatus, and Rhipicephalus turanicus should be investigated as potential vectors in Egypt. The potential for infected ticks to be introduced into Egypt by migrating birds and on imported sheep, camels, and cattle should be considered. An epidemiological investigation of BHA virus in Egypt, which is situated geographically between numerous Eurasian and African foci, should be directed to the local tick fauna, domestic animals, rodents, and humans presenting with febrile illness of unknown origin.

This study included 11 tickborne viruses, 6 of which are known to produce human disease (WAN, THO, DUG, CCHF, QRF, and BHA). Three have been isolated in Egypt from man and/or ticks (WAN, THO, QRF) and antibodies to the other 3 (DUG, CCHF, and BHA) were detected for the first time in Egypt and North Africa in this series of serological surveys (Darwish et al., 1976, 1977, 1978). Three of the 11 are bird- and bird tick-associated viruses (MTY, AH, AM) which were first isolated in Egypt but are apparently not established in the local fauna. DHO virus, isolated from ticks in Egypt, is indicated from serological results to circulate in camels, buffaloes and cattle. WM virus, originally isolated from ticks in the Sudan and widely distributed in Africa and Asia, exhibited one of the highest prevalence rate in the studied Egyptian rodents. Results from investigating rodent sera may point to otherwise obscure epidemiological information since rodents are hosts for larval stages of many tick species. Sero-epidemiological surveys of rodent sera from other Governorates will undoubtedly add much to the basic knowledge of arbovirus epidemiology in Egypt.

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Table 1. Complement-fixing antibodies against certain tickborne viruses in rodent sera from Egypt

Rodent species	No. sera tested	Number positive for antibodies to tickborne viruses																			
		MTY		WM		DHO		WAN		THO		DUG		CCHF		QRF		AH		AM		BHA	
		no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)
Rattus rattus	72	9	(12.5)	7	(9.7)	5	(6.9)	3	(4.2)	3	(4.2)	3	(4.2)	3	(4.2)	1	(1.4)	2	(2.8)	-	-	1	(1.4)
R. norvegicus	176	54	(30.0)	38	(21.6)	14	(7.9)	12	(6.8)	19	(10.8)	19	(10.8)	13	(7.4)	8	(4.5)	2	(1.1)	-	-	-	-
Arvicanthis niloticus	113	9	(7.9)	-	-	9	(7.9)	9	(7.9)	-	-	-	-	6	(5.3)	7	(6.2)	7	(6.2)	4	(3.5)	3	(2.6)
Total	361	72	(19.9)	45	(12.5)	28	(7.7)	24	(6.6)	22	(6.1)	22	(6.1)	22	(6.1)	16	(4.4)	11	(3.0)	4	(1.1)	4	(1.1)

Table 2. Complement-fixing antibodies against certain tickborne viruses in rodent sera from 3 Egyptian Governorates

Governorate	No. sera tested	Number positive for antibodies to tickborne viruses																			
		MTY		WM		DHO		WAN		THO		DUG		CCHF		QRF		AH		AM		BHA	
		no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)
Cairo	142	9	(6.3)	-	-	11	(7.7)	10	(7.0)	-	-	-	-	6	(4.2)	7	(4.9)	9	(6.3)	4	(2.8)	3	(2.1)
Alexandria	166	51	(30.0)	37	(22.2)	13	(7.8)	10	(6.0)	19	(11.4)	18	(10.8)	15	(9.0)	9	(5.4)	2	(1.2)	-	-	1	(0.6)
Suez	53	12	(22.6)	8	(15.1)	4	(7.5)	4	(7.5)	3	(5.6)	4	(7.5)	1	(1.9)	-	-	-	-	-	-	-	-
Total	361	72	(19.9)	45	(12.5)	28	(7.7)	24	(6.6)	22	(6.1)	22	(6.1)	22	(6.1)	16	(4.4)	11	(3.0)	4	(1.1)	4	(1.1)

Table 3. Complement-fixation titers against certain tickborne viruses in rodent sera from Egypt

Virus	No. positive sera	1 : 4		1 : 8		1 : 16	
		No.	(%)	No.	(%)	No.	(%)
MTY	72	34	(47.2)	37	(51.4)	1	(1.4)
WM	45	24	(53.3)	19	(42.2)	2	(4.5)
DHO	28	12	(42.9)	10	(35.7)	6	(21.4)
WAN	24	17	(70.9)	5	(20.8)	2	(8.3)
THO	22	13	(59.1)	8	(36.4)	1	(4.5)
DUG	22	14	(63.7)	7	(31.8)	1	(4.5)
CCHF	22	18	(81.8)	3	(13.7)	1	(4.5)
QRF	16	5	(31.2)	7	(43.8)	4	(25.0)
AH	11	7	(63.6)	-	-	4	(36.4)
AM	4	2	(50.0)	-	-	2	(50.0)
BHA	4	2	(50.0)	2	(50.0)	-	-
Total	270	148	(54.8)	98	(36.3)	24	(8.9)

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUT PASTEUR AND ORSTOM

INSTITUT PASTEUR BP.220

DAKAR - SENEGAL

In 1980, researches were carried on in Senegal at our two field stations in Kedougou to study the yellow fever epidemic situation and in Bandia among rodents and among humans. Also, a short mission was performed in Guinea republic.

1. - VIROLOGICAL STUDIES :

1.1. Human blood samples :

In 1980, 171 specimens were collected from febrile children in Bandia and from adults in Kedougou. Two strains of Zika virus were isolated from febrile entomologists working in Kedougou.
In 1979, one single strain of yellow virus was isolated from a young european girl, unvaccinated and living in Dakar. She was infected in Sine-Saloum country, in the same place as two french tourists who died few days after in Paris hospitals. The young girl, after a benign febrile sickness, has recovered without sequelae.

1.2. Wild vertebrate samples :

In 1980, 423 blood samples collected from various animals (295 rodents and 124 monkeys) were inoculated into suckling mice. One strain of Zika virus was got from an Erythrocebus patas monkey.

Seven strains of Bandia virus were isolated from rodents (3 Mastomys and 4 Taterillus) and 2 strains of Koutango virus from 2 rodents (Mastomys and Taterillus). One strain from Mastomys was yet in outstanding identification.

In 1979, from 635 specimens, 14 strains were isolated (Zika virus from a Cercopithecus aethiops monkey, West Nile virus from a Galago galago bushbaby, 11 strains of Bandia virus and one of Saboya virus from rodents.

1.3. Arthropods :

1.3.1. Mosquitoes :

35.686 mosquitoes caught during 1980, processed in 1276 pools were inoculated. One single strain, not yet identified, was isolated. But many pools are still in progress, because isolations were made at once and the same time by direct intra-cerebral inoculation into suckling mice and by intrathoracic inoculation into breeding mosquitoes and after by I.C. inoculation into suckling mice.

In 1979, 37.687 mosquitoes were inoculated and 72 strains were isolated :

- 2 YF strains from Aedes aegypti (Gambia) in January and one from Aedes gr. furcifer taylori only after enrichment upon mosquitoes, in November.
- 3 strains of Bunyamwera group virus, not yet identified from male Aedes simpsoni and female Aedes neoafricanus and Aedes vittatus.
- 1 strain of Poly Bwamba group virus from Anopheles nili.
- 41 strains of chikungunya virus from A. gr. furcifer taylori, Aedes luteocephalus, Aedes dalzieli and Aedes neoafricanus.
- 24 strains of Zika virus from A. gr. furcifer taylori, A. luteocephalus, A. vittatus and A. neoafricanus.

1.3.2. Ticks :

In 1980, 4185 ticks processed in 98 pools were inoculated and one Koutango strain virus was isolated.

In 1979, from 249 ticks, 6 strains of Bandia virus were isolated.

2. - SEROLOGICAL STUDIES :

2.1. Human sera :

2.1.1. From Kedougou :

Six sera harvested from entomologists were studied and a serological conversion for Zika virus was found in the 2 sera where Zika strains were isolated.

2.1.2. From Bandia :

190 sera from febrile children were harvested, but not yet examinated.

2.1.3. From Madagascar :

43 sera sent recently by the Tananarive Pasteur Institute are going into study.

2.1.4. From North Cameroun :

After an outbreak of febrile jaundices, an investigation was made in the North Cameroon and 350 sera were harvested from children. In 2 towns (Kaele and Mokolo) and one village, 179 were harvested and few have Hi antibodies against chikungunya virus, (16 %) and against flaviviruses (14 %) at a low level. In four other villages, totalizing 158 sera, 20 % have anti-chik. Hi antibodies. In many sera (75 %), we have found Hi antibodies against flaviviruses, but only heterologous reactions.

No evidence for yellow fever virus infection was detected, but a best answer will be given with the CF tests now in progress.

2.2. Wild vertebrate sera :

2.2.1. Rodent sera :

285 sera (111 Mastomys, 168 Taterillus, 5 Tatera and one Myomys) were collected and tested only for Bandia virus by Hi test, to research the influence of this virus on murine population.

2.2.2. Goat sera :

Since 1976, sentinel goats in Bandia area were tested by CF tests against the following viruses : Bhanja, Congo, Dugbe, Jos, Somone, Thogoto and Wad-Medani.
Up to now, a single infection by Congo virus was observed.

By an other hand, experimental inoculations were made into kids to study the CF antibodies kinetic. They became evident about the 15th day, rose up to the highest about the end of the second month and disappeared approximatively about the 7th-8th month.

2.2.3. Monkey sera from Kedougou country :

116 sera were examinated by Hi and CF tests. Their study revealed that yellow fever has disappeared from the Kedougou area and confirmed the 2 epizootic waves of chikungunya virus at first, and then of Zika virus.

INSTITUT PASTEUR :J.J.Salaün, J.P.Digoutte

ORSTOM : M.Germain, M.Cornet, J.L.Camicas

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUT PASTEUR AND ORSTOM BANGUI
CENTRAL AFRICAN REPUBLIC

The use of the mosquito Aedes aegypti to detect arboviruses.

During the years 1978 and 1979 studies were continued out at our field station in Bozo (5° 10' N, 18° 30' E) to collect more information on the life cycle of sylvatic yellow fever. A second forest-gallery situated near the village of Bouboui was prospected in 1979.

68 958 Aedes africanus s.l. collected at the station were divided into 2 398 pools and inoculated into suckling mice. All pools collected during the dry season, (december to may) which were negative by this method were inoculated to living Aedes aegypti. After 7 days the grinded mosquitoes are inoculated into suckling mice. The results of this investigation are mentionned (Table I).

Table I : Strains of virus isolated from Aedes africanus s.l. collected in Bozo and Bouboui in 1978-1979.

Year	Inoculation into suckling-mice.				Intrathoracic inoculation to Aedes aegypti.			
	N° of mosquitoes	N° of pools tested	N° of strains isolated		N° of mosquitoes	N° of pools tested	N° of strains isolated	
1978	24 977	906	Chikungunya	33	1 445	85	yellow fever	4
			Orungo	8				
			Yellow fever	8				
			West-Nile	1				
1979	43 981	1 492	Orungo	1	5 454	202	yellow fever	1
			West-Nile	2				
			Zika	19				
			ArB 7 343(1)	25				

(1) : ArB 7 343 is a new arthropod-borne virus of Bunyamwera group.

The inoculations to Aedes aegypti allowed us to isolate 5 strains of yellow fever virus. 4 of them were isolated at the end of the rainy season. These late isolations show in fact that the epizootic continued over a period of three months instead of the 2 months duration of the former epizootics (1975 and 1977) detected by inoculations into suckling-mice.

The fifth strain was isolated at the beginning of the rainy season and may be the result of a transovarial transmission of yellow fever virus during the preceding dry season.

J.F. SALUZZO, A.J. GEORGES (INSTITUT PASTEUR)
J.P. HERVE, J.P. GONZALEZ, N. DEGALLIFR (ORSTOM)

REPORT FROM THE

NATIONAL INSTITUTE FOR VIROLOGY, SANDRINGHAM, SOUTH AFRICA.

In recent years we have attempted unsuccessfully to demonstrate transovarial transmission of CHIK and RVF viruses by proven mosquito vectors. With CHIK virus this was attempted on the progeny of experimentally infected Ae. furcifer and Ae. aegypti, as well as on field populations of Ae. furcifer-taylori on two occasions when newly emerged males and females were tested for virus following outbreaks of chikungunya the previous summer. In the case of RVF virus, eggs, larvae and adults, the progeny of experimentally infected Ae. aegypti, Ae. caballus, Ae. juppi, Ae. lineatopennis, Cx. theileri, Cx. univittatus and Eretmapodites quinquevittatus, were entirely negative for virus. In all these tests the material was inoculated IC into suckling mice.

These studies were recently extended using modifications in technique. Laboratory reared adult females of Ae. furcifer and Er. quinquevittatus were infected with CHIK and RVF viruses respectively, by feeding on viraemic monkeys or hamsters. Infection rates determined 7 days later showed that 50% of the Ae. furcifer and 90% of the Er. quinquevittatus were infected. The mosquitoes were then offered several sequential blood meals on uninfected animals so that 4-5 layings of eggs were obtained. The sequential egg batches were reared separately, and samples of the progeny at all developmental stages were tested for virus by inoculation of Ae. aegypti cell cultures which were subsequently passed to Vero cells and infant mice to demonstrate the presence of virus. In the case of adults, organs were dissected intact and co-cultivated on monolayers of Ae. aegypti cells. Mosquitoes were held at 26°C and Ae. aegypti cells at 28°C.

Nearly 400 Ae. furcifer at various developmental stages, the progeny of 2nd, 3rd and 4th layings were tested. In the case of RVF-infected Er. quinquevittatus, over 3000 mosquitoes at various developmental stages, the progeny of the 1st to the 5th layings, were tested. In no instance was virus detected. In addition, adult female progeny of various layings of the originally infected Er. quinquevittatus failed to transmit RVF virus to hamsters during a total of approximately 3500 blood meals.

These results indicate that if transovarial and transstadial transmission of these viruses occurs naturally in these particular species, both of which have been implicated as natural vectors, it does so at a low level of efficiency.

However, low level transstadial transmission of CHIK virus from 4th instar larvae to adults was demonstrated in Ae. furcifer but not of RVF virus in Er. quinquevittatus. In these tests larvae were exposed to high concentrations of virus for 2 hours, washed thoroughly, and reared through to adults. Adults of both sexes were tested for virus. One pool containing 65 mosquitoes was positive while 7 pools containing a total of 343 mosquitoes were negative.

J. Peleg (guest worker from Israel Institute for Biological Research), B.M. McIntosh, P.G. Jupp, I. dos Santos, J. Barnard.

REPORT FROM TANDIL VIRUS RESEARCH LABORATORY, FACULTY OF VETERINARY SCIENCES, NATIONAL UNIVERSITY OF CENTRAL BUENOS AIRES PROVINCE (UNCPPA), ARGENTINA

On February 1979 a new virus research laboratory started at Tandil, county located in Buenos Aires province, about 400 km. south from Buenos Aires City (see location in Fig. 1)

We started in a new building of $268m^2$ constructed by the University located in a farm at 7 km. from Tandil City. Another building of $144m^2$ attached to the first is under construction with walls and roof already done.

The staff, all veterinarians getting training in Virology are: Eduardo Esteban, Leticia Igarza, Mónica Di Santo, Adriana Schettino and Daniel Pardo. We also have a veterinarian, Sergio Islas, in charge of the mouse colony and two pretty young girls helping us as technicians. All of them under the leadership of the subscriber.

Our goal is to identify the virus causing endemic and epidemic diseases in Tandil county in animals (cattle, sheeps and horses) and in rural workers.

With this purpose we started by setting up a serum bank that already have 2577 serum samples from different species: humans, bovines, ovines and equines. Search for antibodies were done the first year only against antigens comercialy available and last year with antigens prepared by us.

Screening test for antibodies were done at first for bovine leukosis, equine infectious anemia and a Rickettsial isolated at Yale from human with HUS and TTP that we call Rickettsial yalensis. Currently the search is done against VSI, VSNJ, SLE and Junín. Preliminary results with the last four viruses can be seen in table 1. The final report using aditional tests will take a while.

Other investigations under way are: experimental inoculation of Junín virus in horses, bovines and sheeps to know if there is any reaction. Virus isolation attemps, from sick bovines, ovines and horses stopped until the animal isolation rooms can be used.

Techniques and procedures for virus research followed at this laboratory are the classical ones learned from the Rockefeller Foundation group, with whom I used to work.

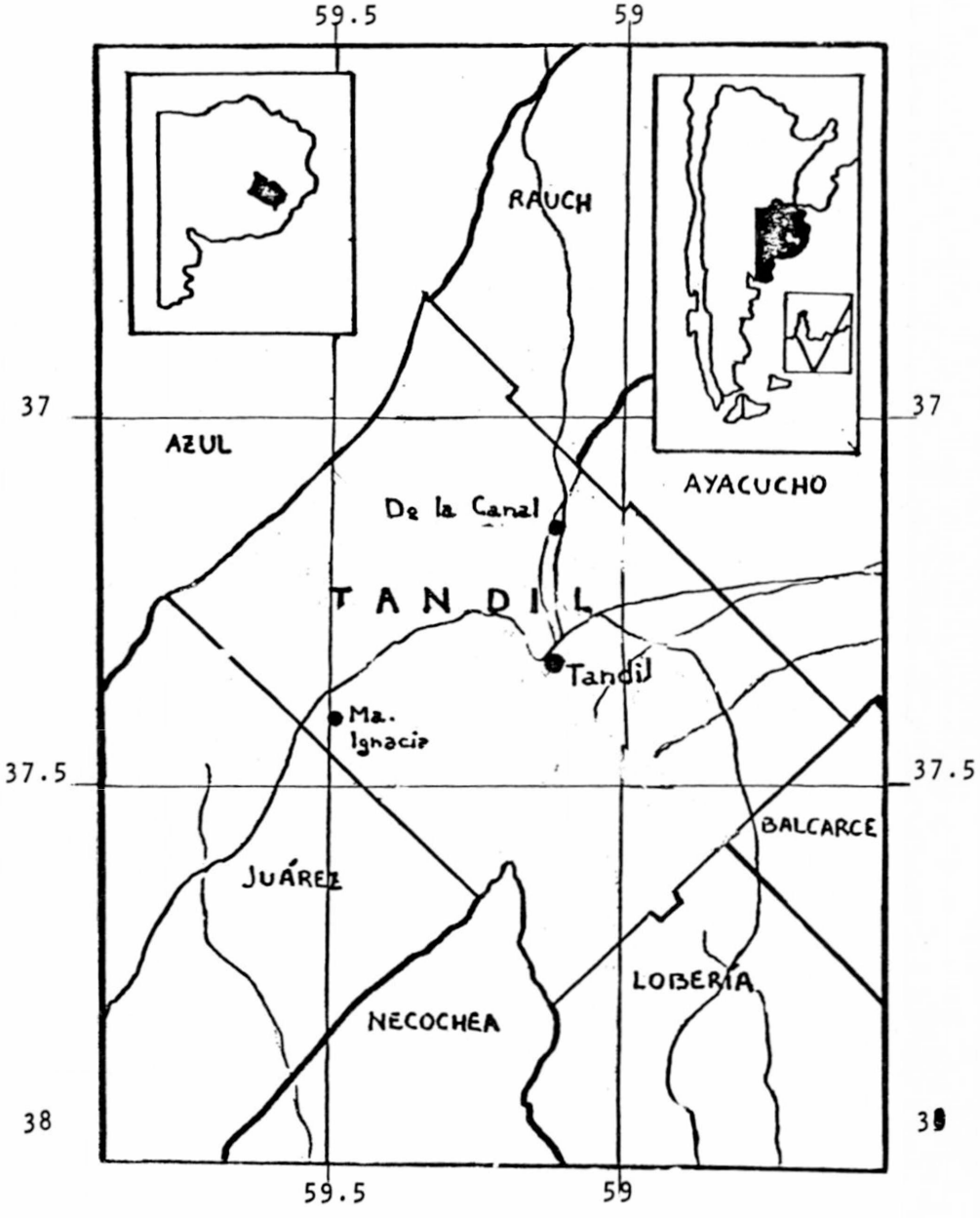
Dr. Norma E. Mettler
Full Professor of Virology
Tandil (Bs.As.) Argentina

Table I:

PRELIMINARY RESULTS WITH SOME OF THE 2577 SERA CORRESPONDING TO DIFFERENT SPECIES

Specie	# Bank of sera	SLE - IH pos/anal.	SLE - CF pos/anal.	VSI - CF pos/anal	VSNJ - CF pos/anal.	JUNIN - CF pos/anal.
Humans	233	3/164	5/216	0/216	-	7/216
Bovines	1706	11/1279	34/1279	0/1279	0/358	21/1279
Equines	282	9/281	22/280	0/279	-	22/279
Ovines	329	1/82	-	-	-	-
Canines	18	0/18	1/13	0/13	-	2/13
Goose	2	0/2	-	-	-	-
Wild Mammals	4	0/3	0/4	0/4	-	0/4
Swines	3	0/3	-	-	-	-

Fig. 1


59.5
59
RAUCH
37
AZUL
AYACUCHO
De la Canal
TANDIL
Tandil
Ma.
Ignacio
37.5
BALCARCE
JUÁREZ
LOBERÍA
NECOCHEA
38
59.5
59

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

PROTEIN SYNTHESIS IN MARITUBA VIRUS INFECTED L-A9 CELLS

Analysis of purified MTB ^{35}S-methionine-labeled virus, by acrylamide gel electrophoresis revealed that the virions contained four structural proteins designated L, G1, G2, and N which had molecular weights of 190.000, 121.000, 22000, and 15.000 respectively. MTB virus grown in the presence of ^{3}H-glucosamine revealed that two of the proteins (G1 and G2) were glycosylated. The time course of virus - induced polypeptides synthesis was followed in experiments with infected cells that were pulse-labeled with ^{35}S-methionine at various times post-infection (p.i.) and the cell extracts analysed by gel electrophoresis and autoradiography. These virus-induced polypeptides could be divided into early and late categories according to their rates of synthesis. The polypeptide L was sinthetized in only small amounts and it was detected late in the virus growth cycle, at about 21h p.i. The synthesis of polypeptide N was first detected at 15h p.i.; its rate of synthesis then increased, reaching a peak at 17h p.i. The kinetics of synthesis of polypeptides G1 and G2 appeared to be similar during 15-19 hours p.i. A pattern of temporal control of virus-induced polypeptide synthesis has been shown in this study but the possibility that the MTB virus polypeptides were derived from a higher molecular wheight precursor is not eliminated.

(I.C. Frugulhetti and M.A. Rebello).

REPORT FROM THE GORGAS MEMORIAL LABORATORY, PANAMA, R.P.*

St. Louis Encephalitis Virus in Panama

During 1980 a project was started to study the mechanisms by which St. Louis Encephalitis (SLE) virus is maintained in tropical ecosystems. To accomplish this objective SLE virus activity was monitored in three ecologically different areas in Panama by exposure of sentinel animals. The surveillance was initiated in February 1980 at Altos de Majé, Bayano, a tropical dry forest area. The project was extended in May to El Llano-Carti, an area of tropical humid forest, and to Puerto Gago, Penonome, a swampy lowland savanna. Hamsters and chickens were exposed at all three sites. In the forested stations, exposures were made in the canopy and on the ground, while in the savanna station, exposures were made in the lower branches of dwarf trees in gallery forest. A few sentinel monkeys also were exposed in the canopy at Altos de Maje and El Llano-Carti.

Blood samples were collected from all sentinels once a week and the serum separated in the field. These samples as well as tissues from dead or dying sentinels were frozen in liquid nitrogen and returned to the laboratory in Panama City each week for processing. The sera were tested for antibodies to SLE virus by plaque reduction neutralization using Vero cell cultures grown in 96-well panels. Each sample was screened at dilutions of 1:4 and 1:16 against about 100 plaque forming units of virus, and those sera giving $\geq$90% reduction in plaque count at the 1:16 dilution were considered positive for antibodies to SLE virus. The SLE antibody positive sera also are screened against the indigenous flaviviruses, Ilheus and Bussuquara to exclude the presence of cross-reacting antibody. For virus isolation, the tissues from dead animals were inoculated into tubes of Vero cell cultures.

SLE virus antibody was detected at all three surveillance sites (Table 1). At Puerto Gago, ten chickens seroconverted between July and November; five chickens from Altos de Maje also developed antibodies to SLE virus during this same period. A single hamster stationed at El Llano - Carti seroconverted in October. Tissues dissected from 135 dead sentinels were processed for virus isolations. A strain of virus recovered from a hamster that died in late October at Puerto Gago has been identified as SLE. An additional eight viral or presumptive viral isolates were recovered from sentinel animal tissue. Two of these, recovered from hamsters stationed at Altos de Maje, have been identified as strains of Punta Toro virus. The remaining six are in the process of being classified.

In response to the SLE virus activity detected in sentinels at Puerto Gago, mosquito collections were initiated at this site in September. Adults were collected in traps baited with chickens and hamsters placed at each of the 12 sentinel exposure sites at Puerto Gago. A horse-baited trap also was stationed in the area. A total of 29,204 adult female mosquitoes comprising 30 different species were collected. Culex nigripalpus and Aedes

taeniorhynchus were the most abundant mosquitoes. Other species frequently collected were Cx. declarator, Mansonia titillans, Anopheles albimanus, Cx. dunni and Cx. (Melanoconion) sp. All specimens were pooled by species and inoculated into tubes of Vero cell cultures for virus isolation. Four presumptive viral isolates have been established and are in the process of being identified. These isolates were obtained from pools of Cx. dunni, Ma. titillans, Cx. (Melanoconion) sp. and Ae. taeniorhynchus.

The field studies on SLE virus ecology in Panama will be continued over the next two years. In addition, laboratory experiments on the vector capability of suspected mosquito vectors of SLE virus in Panama such as Ma. dyari, Sabethes chloropterus, Haemagogus lucifer and Cx. nigripalpus and on the viremic response of suspected vertebrate hosts such as sloths, the Olivaceous Cormorant, Keeled billed Toucan, and the spider monkey are in progress or will be initiated shortly.

(C. G. Hayes, A. J. Adames, P. H. Peralta and P. Galindo)

Table 1. Chronologic distribution of seroconversion to SLE virus in sentinel animals stationed at three surveillance sites in Panama.

	Puerto Gago		Altos de Maje		El Llano - Carti	
	Chickens	Hamsters	Chickens	Hamsters	Chickens	Hamsters
July	61+(1)	45	110	111	58	41
August	45 (2)	42	91 (2)	63	41	46
September	49 (3)	48	71 (2)	70	48	49
October	85 (3)	32	99 (1)	114	57	57 (1)
November	58 (1)	21	73	60	48	45
Total	298 (10)	188	444 (5)	418	252	238 (1)

+ Number of blood samples collected and tested for antibodies to SLE virus. ( )= Seroconversions to SLE virus.

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REPORT FROM THE NIH AND WHO COLLABORATING CENTER FOR REFERENCE AND RESEARCH IN SIMIAN VIRUSES, SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION, SAN ANTONIO, TEXAS

Nonhuman primates have been associated with a number of arthropod-borne virus diseases, as well as several other apparently nonarboviral diseases of interest to the "arbovirologist." Of the latter, certain of the hemorrhagic diseases, Marburg in particular, may involve nonhuman primates. The Simian Virus Center is currently assaying simian sera, especially those from African animals, for the presence or absence of antibody to this group of viruses, which comprise Congo-Crimean hemorrhagic fever, Rift Valley fever, Ebola, Lassa and Marburg (CRELM).

Those laboratories with animals suspected of having had contact with any of these viruses, and who may wish to have the sera tested (immunofluorescence), may contact this Center.

(S. S. Kalter)

REPORT FROM THE RABIES-ARBOVIRUS UNIT, TEXAS DEPARTMENT OF HEALTH, AUSTIN, TEXAS

January 1, 1980—December 31, 1980

MOSQUITO ISOLATES

For the period indicated above, 2023 liters of mice were inoculated for arbovirus isolation. This represents 3875 pools totaling 53,163 mosquitoes.

Locality	Collection Date	Pooled Species	Number of Isolations	Virus
Dallas	3/18/80	C. tarsalis	1	HART PARK
Beaumont	5/20/80	C. (Melanoconion) sp. C. salinarius C. quinquefasciatus	1	HART PARK
Beaumont	5/21/80	C. quinquefasciatus C. salinarius	2	HART PARK
Dallas	5/22/80	C. quinquefasciatus C. restuans C. tarsalis	1	HART PARK
Dallas	5/27/80	A. quadrimaculatus C. quinquefasciatus C. tarsalis	1	HART PARK
Dallas	6/2/80	C. tarsalis C. territans C. quinquefasciatus C. restuans C. salinarius	2	HART PARK
Alvin	6/3/80	C. quinquefasciatus	1	HART PARK
Dallas	6/9/80	C. quinquefasciatus	1	HART PARK
Port Arthur	6/10/80	C. quinquefasciatus	1	HART PARK
Dallas	6/11/80	C. quinquefasciatus A. vexans	1	HART PARK
Brazos Co.	6/12/80	A. quadrimaculatus C. quinquefasciatus	1	HART PARK
Dallas Co.	6/12/80	C. (Melanoconion) sp. C. quinquefasciatus	1	HART PARK
Dallas Co.	6/12/80	C. restuans C. salinarius	1	HART PARK
Grayson Co.	6/16/80	C. quinquefasciatus	1	HART PARK
Dell City	6/16/80	A. pseudopunctipennis C. tarsalis	1	HART PARK

Locality	Collection Date	Pooled Species	Number of Isolation	Virus
Hidalgo Co	6/17/80	C. quinquefasciatus	2	HART PARK
Dallas	6/17/80	A. aegypti C. (Melanoconion) sp. C. quinquefasciatus	1	HART PARK
Corpus Christi	6/18/80	C. salinarius C. quinquefasciatus A. sollicitans	1	HART PARK
		A. taeniorhynchus C. quinquefasciatus	1	SLE
Hidalgo Co.	6/23/80	A. sollicitans C. quinquefasciatus	3	HART PARK
Dallas	6/24/80	C. restuans C. quinquefasciatus	1	HART PARK
		A. quadrimaculatus C. quinquefasciatus C. tarsalis	1	HART PARK
		A. quadrimaculatus A. punctipennis C. quinquefasciatus	1	HART PARK
		C. quinquefasciatus C. (Melanoconion) sp. C. resturans	1	HART PARK
Dallas	6/30/80	A. vexans A. punctipennis	1	HART PARK
		C. quinquefasciatus	2	HART PARK
		C. quinquefasciatus C. tarsalis	1	HART PARK
Amarillo	6/30/80	C. tarsalis A. vexans Psorophora discolor	1	HART PARK
Willacy Co.	7/1/80	C. quinquefasciatus	1	HART PARK
Beaumont	7/1/80	A. quadrimaculatus C. quinquefasciatus C. salinarius	1	HART PARK
Galveston Co.	7/1/80	A. quadrimaculatus C. quinquefasciatus	1	SLE
Dallas	7/1/80	C. salinarius C. quinquefasciatus	3	HART PARK
		A. vexans C. quinquefasciatus C. restuans	2	HART PARK

Locality	Collection Date	Pooled Species	Number of Isolation	Virus
		C. restuans C. salinarius	1	HART PARK
Collin Co.	7/2/80	C. quinquefasciatus	1	HART PARK
Corpus Christi	7/2/80	C. quinquefasciatus	1	SLE
Dell City	7/7/80	A. vexans C. tarsalis	2	HART PARK
		C. tarsalis C. inornata	1	HART PARK
		A. pseudopunctipennis C. tarsalis	1	HART PARK
		A. sollicitans A. pseudopunctipennis C. tarsalis	1	WEE
Corpus Christi	7/7/80	C. quinquefasciatus	1	SLE
Port Arthur	7/8/80	A. quadrimaculatus C. quinquefasciatus	1	HART PARK
Brazos Co.	7/16/80	A. aegypti C. quinquefasciatus C. tarsalis C. quinquefasciatus	1	HART PARK
Harlingen (Region 8)	7/29/80	C. quinquefasciatus	1	HART PARK
Dallas	11/19/80	C. (Melanoconion) sp. C. restuans C. quinquefasciatus	1	HART PARK

BIRD BLOODS FOR ISOLATION

A total of 120 wild bird bloods were submitted for arbovirus isolation from Dallas, Lubbock, San Antonio, Harlingen, and Region 11. All were negative for viral isolation in suckling mice. (Dallas, 82; Lubbock, 5; San Antonio, 11; Region 11, 22.)

BIRD BLOODS FOR SEROLOGY

Hemagglutination inhibition tests were performed on a total of 2078 sera submitted from the following areas: Lubbock, Dallas, San Antonio, Harlingen, and Tyler. Positive results are as follows:

Locality	Collection Date	Species	# Positives	Antibodies Detected
Lubbock (Region 2)	1/1/80	Chicken	2	WEE (≥ 1:40 & ≥ 1:80)
	7/15/80	Chicken	1	WEE (≥ 1:80)
	8/1/80	Chicken	1	SLE (≥ 1:80)
	9/1/80	Chicken	16	WEE 6 (1:10) 2 (1:20) 4 (1:40) 8 (≥ 1:80)
			2	SLE (1:10 & ≥ 1:80)
Dallas (Region 5)	8/1/80	Chicken	1	WEE (≥ 1:80)
Jefferson Co.	9/1/80	Chicken	3	SLE 2 (1:10) 1 (1:80)

HUMAN CASES

Five suspected or confirmed cases of St. Louis Encephalitis, resulting in one death, have had paired serum specimens tested by our laboratory. One case of imported Dengue Fever has been identified.

Patient 1: A 66 y.o.m. from Dallas with suspected St. Louis Encephalitis (SLE).

Serum 1.	6/25	HI	1:160	CF	Negative
Serum 2.	7/7	HI	1:160	CF	Negative
Serum 3.	7/15	HI	1:160	CF	Negative

Patient 2: A 55 y.o.m. from Wichita Falls classified as a case of SLE.

Serum 1.	8/2	HI	Negative
Serum 2.	8/7	HI	1:160

Patient 3: A 8 y.o.m. from Port Arthur classified as a case.

Serum 1.	8/13	HI	1:20
Serum 2.	8/19	HI	1:80

Patient 4: A 35 y.o.m. from Galveston whose death is being listed as a result of SLE.

Serum 1.	8/5	HI	1:10
Serum 2.	8/13	HI	1:1280
Serum 3.	8/25	HI	1:1280
(Post Mortem)			

A virus, identified as SLE by the CF test, was isolated from the brain of this patient.

Patient 5: A 32 y.o.m. from Galveston with a suspected SLE case.

Serum 1.	8/22	HI	1:80
Serum 2.	9/2	HI	1:80

CF testing in progress.

Patient 6: A 30 y.o.m., a native of the Philippine Islands who is currently living in Houston, returned to Houston in early August after a trip to the Phillipines where he became ill with Dengue-like symptoms.

		HI		CF			
		SLE	Dengue 1	Deng 1	Deng 2	Deng 3	Deng 4
Serum 1.	8/13	>1:10,240	>1:10,240	1:2048	1:1024	1:1024	1:2048
Serum 2.	8/19	>1:10,240	>1:10,240	1:1024	1:1024	1:1024	1:1024

(Ronald W. Johns, Chief, Rabies-Arbovirus Unit)

REPORT FROM THE OFFICE OF LABORATORY SERVICES AND ENTOMOLOGY

DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES

JACKSONVILLE, FLORIDA

Arbovirus surveillance in Florida was conducted for the entire year of 1980. 6,519 sera from patients with CNS symptoms were tested by HI against SLE, EEE, VE, Dengue, and CAL antigens. There were a total of 9 confirmed cases of SLE and 4 confirmed EEE. There was also one confirmed case of Dengue (patient from Texas). It is interesting to note that the onset of illness in the last confirmed SLE case was on December 1, 1980. There were over 100 patients with constant titers to Group B antigens, indicating previous infection at some undetermined time.

6,293 chicken sera were tested in 1980. 98 chickens developed antibody to SLE and 21 chickens developed antibody to EEE.

A mammal study in the Tampa Bay area yielded 11.5% (37/323) of raccoons and 10% (26/261) of oppossums reactive to SLE antigen. In the Panhandle area, 2 out of 4 raccoons tested were positive, and 6 of 19 oppossums. 3% of the avians tested, from the Tampa Bay and Panhandle areas combined, also demonstrated antibodies to SLE antigen.

There were 3,369 pools of C. nigripalpus mosquitoes tested. There were 3 EEE isolated (2 from 1979 collections) and 3 SLE isolated (1 from 1979 collection). The last SLE virus was isolated from a pool of C. nigripalpus mosquitoes collected November 26, 1980.

The sentinel chicken system appears to be working well and the State of Florida will continue this early warning system for 1981.

(N. J. Schneider, F. M. Wellings, E. E. Buff, J. A. Mulrennan, R. A. Gunn, and H. Janowski)

REPORT FROM THE CENTER FOR INFECTIOUS DISEASES,
CENTERS FOR DISEASE CONTROL,
ATLANTA, GEORGIA

Surveillance for Human Arbovirus Infection, United States, 1980--Preliminary Data

The 1980 arboviral season has been an active one for St. Louis encephalitis (SLE) especially at locations along the Gulf Coast. The first indigenous transmission of dengue in the continental United States since 1945 was documented in Texas. Eastern Equine Encephalitis (EEE) was reported in humans for the first time by Michigan and New Hampshire. Details of the preliminary reports for 1980 are given below.

St. Louis Encephalitis

A total of 115 cases were reported from 13 states. A total of 52 confirmed cases were reported from Houston and surrounding areas where an outbreak extended from July through October. A remarkably early St. Louis encephalitis (SLE) illness occurred in a Houston resident the second week of March, but almost 4 months intervened before further cases occurred. The remainder of the 69 cases reported from Texas were associated with Port Arthur or other locations on the Gulf Coast. Louisiana reported a total of 15 SLE cases, 14 from a focal outbreak in the North Ward of New Orleans. Surveillance for SLE virus activity in avians detected antibodies early in the outbreak so that control measures were begun concurrently with the onset of first human case and long before a laboratory diagnosis was available. Other Gulf Coast states with SLE activity include Florida (10 cases), Mississippi (2 cases), and Alabama (2 cases). The remaining SLE cases were reported from Illinois and Indiana (5 cases each), Ohio (2 cases), and 1 case each from Arkansas, Iowa, Kansas, Nevada, and Tennessee.

California Encephalitis

A preliminary total of 50 cases of California encephalitis (CE) was reported by 7 states: 5 traditional CE locations, Illinois (15 cases), Wisconsin (12 cases), Iowa (8 cases), Indiana (7 cases), Ohio (6 cases), and 2 southern states, North Carolina and Tennessee (1 case each). The 1980 total was less than half that of the preliminary 1979 total. Reduced case ascertainment and delayed reporting by several states were probably major factors in the lower number of cases.

Eastern Equine Encephalomyelitis

A total of 9 cases of eastern equine encephalitis (EEE) were reported from 5 states: 5 cases from Florida and 1 case each from Georgia, Michigan, New Hampshire, and South Carolina. The Michigan and the New Hampshire cases were the first ever reported in residents of these states. The Michigan case was associated with an outbreak of equines from the southeast corner of the state. A total of 94 clinical equine cases was reported from July through mid-September. In contrast, the New Hampshire case was not associated with an equine outbreak.

Western Equine Encephalomyelitis

There were no reports of western equine encephalomyelitis cases from the states for 1980.

Dengue

A total of 69 cases of dengue were reported from 9 states. For the first time since 1945, indigenous transmission was documented in the United States when CDC's San Juan Laboratory reported the isolation of dengue 1 virus from a 5-year-old girl in Brownsville, Texas. All the indigenous cases occurred in Texas where travel histories showed that at least 17 residents had acquired dengue 1 infection. Texas reported 54 cases of dengue infection based on serologic confirmation or isolation of dengue 1 virus. Although the cases were concentrated in south Texas near the locations of outbreaks in Mexican cities, active surveillance documented only low level or sporadic occurrence of dengue in Texas and no major outbreak was detected. Imported dengue was reported from 8 states: New York (6), Louisiana (2), South Carolina (2), and California, Florida, Hawaii, North Carolina, and Oregon (1 case each).

Karl Kappus, Cornelia Davis, Jonathan Kaplan, Charles Calisher, Gladys Sather, Peter Katona, Lawrence Schonberger

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

Arbovirus Surveillance in Pennsylvania, 1980

The Departments of Health and Environmental Resources, in a cooperative program, conducted an arbovirus surveillance program from July 7 to October 10, 1980.

Sentinel flocks of four (4) cockerels were placed at 68 sites throughout the state at the locations shown in the Figure. This compares with 50 sentinel sites in 1979. This year, there was at least 1 sentinel flock in 36 of the 67 counties as compared with coverage in 28 counties in 1979. The cockerels were bled weekly and the sera tested, after protamine sulfate-acetone extraction, for hemagglutination-inhibition (HI) antibodies against St. Louis Encephalitis (SLE), Western Equine Encephalitis (WEE), Eastern Equine Encephalitis (EEE), and California Encephalitis (CE) viruses. There were no seronconversions in 11,080 HI tests performed. This is the first year that the program in this format has revealed no arbovirus activity (Table).

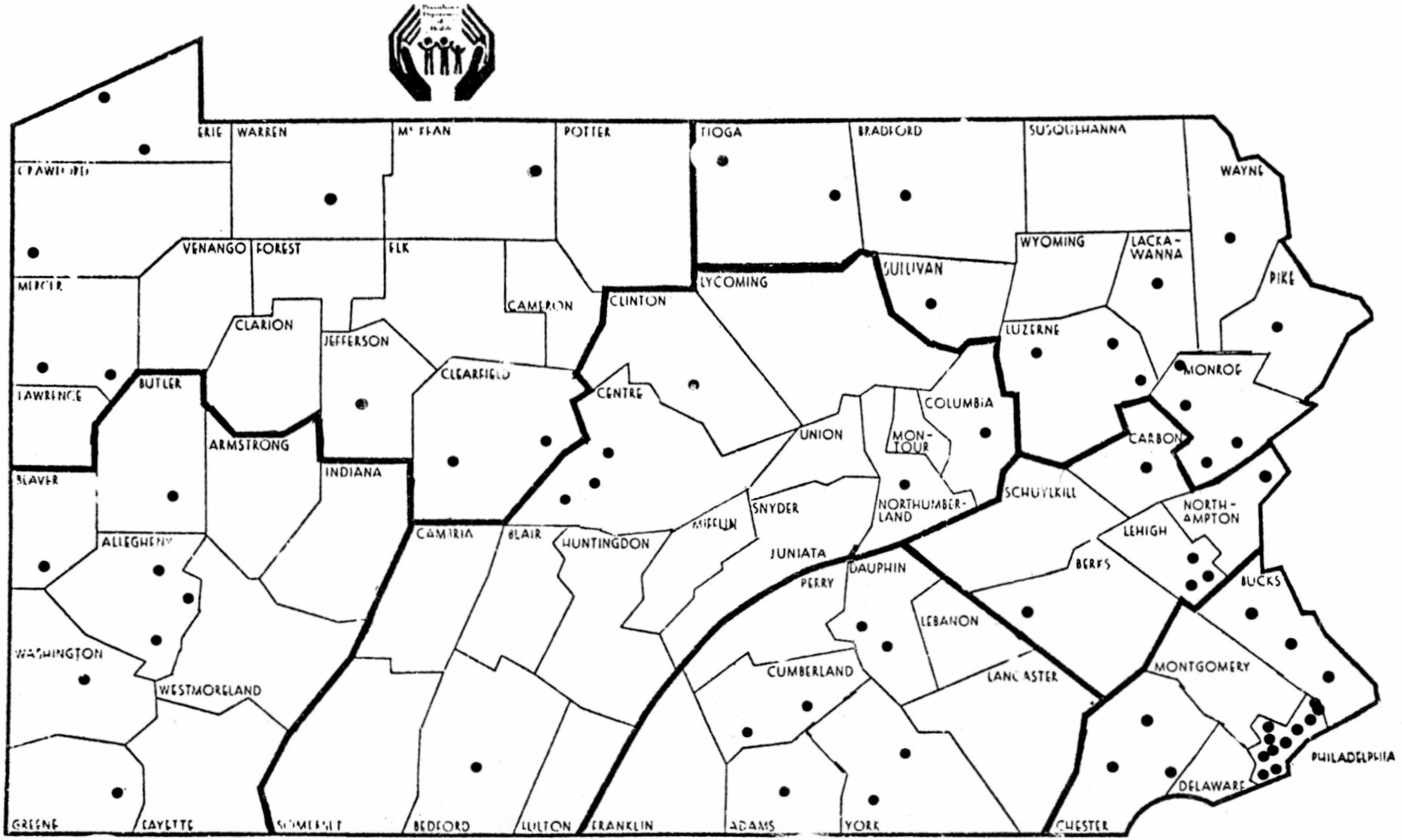
Results of Hemagglutination-Inhibition (HI) Testing, Arbovirus Surveillance Program, Pennsylvania, 1978-1980.

Year	No. of serum samples tested	No. with HI antibodies against			
		WEE	SLE	EEE	CEV
1978	2493	1	0	0	0
1979	2364	3	1	0	0
1980	2795	0	0	0	0

Seventy-nine (79) serum samples from 46 patients with a clinical diagnosis compatible with arbovirus infection were tested for serologic evidence of infection with SLE, WEE, EEE, or CEV. A 7-year-old boy who died 9 days after onset of disease had a titer of 1:10 against SLE in serum samples collected 2 and 9 days after onset; no further specimens were available from this patient. None of the other 45 patients demonstrated evidence of arbovirus infection.

(Bruce Kleger, Philip Nash, and Vern Pidcoe)

SENTINEL POULTRY FLOCK LOCATIONS
Encephalitis Surveillance Program, Pennsylvania, 1980



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

Human Responses to a Dengue Type 2 Vaccine

The candidate dengue type two (DEN-2) vaccine virus, designated PR-159/S-1, is a temperature sensitive, small-plaque varient which shows reduced virulance for suckling mice. Primate studies showed that the vaccine virus is attenuated in rhesus monkeys and initial human studies demonstrated acceptable safety and immune responses in five DEN-2 susceptable recipients. All five vaccine recipients had previously been immunized with 17D yellow fever vaccine and had yellow fever neutralizing antibodies at the time of dengue immunization.

We report studies undertaken to compare the safety and immunogenicity of the candidate DEN-2 vaccine in yellow fever immune and non-immune recipients. To date the DEN-2 vaccine has been administered to 46 volunteers in six studies (Table 1); 21 were yellow fever immune and 25 were yellow fever non-immune. Thirty-five volunteers had no complaints while 11 experienced mild symptoms which may have been related to vaccination. The symptoms included low-grade fevers ($<38.5^{o}c$) which occurred in two yellow fever immune recipients and transient erythematous rashes which occurred in one yellow fever immune and two yellow fever non-immune recipients. Mild non-specific headaches and/or muscle aches occurred in six people, equally distributed in the two groups (Table 2). Three persons missed duty for up to two days because of these complaints but no one developed a serious illness.

Virus isolation rates were approximately equal for the yellow fever immune and non-immune groups and all isolates retained the temperature sensitive characteristics of the vaccine virus. A dose response was sought in both the yellow fever immune and non-immune groups (Table 3). For those that had preexisting yellow fever antibody a dose response was observed with a 50 per cent immunizing dose, of $10^{3.3}$ PFUs based on neutralizing antibody titers. For the yellow fever non-immunes the 50 per cent immunizing dose could not be determined as some recipients of each dose developed antibody and infection did not appear to be solely dependent on dose. It also appeared that prior yellow fever immunization also stimulated higher and more durable DEN-2 antibody titers following vaccination (Figure).

Further studies are underway to comfirm or refute the differences in the immune responses observed between the yellow fever immune and non-immune recipients of the Den-2 vaccine.

(R.M^{c}N. Scott, W.H. Bancroft, J.M. M^{c}Cown, K.H. Eckels, P.K.Russell)

TABLE 1

DEN-2 Vaccine
Human Volunteer Studies

Study	Purpose	Recipients
1	Safety, Immune Response of Yellow Fever Immunes	6
2	Dose response in Yellow Fever nonimmunes	19
3	Dose response in Yellow Fever immunes	15
4	Response to revaccination	(12)
5	Intradermal inoculation	6
	Total Recipients	46

Table 2

Clinical Responses to
DEN-2 Vaccine

Volunteers	No. Infected	Leukopenia (<4000 WBC)	Fever (>38°C)	Rash
YF Immune	10	6	2	1
YF Nonimmune	10	3	0	2

TABLE 3

Responses to Varying Doses
of DEN-2 Vaccine

Approx Dose (pfu)	Freq. of Seroconversion (N antibody)	
	YF-Immune	YF-Nonimmune
$10^{5.5}$	5/5	2/5
$10^{4.5}$	N.D.	3/5
$10^{3.5}$	3/5	2/5
$10^{2.5}$	2/5	1/4
$10^{1.5}$	0/4	N.D.
Combined	10/19	8/19

THE EFFECT OF YELLOW FEVER IMMUNITY ON DEN-2 NEUTRALIZING ANTIBODY FOLLOWING DEN-2 IMMUNIZATION

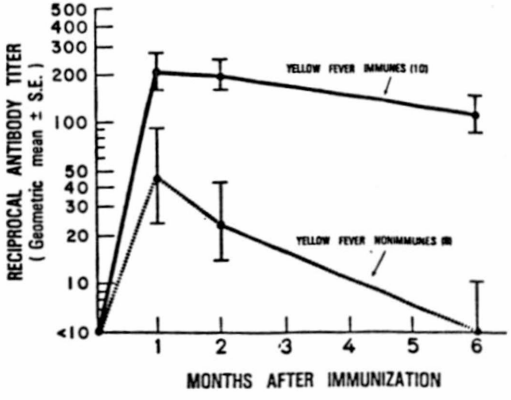


Table 1

Isolations from Arthropods in New Jersey*
4th Quarter 1980

Group	Virus and No. of Strains EE	WE	SLE	Isolated From	Collected In	Month
A	7	2		C.melanura	Bass River	September
A	1			C.melanura	Burlington Co.	September
A	20	3		C.melanura	Dennisville	Aug. Sept. Oct.
A	1			C.restuans	Dennisville	September
A	3	1		C.melanura	New Gretna	September
A	15	4		C.melanura	Woodbine	Aug. Sept. Oct.
A		1		A.quadrinaculatus	Woodbine	August
A	1			C.pipiens	Middlesex Co.	August
B			2	C.pipiens	Middlesex Co.	August
Totals:	48	11	2			

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

Table 2

Isolations from Avian Blood in New Jersey *

Species	Isolate	Date Collected	Location
Robin	EE	8/28/80	Dennisville
Robin	EE	9/3/80	Dennisville
Red Start	EE	9/3/80	Dennisville
Black & White Warbler	EE	9/10/80	Dennisville
Myrtle Warbler	EE	10/10/80	Dennisville

* Collected by the New Jersey Agricultural Experiment Station, Rutgers University.

Table 3

Isolations from Horses in New Jersey

Location	Isolate	Date Collected	Isolation Data Organs	Isolation Data Blood	Serology Data HI	Serology Data Neut.
Clarksburg	EE	8/30/80	Brain EE	Neg.	1280	ND
Penns Grove	WE	8/9/80	-	WE	B_1 <10	1.0
					B_2 20	2.0

Sentinal chicken flocks containing ten birds each were maintained in Burlington, Camden, Mercer and Middlesex Counties. One chicken each in Burlington and Mercer Counties demonstrated antibody conversions to SLE in mouse neutralization tests.

Very truly yours,

Wayne Pizzuti

Wayne Pizzuti
Chief Virologist
Virology Program

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

Studies of intestinal infection and transmission thresholds of VE virus in an enzootic vector mosquito, Culex (Melanoconion) opisthopus, began during 1979 following the demonstration in 1977 that this mosquito is a natural vector species of VE virus at a marsh focus of virus on the Pacific coast of Guatemala. This species was only the third mosquito species proven to be an enzootic vector of VE virus; the other two species are Cu. (Mel.) portesi in Trinidad, West Indies and aikenii in Panama. Determinations of the intestinal thresholds of VE virus (minimal intestinal dose of virus) in Cu. (Mel.) opisthopus began by examining an enzootic virus strain (68U201) from the Pacific coastal marsh focus in Guatemala. These experiments revealed that intestinal thresholds of this VE strain were low (<5 pfu) for both infection and transmission of virus by wild-caught Cu. (Mel.) opisthopus from Guatemala. Subsequently a second strain of VE virus (69U332) was tested and found to have higher intestinal thresholds for infection and transmission of virus by Cu. (Mel.) opisthopus; the 50% intestinal infection threshold exceeded 16,000 pfu. This VE strain was isolated from a sentinel hamster at the Pacific focus in Guatemala in October 1969, about two months after the VE epidemic-epizootic had terminated in that region. It was identified as VE virus by plaque-reduction neutralization and by complement-fixation tests, and had equine-virulence, hemagglutination-inhibition, hemagglutination-pH and Vero plaque properties like epizootic VE virus. Obviously further studies are required to learn whether these results reflect fundamental differences between enzootic and epizootic VE strains.

Cu. (Mel.) opisthopus propagated through four generations in the insectary at Cornell, Ithaca, New York have retained their susceptibility to two enzootic strains of VE virus (68U201 and 70U80). Intestinal infection and transmission thresholds of an enzootic VE strain in Cu. (Mel.) opisthopus from Florida,U.S.A. (kindly provided by personnel at CDC-Fort Collins from their colony) were low like those of wild-caught Cu. (Mel.) opisthopus from Guatemala.

Intestinal threshold measurements have begun with Aedes taeniorhynchus a known epizootic vector of VE virus, which is also present at the Guatemalan enzootic focus, but is of unknown vector activity there. Cu. (Mel.) opisthopus brought from Guatemala to Cornell (Ithaca, New York) have been successfully reared through the fourth generation to date. Aedes taeniorhynchus started from Guatemalan mosquitoes in 1978 is in the fifth generation.

Mosquito population measurements using several methods for collecting mosquitoes at the Guatemalan VE focus during 1979 showed that Cu. (Mel.) opisthopus continued to be a prominant mosquito. Aedes taeniorhynchus and Mansonia titillans were also present, but Culex nigripalpus populations were significantly smaller than in 1978. Host feeding patterns of Cu. (Mel.) opisthopus were examined during 1977-1979 by precipitin tests. 70% of 415 Cu. (Mel.) opisthopus contained mammalian blood, 22% reptilian, 8% avian and less than 1% amphibian. Among the mammalian bloodmeals, 28% were bovine, 13% horse, 9% rodent, 7% guinea pig, 5% hamster/Oryzomys, 2% rabbit, 1% opossum, 1% human and less than 1% swine.

Sentinel hamsters and sentinel guinea pigs exposed at the Pacific coastal enzootic focus of VE virus in Guatemala showed that VE virus was cycling in 1979, though probably at a lower level than during 1977 and 1978. In contrast, group C-probably Nepuyo virus was more prevalent. Exposures of sentinel hamsters at an enzootic marsh habitat at Puerto Barrios on the Atlantic coast of Guatemala during 1979 showed that VE virus was still actively cycling as it had been during 1968 and 1970. Humans employed there had detectable VE HI antibodies in serum in 1979 (37% of 109 persons).

Toxorhynchites amboinensis inoculated intrathoracially were compared with primary chicken embryonic cell cultures for their sensitivity to detect seven strains of VE virus. Virus titers were slightly higher in these mosquitoes for three strains, were equal for two strains and were slightly lower for two strains. Each strain of VE virus reached concentrations of $10^{2.7-5.7}$ pfu in individual mosquitoes after extrinsic incubation periods of 12-19 days. For four strains, the source of the virus suspension (i.e., from infected vertebrate cells or infected invertebrate (Toxorhynchites amboinensis) cells) had no effect on comparative titers. No temperature-sensitive virus was detected in Toxorhynchites amboinensis infected by six VE strains.

A strain of group C bunyavirus, isolated from Cu. (Mel.) opisthopus in Guatemala during 1977, was identified as Nepuyo virus. This is the first isolation of NEP virus from this mosquito species at the Pacific coastal enzootic focus in Guatemala.

The hypothesis that the strains of equine-virulent Venezuelan encephalitis (VE) virus that cause equine epizootics and epidemics are present as subpopulations within enzootic strains is being directly tested. Isolates from Guatemala and neighboring regions of Belize and Honduras obtained before the 1969 Central American outbreak, and from Guatemalan enzootic habitats after the outbreak have been examined for the presence of epizootic VE virus by the combined techniques of hydroxylapatite (HT) chromatography and selection of small plaques in Vero cells. Likewise, isolates from the Gulf coast of Mexico obtained before and after the 1966 outbreak in Tampico have been studied. Fifty of fifty-nine isolates or isolate pools (representing 91 of 104 isolates) contained various quantities of virus which eluted from hydroxylapatite and produced small plaques in Vero cells like epizootic virus. Candidate epizootic VE virus clones from 32 isolates or isolate pools have been shown to be stable and homogeneous with respect to plaque size in Vero cells. Twenty-three of these clones have been cultured to high titer and remain stable.

Examination of candidate-epizootic clones for viral characteristics which distinguish epizootic from enzootic VE virus strains is now in progress. Guinea pig virulence has been used as a screen test of candidate clones for equine-virulence. Of the 50 guinea pigs inoculated with the 23 candidate clones, only one died. The clone used to inoculate this animal was inoculated into 5 other guinea pigs which all survived the infection. Therefore, if guinea pig virulence can be used as a valid indicator of equine-virulence, results to date would suggest that none of the 23 clones is equine-virulent. Antigenic characterization of these clones by virus-dilution, short-time hemagglutination-inhibition tests is now in progress.

By coinfecting suckling mice, i.c., with artificial mixtures of epizootic and enzootic VE viruses, it was shown that both viruses in the pair replicate well, and that the highest degree of alteration in ratio was 5-fold. These experiments indicate that passage of enzootic VE virus isolates through suckling mice does not significantly decrease the chance of detecting possible subpopulations of epizootic VE virus in the isolate.

HT chromatography of mosquito passaged epizootic and enzootic VE virus strains showed that they eluted at distinctively different phosphate concentrations than when passaged through vertebrate cells. The phosphate concentration at which the virus eluted was dependent upon both the virus strain and the species of host mosquito.

(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, 1980

Serum samples of 469 patients with signs and symptoms of central nervous system infection or with fever of unknown origin were tested for hemagglutination-inhibition antibodies to EEE, WEE, SLE, POW and CE viruses. In contrast to preceding years when CE infections were detected frequently in New York State residents, no confirmed or presumptive cases of these or other arbovirus infections were diagnosed.

During the summer of 1980, a total of 3,359 pools of 155,569 adult female mosquitoes, collected from 4 regions in New York State, were tested for viruses. Forty-three virus isolates, 2 EEE, 31 CAL and 10 FLA, were obtained (Table 1). Both EEE isolates were from the Syracuse region. Five FLA isolates each were detected in the Syracuse region and in Suffolk County of the White Plains region. More than half of the CAL isolates were from the Albany region and 11 of the CAL isolates were acquired from the Buffalo region where this serogroup was infrequently found in previous years. Most of the CAL viruses were obtained from Aedes communis with only 3 isolates from Aedes triseriatus, the most common vector of La Crosse virus in the upper midwestern United States.

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

Laboratories for Virology
Division of Laboratories and Research
New York State Department of Health
Albany, New York 12201

Table 1

Arbovirus Surveillance, 1980

Wild-caught Mosquitoes

	No. Tested		No. Isolates		
	Pools	Specimens	EEE	CAL	FLA
Region:					
Buffalo	682 (494)*	19,261 (17,305)*	0	11	0
Syracuse	559 (149)	37,553 (10,196)	2	1	5
Albany	1,528 (1,144)	46,806 (42,117)	0	18	0
White Plains	590 (325)	51,949 (29,330)	0	1	5
Total	3,359 (2,112)	155,569 (98,948)	2	31	10
Genus and species:					
Aedes aurifer	22	2,156			
Aedes cantator	60	4,977		1	
Aedes canadensis	301	15,504		2	
Aedes cinereus	88	3,200		1	
Aedes communis	381	20,702		17	
Aedes dorsalis	17	954		1	
Aedes excrucians	43	1,008		1	
Aedes sollicitans	208	20,460		2	
Aedes stimulans	336	14,983		2	
Aedes triseriatus	169	3,272		3	
Aedes trivittatus	60	1,760		1	
Aedes vexans	179	4,899			
Aedes spp.	132	4,026			
Other Aedes spp.	116	1,627			
Culex spp.	274	12,757			4
Culiseta spp.	370	19,811	2		6
Coquillettidia spp.	347	20,881			
Anopheles spp.	249	2,511			
Other mosquitoes	7	81			
Total	3,359	155,569	2	31	10

*( ) Aedes spp.

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE,

DEPARTMENT OF MEDICAL MICROBIOLOGY,

UNIVERSITY OF TORONTO,

TORONTO, ONTARIO, CANADA.

Arbovirus Infections in Travellers Returning to Canada, 1974 - 1980.

Between January 1, 1974 and December 31, 1980 sera were received from 358 patients who had a history of recent travel outside of Canada. These sera were screened by hemagglutination inhibition against the following antigens: alphaviruses - eastern equine encephalitis, western equine encephalitis and Chikungunya, flaviviruses - Powassan, St.Louis encephalitis, dengue and Banzi, and a California group virus - Snowshoe hare. All sera showing positive hemagglutination inhibition serology were subsequently tested by complement fixation against the corresponding antigens.

Forty-eight arbovirus infections were diagnosed. These included 31 infections that were identified by a diagnostic (four fold or greater) increase in antibody titres and 17 infections that were identified by a diagnostic (four fold or greater) decrease in antibody titres. In addition, in 1977, dengue I virus was isolated from the acute serum of one patient recently returned from Jamaica who subsequently yielded a diagnostic increase in titres to flavivirus antigens.

A breakdown of the infections diagnosed is presented in Table 1. These included one alphavirus infection, likely due to Chikungunya or O'Nyong-Nyong virus and 47 flavivirus infections. The flavivirus infections included one imported case of St. Louis encephalitis from Ohio in 1975 and one imported case of Powassan encephalitis from New York state in 1978. Twenty-two flavivirus infections were identified as dengue due to the fact that the patients were recently returned from areas of known dengue activity and had symptoms consistent with dengue fever. Twenty-three other flavivirus infections were diagnosed for which no definite etiological agent could be established.

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

Table 1. Arbovirus Infections Contracted Outside Canada and Diagnosed or Confirmed by the National Arbovirus Reference Service 1974 - 1980.

YEAR	VIRUS	AREA OF TRAVEL	NO. CASES
1974	Chikungunya or O'Nyong-Nyong	Uganda	1
1975	St.Louis encephalitis	United States	1
1976	Flavivirus	Nigeria, Ghana, Dohomey	1
1977	Dengue	Jamaica	12
		Haiti	1
	Flavivirus	India, Thailand, Indonesia	1
		India	1
		Sri Lanka	1
		Unknown	4
1978	Dengue	Trinidad	3
		Jamaica	1
		Tahaita	1
	Powassan	United States	1
	Flavivirus	French Guiana, Guadeloupe	1
		Southeast Asia	1
		Nigeria	1
		Unknown	3
1979	Dengue	Tahiti	1
		Barbados	1
		Haiti	1
	Flavivirus	Sri Lanka	2
		Viet Nam	1
		Far East	1
		Unknown	1
1980	Dengue	Jamaica	1
	Flavivirus	Guyana	1
		India	1
		Fiji	1
		Unknown	1

REPORT FROM THE DIVISION OF LABORATORIES, ILLINOIS

DEPARTMENT OF PUBLIC HEALTH, CHICAGO, ILLINOIS

Report from the Arbovirus Surveillance Program

The first laboratory confirmed human cases of St. Louis Encephalitis (SLE) in Illinois since 1977 were identified during the late summer and early fall of 1980. The first case, with onset of illness on August 29, involved a 73-year-old female who lived near the Madison-St. Clair County line. The second case involved a 68-year-old female from Chicago (Cook County) with onset of September 6. The final two cases, 53-and 68-year-old females, were from St. Clair County and had onsets of September 24 and 26, respectively. None of these cases was fatal.

Based on results of sequential collection and testing of serum from juvenile house sparrows (Passer domesticus), occurrence of detectable human disease in southern Illinois was not wholly unexpected last year (Fig.1). In St. Clair County, adjacent to St. Louis, Missouri, we found hemagglutination-inhibition (HI) antibodies to SLE virus (titer=1:20 or greater) in a total of 18 juvenile birds (55% of all juvenile positives in the state) during collection intervals from June 23-24 to September 23 (4.2%). These birds originated at two principal sites within the county, one just north of East St. Louis and the other in Belleville, about 14 miles to the southeast. At the former site, 13 of 377 (3.4%) juvenile bled between May 5 and September 23 had SLE antibodies while 5 of 332 (1.5%) from Belleville were positive. Of great interest to us was the fact that the three human cases from south-western Illinois were from residential areas located between these two collection sites.

In 1979, eight (62%) of the 13 positive birds identified from the 3,788 tested in the state were from St. Clair County. One case of SLE was confirmed there in 1977 after 21 cases (two fatalities) were reported there in 1975. This series of results over the last 6 years strongly suggests that an endemic focus of SLE virus transmission may exist in this densely-populated area.

In 1980, 15 human cases of California (LaCrosse) Encephalitis were diagnosed in the laboratory. This is the same number reported in both 1977 and 1978 but four more than the 11 seen in 1979. All of the 1980 infections occurred in children, ages 25 months to 14 years. Cases originated in five counties including the first reported case from Stephenson County, on the Illinois-Wisconsin state line, and McLean and Macon Counties, in central Illinois. Onset of illness ranged from June 20 to September 15. Eleven (73%) of the cases were from Peoria County, a perennial source of this disease. In fact, 57(50%) of the 113 cases reported in Illinois since 1966 have been in Peoria County children.

Epidemiological follow-up on the 1980 cases further documented the focal nature of this disease. In each of two Peoria County families, two children were hospitalized and confirmed cases. One house away from one of the families, a third child was afflicted. Ongoing field investigations in our study area there have resulted in isolation this year of LaCrosse-like virus from larvae collected from a treehole that also yielded virus-infected larvae in 1978 and 1979.

Recent definitive laboratory testing by Dr. Charles H. Calisher, Vector-Borne Disease Division, Center for Disease Control, has revealed that nine representative "LaCrosse-like" isolates that we have made from Aedes triseriatus complex mosquitoes were LAC virus. This group included four isolates from laboratory-reared larvae collected from treeholes in our Peoria County study area from 1978 through 1980. The other five isolates were from laboratory-reared adult mosquitoes that had been collected as eggs in ovitraps placed at four separate sites in Peoria County and one in a suburban forest preserve in Cook County. Preliminary analysis of 5,419 A. triseriatus mosquitoes from human biting collections at the Peoria County study area in 1979 were tested in 218 pools and yielded 48 isolates of what is undoubtedly LAC virus.

(Gary G. Clark and Harvey L. Pretula)

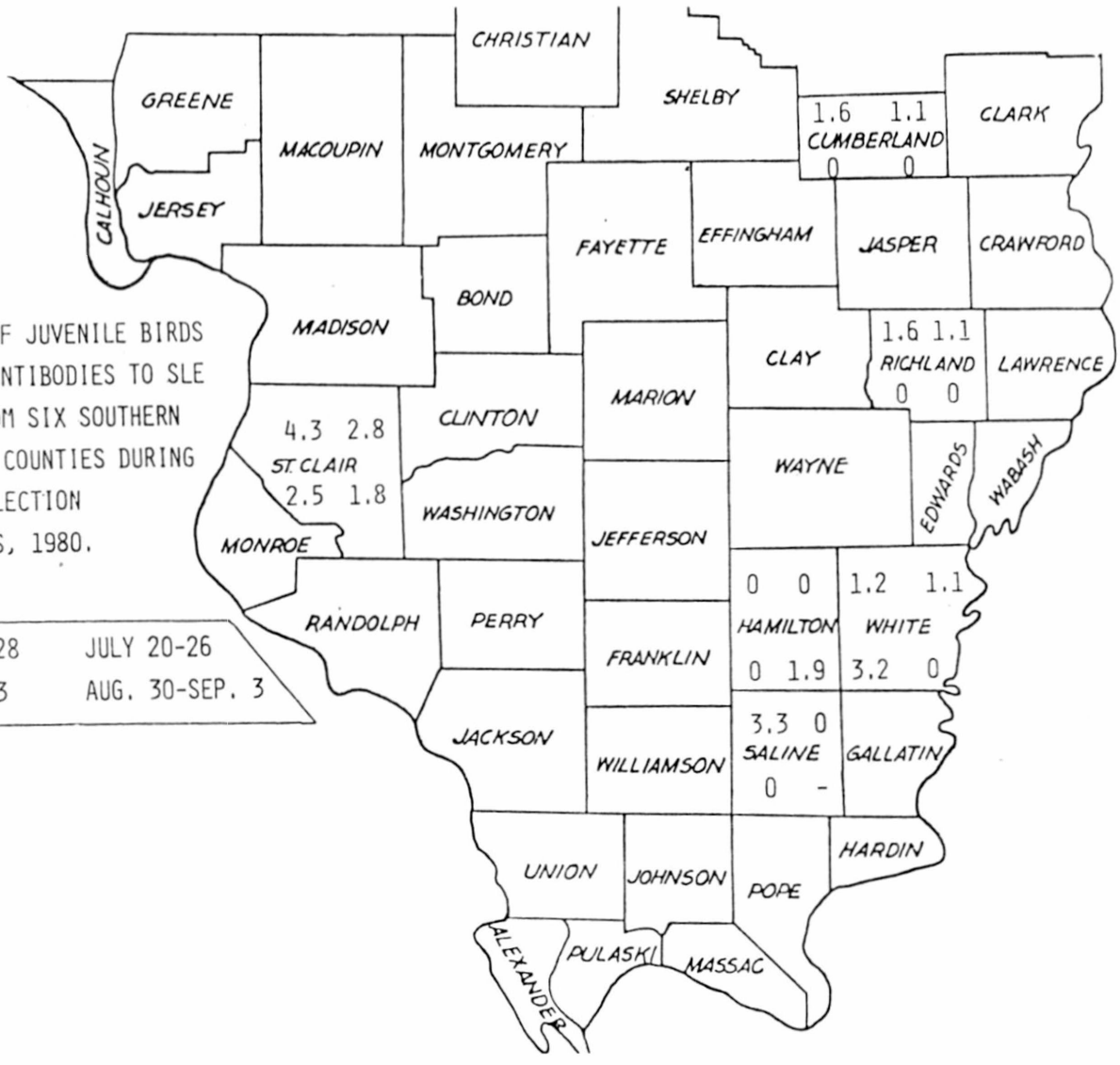


Figure 1.

PERCENT OF JUVENILE BIRDS WITH HI ANTIBODIES TO SLE VIRUS FROM SIX SOUTHERN ILLINOIS COUNTIES DURING FOUR COLLECTION INTERVALS, 1980.

JUNE 22-28	JULY 20-26
AUG. 9-13	AUG. 30-SEP. 3

REPORT FROM THE ZOONOSES RESEARCH LABORATORY, DEPARTMENT OF PREVENTIVE MEDICINE, UNIVERSITY OF WISCONSIN, MADISON 53706.

CALIFORNIA ENCEPHALITIS (CE) VIRUS ANTIBODIES IN WISCONSIN PATIENTS.

Antibodies to La Crosse (LAC) and other CE group arboviruses were detected in sera from patients with febrile or CNS disease in Wisconsin during 1979 and 1980 by comparative neutralization tests in BHK_{21} cells. Tests were promptly conducted on portions of either acute or convalescent serums as received from collaborating physicians and through the virus diagnostic section of State Laboratory of Hygiene, which also conducted the routine diagnostic tests on paired serums for other viral agent on paired serums, including HI and CF tests for LAC and other arboviruses. Counter-immuno-electrophoresis (CIE) and indirect FA tests were also conducted on sera from some of these patients in collaboration with Dr. Cameron Gundersen in La Crosse and Dr. Barry Beaty of the Yale Arbovirus Research Unit, Donald Nelson and Dr. Jeff Davis of SHD, and two medical students.

Antibodies neutralizing CE group arboviruses were found in serums from 148 (16.4%) of 902 patients tested in neutralization tests during 1979 and 1980. The distribution of antibodies in 856 patients with known age, sex, and geographic distribution is given in the table one. Sera from 82 (9.6%) of the 856 patients neutralized LAC virus in highest titer, 57 (6.7%) neutralized Jamestown Canyon (JC) virus, and 2 (0.2%) neutralized Trivittatus (TVT) virus.

Antibodies to LAC virus were found mostly in patients from the LAC endemic region (the southwest third) of Wisconsin. Thirty-six percent (64/179) of the patients under 15 years of age from the endemic region had antibodies neutralizing LAC, while only 11% (12/114) of those 15 or more from the endemic region had detectable antibody. In patients tested from the other two-thirds of Wisconsin antibodies neutralizing LAC virus were found in 2.6% (5/194) of children and in only 0.3% (1/369) of those 15 or more years of age.

Antibodies neutralizing JC virus were found in 6.7% (57/856) of those tested, throughout Wisconsin with higher rates in adults than in children. Antibodies to JC were found in 9% (10/114) of adults tested during 1979 and 1980 from the SW third of Wisconsin and in 10% (36/369) of those from northern and eastern Wisconsin. Lower antibody rates were found in children, in only 2% (3/179) of the children tested from the southwest third, and in 4% (8/194) of children from the rest of the state.

Antibodies to Trivittatus (TVT) virus were found in serums from two (0.2%) of the patients tested, both in young men from the southeast corner of the state, one during 1979 and the other in 1980.

Sixty cases of California (La Crosse) encephalitis were found in these patients during the past two years by rises in titer to LAC virus, table two. Further studies of those with antibodies to JC and TVT viruses are underway for recency of infection and possible relation to disease.

(Wayne Thompson)

TABLE 1.

DISTRIBUTION OF ANTIBODIES NEUTRALIZING LA CROSSE (LAC), JAMESTOWN CANYON (JC), AND TRIVITTATUS (TVT) CALIFORNIA GROUP ARBOVIRUSES IN PATIENTS WITH FEBRILE OR CNS DISEASE IN WISCONSIN - 1979 AND 1980.

	SOUTHWEST THIRD WISCONSIN				OTHER TWO-THIRDS				WISCONSIN TOTALS	
	UNDER 15		15 & OVER		UNDER 15		15 & OVER		ALL AGES	
	1979	1980	1979	1980	1979	1980	1979	1980	1979	1980
LAC	(31%) 38/122	(46%) 26/57	(9%) 7/77	(14%) 5/37	(1%) 1/109	(5%) 4/85	(0.5%) 1/222	(0%) 0/147	(9%) 47/530	(11%) 35/326
JC	(0%) 0/122	(5%) 3/57	(9%) 7/77	(8%) 3/37	(6%) 6/109	(2%) 2/85	(11%) 24/222	(8%) 12/147	(7%) 37/530	(6%) 20/326
TVT	(0%) 0/122	(0%) 0/57	(0%) 0/77	(0%) 0/37	(0%) 0/109	(0%) 0/85	(0.5%) 1/222	(0.7%) 1/147	(0.2%) 1/530	(0.3%) 1/326
TOTALS:	(31%) 38/122	(51%) 29/57	(18%) 14/77	(22%) 8/37	(6%) 7/109	(7%) 6/85	(12%) 26/222	(9%) 13/147	(16%) 85/530	(17%) 56/326

TABLE 2.

AGE-SEX-GEOGRAPHIC AND SEROLOGIC DETERMINATIONS OF PATIENTS WITH ANTIBODY TO LA CROSSE VIRUS IN WISCONSIN-1979 AND 1980.

WISCONSIN:	SOUTHWEST THIRD				NORTH AND EAST PORTION				TOTALS 1979-80	
	Under 15 yrs		15 & Over		Under 15 yrs		15 & Over			
1979 ♂	(28%)	(36%)	(0%)	(7%)	(3%)	(2%)	(0%)	(0%)	(7%)	(10%)
	19 c	25/69 ab	0 c	3/41 ab	1 c	1/64 ab	0 c	0/114 ab	20 c	29/288 ab
1979 ♀	(21%)	(25%)	(6%)	(11%)	(0%)	(0%)	(0%)	(9%)	(6%)	(7%)
	11 c	13/53 ab	2 c	4/36 ab	0 c	0/45 ab	0 c	1/108 ab	13 c	18/242 ab
1980 ♂	(42%)	(47%)	(0%)	(15%)	(4%)	(4%)	(0%)	(0%)	(12%)	(15%)
	19 c	21/45 ab	0 c	3/20 ab	2 c	2/53 ab	0 c	0/59 ab	21 c	26/177 ab
1980 ♀	(33%)	(42%)	(6%)	(12%)	(3%)	(6%)	(0%)	(0%)	(4%)	(6%)
	4 c	5/12 ab	1 c	2/17 ab	1 c	2/32 ab	0 c	0/88 ab	6 c	9/149 ab
TOTALS 1979-80	(30%)	(36%)	(3%)	(11%)	(3%)	(3%)	(0%)	(0.3%)	(7%)	(10%)
	53 c	64/179 ab	3 c	12/114ab	4 c	5/194 ab	0 c	1/369 ab	60 c	82/856 ab

c : serologically confirmed cases of La Crosse encephalitis.

ab: total with antibody to LAC virus (including confirmed cases)/number of patients tested.

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

Susceptibility of U.S. and Caribbean Strains of Aedes aegypti to Oral Infection with Dengue Viruses.

Dengue viruses have been documented in Central America as early as 1942, and evidence suggests that dengue 2 and 3 have remained endemic in the Caribbean region since that time. Both serotypes have been responsible for recent epidemics. In February 1977, dengue 1 was introduced into the Caribbean for the first time. This virus spread from Jamaica, where it was first reported, to Mexico in the west and Barbados, Trinidad, and Surinam in the east. In 1980 dengue 1 moved up the east coast of Mexico, and the first indigenous cases were reported in Texas in August. Since the principal vector mosquito, Aedes aegypti, occurs in most southern port cities of the U.S., there is concern that epidemic dengue may again be introduced into the United States. The purpose of this project is to determine whether variation in susceptibility of Aedes aegypti for dengue viruses occurs among selected geographic strains from the southern United States and the Caribbean.

The viruses used in these experiments were the prototype dengue 2, which had never been passed in suckling mice or tissue culture, and the Mexican dengue 1, which was isolated from human sera and had had 2 passages in a Toxorynchites cell line.

The origin and brief history of the mosquito strains used are shown in Table 1. The majority were colonized in the Bureau of Tropical Diseases Laboratory, Centers for Disease Control, Atlanta, from either field-collected larvae or eggs. All colonies were maintained in the laboratory according to standard rearing procedures for Aedes aegypti.

The oral infection experiments were done by feeding newly emerged female mosquitoes on a hanging drop virus suspension consisting of equal parts of virus, washed human erythrocytes and 10% sucrose. Only mosquitoes which took at least three-fourths of a full blood meal were selected and held for 14 days at 30°C. They were then tested for dengue virus infection by the presence or absence of viral antigen in brain squashes by DFAT. In each experiment, a small aliquot of the feeding suspension was held at room temperature for the duration of the mosquito feeding, which usually lasted 1-2 hours. It was subsequently titrated to determine the minimum virus titer of the feeding suspension. These are the titers listed in each table.

In the first experiment, available strains of Ae. aegypti were tested for susceptibility to oral infection with prototype dengue 2 virus since the Caribbean dengue 1 strain was not available. The results in Table 1 show considerable variation among the strains tested. With the exception of the Port Au Prince, Ae. aegypti strains from the Caribbean showed the highest susceptibility with infection rates of 42 to 45 percent. The Miami colony had a high infection rate, but only 15 mosquitoes were tested. This colony was started from only 4 females. The Gambia, New Orleans colony, and Port Au Prince strains had the lowest susceptibility.

Table 3 shows the comparative susceptibility of U.S. and Caribbean strains of Ae. aegypti to oral infection with dengue 1 virus. In general, the strains of Ae. aegypti from the Gulf Coast of the U.S. had very low infection rates. An exception was the strain from Sarasota, Florida, which had an infection rate of 21%. The Caribbean strains were slightly more

susceptible with the highest infection rate observed in Ae. aegypti from Villalba, Puerto Rico, an area where dengue transmission has been documented continuously for several years. Of interest was the relatively low infection rate in the Mexican Ae. aegypti which came from Montemorellos, an area where an outbreak occurred in 1980.

The data suggest that there may be considerable variation in susceptibility to oral infection with dengue 1 virus among U.S. and Caribbean strains of Ae. aegypti. These differences could be important in helping to explain the pattern of epidemic dengue in the region over the past few years. For example, the lack of epidemic activity in the U.S. agrees with the low susceptibility of most U.S. strains of Ae. aegypti. Obviously, other factors are also important and more work should be done to identify the factors responsible for the presence or absence of epidemic dengue in the area.

(D.J. Gubler, R. Novak*, D.B. Francy, D. Eliason*, and E. Campos)

*Division of Tropical Diseases, CDC, Atlanta, Georgia

Table 1

History of Ae. aegypti Strains Used in Susceptibility Studies

STRAIN	HISTORY
Jakarta, Indonesia	Laboratory colony 1975 - F?
Gambia, West Africa	Laboratory colony 1979 - F?
Montemorellos, Mexico	F_2 adults from field-collected larvae - 1980
Brownsville, TX	F_1 adults from field-collected eggs - 1980
Corpus Christie, TX	F_1 adults from field-collected eggs - 1980
San Antonio (Harlendale), TX	F_1 adults from field-collected larvae - 1980
San Antonio (Balcones), TX	F_1 adults from field-collected larvae - 1980
New Orleans (Colony), LA	F_3 adults from field-collected eggs - 1980
New Orleans (Airport), LA	F_1 adults from field-collected larvae - 1980
New Orleans (Magazine), LA	F_1 adults from field-collected larvae - 1980
Sarasota, FL	F_1 adults from field-collected eggs - 1980
Vero Beach, FL	F_3 adults from field-collected larvae - 1980
Miami (Colony), FL	F_4 adults, colony started from 4 females hatched from field-collected eggs - 1980
Miami (Gould), FL	F_1 adults from field-collected larvae - 1980
Miami (Central), FL	F_1 adults from field-collected larvae - 1980
San Juan, PR	F_2 adults from field-collected larvae - 1980
Villalba, PR	F_2 adults from field-collected larvae - 1980
Les Cayes, Haiti	F_4 adults from field-collected larvae - 1980
Port Au Prince, Haiti	F_2 adults from field-collected eggs - 1980

Table 2

Comparative Susceptibility of Geographic Strains of *Aedes aegypti* to Oral Infection with Dengue 2 Virus*

Mosquito Strain	Number Tested	% Infected
Jakarta, Indonesia	15/55	27.3
Gambia, West Africa	2/36	5.5
Miami (Colony), FL	9/15	60.0
Vero Beach, FL	9/38	23.7
New Orleans (Colony), LA	3/32	9.4
San Juan, PR	10/24	41.7
Villalba, PR	10/22	45.4
Les Cayes, Haiti	15/34	44.1
Port Au Prince, Haiti	3/33	9.1

*--Prototype dengue 2. Titer of feeding suspension was $10^{7.6}$ MID_{50} per ml.

Table 3

Comparative Susceptibility of U.S. and Caribbean Strains of *Aedes aegypti* to Oral Infection with Dengue 1 Virus*

Mosquito Strain	Number Tested	% Infected
Montemorellos, Mexico	4/29	13.8
Brownsville, TX	2/39	5.1
Corpus Christie, TX	3/46	6.5
San Antonio, TX	3/26	11.5
New Orleans, LA	3/35	8.6
Miami, FL (Colony)	2/12	16.7
Miami, FL (Central)	2/14	14.3
Sarasota, FL	9/42	21.4
San Juan, PR	8/32	25.0
Villalba, PR	11/24	45.8
Les Cayes, Haiti	9/42	21.4
Port Au Prince, Haiti	12/64	18.7

*--Mexican dengue 1. Titer of feeding suspension was $10^{7.3}$ MID_{50} per ml.

Diapause Termination, Gonoactivity, and Differentiation of Host-Seeking Behavior from Blood-Feeding Behavior in Hibernating *Culex tarsalis* Mosquitoes

Diapausing *Culex tarsalis* were collected from mine tunnels in Boulder County, Colorado, during late September and early October 1980 and studied in the laboratory. Exposure to a long photophase (L:D 15:9) resulted in diapause termination in essentially all mosquitoes by day 7 at 25°C, but warming alone (at 25°C) for up to 8 days did not terminate diapause in a significant proportion of females maintained at typical autumn day lengths (Fig. 1). Diapause was terminated in *Culex tarsalis* by topical application of methoprene (Fig. 1), a juvenile hormone (JH) mimic, suggesting that the physiological pathway for diapause termination involves secretion of JH by a reactivated corpus allatum. Host seeking and blood feeding were shown to represent discrete stages in the biting cycle of *Cx. tarsalis* and these were differentiated in diapausing females by varying the size of the feeding chamber (Table 1). Diapausing females could be induced to take a blood meal only when the host-seeking step in the biting cycle was bypassed by placing the females in close proximity to a host. Gonotrophic dissocation occurred in a significant proportion of females only when three conditions were met: females had to be in a state of diapause when fed, and the fed females had to be incubated at a cool temperature (15°C) and under short-day conditions during the time of bloodmeal digestion (Table 2). The demonstration that gonotrophic dissociation can occur under experimental conditions probably has no relation to what occurs in nature. Warm Indian summer conditions notwithstanding, typically short autumn day-lengths are sufficient to suppress host-seeking behavior in diapausing populations and host-seeking is a requisite for blood feeding in nature.

(Carl J. Mitchell)

Fig. 1. The effects of photophase and treatment with a juvenile hormone mimic on feeding rates in Cx. tarsalis collected from hibernaculae and maintained at 25°C.

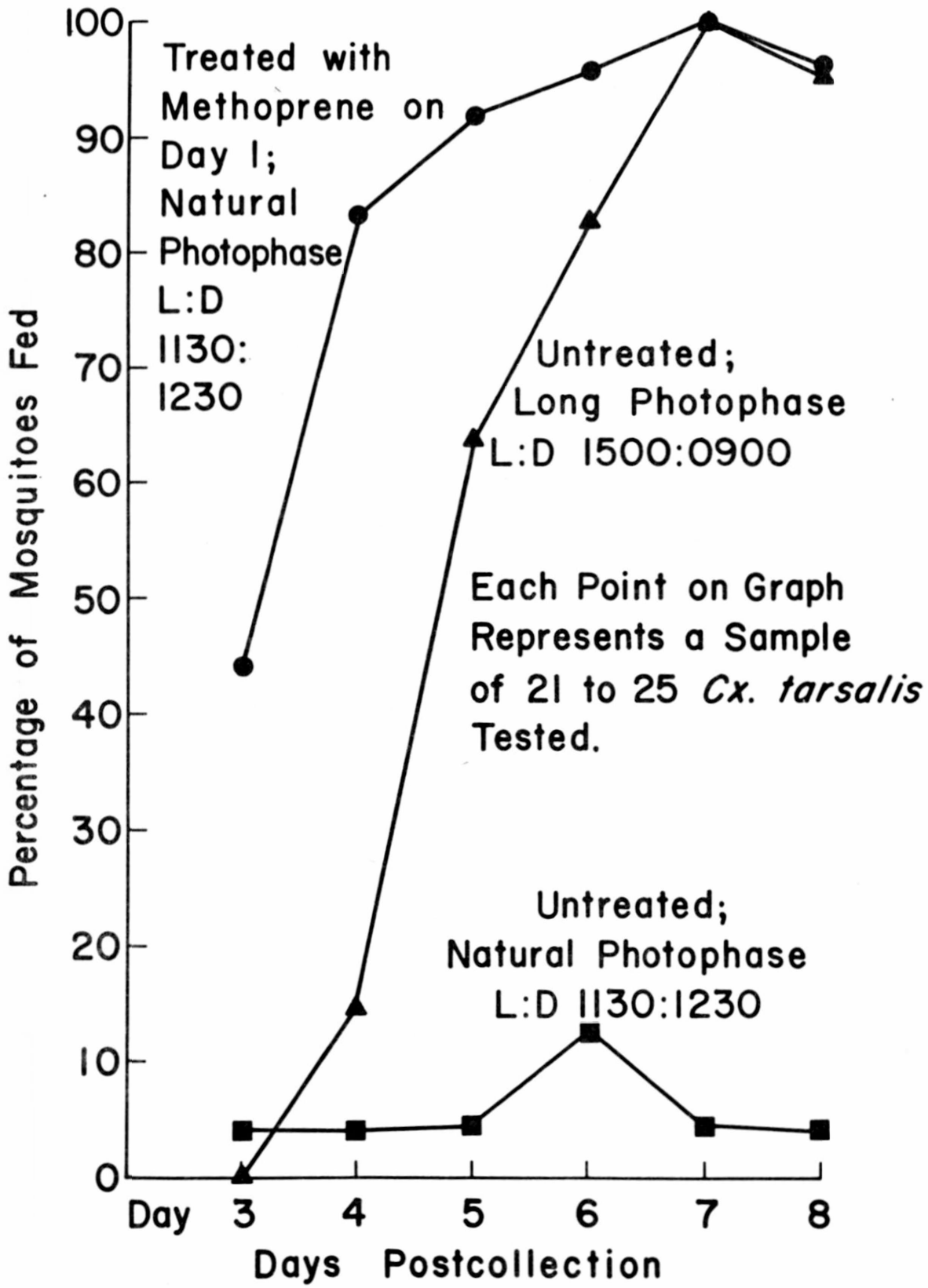


Table 1. The effects of feeding chamber size on feeding rates in diapausing Cx. tarsalis and gonotrophic dissociation rates in mosquitoes incubated at 15°C and L:D 1055:1305 following feeding.

Day Feeding Trial Begun	Feeding Chamber Size	Photoperiod Exposure From Time of Collection Through Feeding: L:D 15:9 Feeding Rate*	L:D 15:9 Gono. Diss. Rate**	L:D 1055:1305 Feeding Rate*	L:D 1055:1305 Gono. Diss. Rate**
Day of Collection	1/2 pint	7/25 (28)	7/7 (100)	10/25 (40)	7/10 (70)
	1 gal.	0/25 -	- -	-	- -
Day 1 post-Collection	1/2 pint	7/25 (28)	6/7 (85.7)	13/25 (52)	8/11 (72.7)
	1 gal.	0/25 -	- -	0/25 -	- -
Day 2 post-collection	1/2 pint	Chick died; No data		8/25 (32)	7/8 (87.5)
	1 gal.	0/25 -	- -	0/25 -	- -
Day 3 post-collection	1/2 pint	10/25 (40)	1/9 (11.1)***	9/25 (36)	6/8 (75)
	1 gal.	0/24 -	- -	1/25 (4)	Not done

* Number fed/number tested (%).

** Number with ovarian follicles in the resting stage/number that took more than 1/2 of a full bloodmean (%).

*** Statistically significant ($p < 0.01$) difference (according to Fisher's exact test) when compared to other rates in same column.

Table 2. A summary of observations on the occurrence of gonotrophic dissociation in Cx. tarsalis incubated under a variety of conditions.

Light and Temp. Regimen During Bloodmeal Digestion	Diapausing When Fed: Frequency of Gonotrophic Dissociation	Diapause Terminated by Exposure to Long Photophase or Treatment with a JH mimic* Prior to Feeding: Frequency of Gonotrophic Dissociation
Natural Photophase 15°C	28/37** (75.7%)	1/23 (4.3%)
Natural Photophase 25°C	1/15 (6.7%)	0/109* (0%)
Long Photophase 25°	0/9 (0%)	0/79 (0%)

* Treated with methoprene.

** Number undergoing gonotrophic dissociation/number tested (%).

Comparison of yellow fever 17D vaccine preparations by RNA oligonucleotide mapping

At a recent PAHO-sponsored meeting on yellow fever in Belem, Brazil (April, 1980) it was recommended that YF 17D vaccine manufacturing capabilities be modernized and that, as part of this program, research be conducted to evaluate cell culture substrates for virus production. In this regard, it was recognized that new methods were needed to characterize both the present 17D vaccines and any new vaccine candidates produced in cell culture. A recognized potential problem is the disparity between the origins and passage pedigrees of vaccines produced in different countries. Although all of the world's vaccines have a common ancestry (original 17D virus), two currently used vaccines (made in Brazil and Dakar) were derived from the 17DD substrain, whereas in other countries, vaccines were derived from the 17D-204 substrain. Regardless of substrain origin, all vaccines in use have had variable histories of passage in embryonated eggs, resulting in total viral subcultures ranging from 233 to 287, and further manipulations have been done to achieve freedom from avian leukosis virus (ALV).

A project was initiated, with the assistance of PAHO/WHO and each of the 12 vaccine producers, to characterize present 17D vaccine lots by RNA oligonucleotide fingerprinting. Vaccine, secondary seed, and (when available) primary seed were obtained through the kindness of each institute.

A working seed of vaccine was prepared in fluid cultures of SW-13 cells. Each virus seed was inoculated at MOI approx. 1.0 into ten 150 cm^2 flasks of SW-13 cells, fluid medium collected 72 hours later, and the virus purified by PEG precipitation and velocity and isopycnic density gradient centrifugation. The viral RNA was isolated, and digested with T_1 RNase. The oligonucleotide fragments were labeled in vitro at the 5' end by use of a polynucleotide kinase - ^{32}P - gamma - ATP reaction mixture, as described by Pedersen and Haseltine (J. Virol. 33:349-365, 1980). Fingerprints were prepared by two-dimensional gel electrophoreses, as previously described by Trent, et al. (J. Gen. Virol. 43:365-381, 1979).

One lot of 17D vaccine designated 2654LE and produced by Connaught Laboratories, Inc., Swiftwater, PA, USA was used to confirm reproducibility of results by repeated analyses, and was then used as a reference strain for comparison with other strains. A total of 52 of the larger oligonucleotides were compared in these analyses.

To date, 10 additional vaccine lots have been studied (Table 1). A very high degree of homology was demonstrated. Three vaccines (South African Lot 10802, Brazilian Lot 997, and Colombian Lot 292) had minor differences from the reference strain. The South African vaccine lacked oligonucleotide #5 of the reference strain and possessed 2 new oligonucleotides, one of which

appeared to be a leftward shift of oligo #5, due to a change in charge (e.g. a single base shift). The Brazilian vaccine was missing oligo #16 and possessed a new oligonucleotide, which, as above, appeared to be a shift (due to a change in charge) of oligo #16. The genome differences did not correlate with substrain origin, since the South African and Colombian vaccines originated from substrain 17D-204 and the Brazilian vaccine originated from substrain 17DD.

The secondary seed (Lot S101) used to prepare the Braizilian Lot 997 vaccine was examined and found to be identifical with it (missing oligonucleotides #13 and 16 of the reference strain). Therefore, the genome differences in the Brazilian vaccine occurred before the level of the secondary seed and were stable through egg passage to prepare Lot 997 vaccine. The supply of primary seed used to prepare S101 has been exhausted and is not available for study.

The results so far indicate a high degree of stability of 17D despite the different pedigrees, at least at the level of sensitivity of the fingerprinting technique (examination of approximately 10% of the genome).

Further studies are in progress on the oligonucleotide patterns of other vaccines, to determine whether the changes in the South African vaccine occurred at or before the levels of the primary or secondary seed lots, and to define whether biological differences in neurovirulence between certain YF strains have covariant oligonucleotide map markers.

(T. P. Monath and R. Kinney)

Table 1. Yellow fever 17D vaccines studied by RNA oligonucleotide fingrprinting.

Producer	Lot Designation	ALV		Substrain Origin	Subculture Level	No. (%) RNA oligos shared with reference strain Connaught Lot 2654 LE	Designation of the 2654 LE oligonucleotides missing	Number of additional oligonucleotides not found in standard
		Contam.	Free					
Connaught (USA)	2834	+		17D-204	234	52/52 (100%	-	-
Connaught (USA)	2090		+	17D-204	237	52/52 (100%)	-	-
Commonwealth Serum Lab (Australia)	905.13-002-1	+		17D-204	235	52/52 (100%)	-	-
Wellcome (UK)	YF/1/188		+	17D-204	235	52/52 (100%)	-	-
Nat'l Inst. Virol. (So. Africa)	10802	+		17D-204	234	51/52 (98%)	#5	2
Inst. Pasteur (Dakar, Senegal)	672		+	17D-204	235	52/52 (96%)	-	-
Inst. Nacional de Salud, Bogota (Colombia)	292	+		17D-204	234	5 /52 (100%)	-	3
Inst. Pasteur (Dakar, Senegal)	659	+		17DD	287	52/52 (100%)	-	-
Oswaldo Cruz (Brazil)	997	+		17DD	286	50/52 (96%)	#13,#16	1

Field Studies Conducted during an Eastern Equine Encephalomyelitis Epizootic in Southwestern Michigan during August and September, 1980

During late summer an outbreak of equine encephalitis became apparent in southwestern Michigan. On August 19, 1980 the Michigan Department of Agriculture confirmed reports of 3 probable Eastern equine encephalomyelitis cases in horses from the Vicksburg area, Kalamazoo County. The probable diagnoses were made on the basis of characteristic clinical presentation and histopathology of brain tissue. Several additional suspect cases were discovered at which time the Michigan Department of Agriculture sent letters to veterinarians in the state requesting information and specimens for diagnosis on suspect horse cases. Upon request for assistance by the Michigan Department of Public Health, staff from the Vector-borne Diseases Division, Viral Diseases Division, and the Bureau of Tropical Diseases, CDC joined Department of Public Health personnel in field investigations.

Ultimately, a total of 94 probable and confirmed cases of EEE in horses were reported from a 9-county area in southwestern Michigan. At least 1 human case was confirmed in a 13-year-old male who still remains hospitalized and comatose, dependent on life-support systems. The focus of activity was in southern Kalamazoo and northern St. Joseph counties where field studies were conducted to determine the extent of virus activity and to make recommendations for control.

Mosquito collections were made at 63 sites throughout the affected area between August 25 and September 9. Collections were principally made using CDC light traps with CO_2; supplemental collections using bird-baited cone traps and human and horse landing and biting collections were also made.

Bird and small mammals were sampled at multiple sites in the area. Domestic birds, primarily chickens and pheasants, were also bled.

Wild birds were captured using mist nets, and small mammals were live trapped. Where possible, pasture or stable mates of clinically ill horses were bled.

Mosquitoes and vertebrate sera were tested for virus by inoculation of primary embryonic duck embryo (DE) and Vero cell cultures. Serological testing of vertebrates was done by serum dilution plaque-reduction neutralization tests (SDNT) in Vero cell culture.

Approximately 103,000 mosquitoes representing 33 species were tested for virus. The predominant species were Aedes vexans and Coquillettidia perturbans. Those species from which 1 or more virus strains were recovered are summarized in Table 1. Although the numbers collected were not large, the greatest number of EEE virus isolations were from Culiseta melanura, the classical enzootic vector of this virus. A primary objective of this study was to determine the probable vector of EEE virus to equines and humans. Several strains of EEE virus were recovered (not all are definitively identified) from Coq. perturbans. The minimal infection rate in this species was much lower than for Cs. melanura; however, the exceedingly large populations of this mosquito coupled with EEE virus infection and its feeding pattern makes it highly probable that this species served as the principal vector to equines. This conclusion is supported by collections from bird-baited cone traps, in which Coq. perturbans was taken in relatively large number (Table 2) and by the landing and biting collections from horses (Table 3). The other predominant mosquito feeding on horses as well as humans (Table 4) was Aedes vexans. This species was collected far less frequently in the bird-baited traps, suggesting a decreased opportunity for EEE virus infection as compared with Coq. perturbans.

Four horses bled during field studies were acutely ill with EEE symptoms, and all 4 had EEE SDNT antibody. An additional 16 horses had EEE antibody for an overall prevalence rate of 29.9% (Table 5). It was not possible to separate naturally acquired infections from antibody induced by vaccination. None of 24 unimmunized draft horses from an area a few miles southeast of the epizootic region in St. Joseph County had EEE antibody, although Cache Valley virus was isolated from 1 of the horses (Table 5).

No virus or EEE antibody was demonstrated in the 28 small mammals tested. Most were small rodents, and all were captured at sites with evidence of relatively intense EEE virus transmission.

Six strains of EEE, 5 of Highlands J (WEE complex) and 1 of Flanders viruses were isolated from blood of 401 wild birds of 42 species sampled (Table 5). EEE SDNT antibody prevalence in wild birds was 29.9%. Six EEE virus strains were recovered from ring-necked pheasants, including 3 isolations from brain tissue of dead birds. These isolations were from 2 small flocks (about 50 and 200 birds) in Kalamazoo County raised in backyard pens. Both flocks had sick and dead birds, and cannibalism occurred despite the universal use of blinders on birds. About 12% of 50 pheasants tested from these 2 flocks and 11% of 99 pheasants tested from a large flock in St. Joseph County had EEE antibody. Eight chickens (11.8%), but no other domestic fowl, had EEE antibody and no virus was isolated from 68 domestic bird bloods tested.

As a result of epidemiologic studies on the horse cases and the results from field studies on mosquitoes, an area of approximately 250,000 acres of intense epizootic transmission was delineated. Aerial spraying with malathion was done during a 3-day interval in September which resulted in an overall reduction of mosquito populations of 97%.

TABLE 1

Arbovirus Isolations from Mosquitoes Collected during Investigation of an Eastern Equine Encephalomyelitis Outbreak in Southwestern Michigan, 1980

Mosquito Species	No. Mosq.	No. Pools	EEE	MIR	Other	Unid.
Aedes canadensis	2,918	84	1	0.3		1
Aedes cinereus	1,114	79				1
Aedes stiticus	1,021	77				1
Aedes triseriatus-hendersoni	389	57	1	2.6		1
Aedes trivittatus	7,828	153			1 LAC[a]	
Aedes vexans	28,755	337	1	0.03	1 BUN[a]	1
Aedes spp.	18,397	261	1		1 LAC	2
Anopheles spp.	1,101	55	1	0.9		
Coquillettidia perturbans	18,286	258	2	0.1	3 BUN	4
Culex (Culex) spp.	1,372	87				2
Culiseta inornata	7	4				1
Culiseta melanura	1,397	81	9	6.4	2 HJ	4
Others (includes 21 species)	20,242	740				

[a]--LAC=LaCrosse (California) encephalitis and BUN=Bunyamwera group

TABLE 2

Mosquitoes Collected in Bird-Baited, Cone-Type Traps from Kalamazoo and St. Joseph Counties, Michigan, 1980

Mosquito Species	No. Tested	
	Mosq.	Pools
Aedes canadensis	37	7
Aedes cinereus	2	2
Aedes sticticus	26	5
Aedes trivittatus	152	8
Aedes vexans	4	3
Aedes spp.	75	9
Coquillettidia perturbans	132	10
Culex pipiens	635	13
Culex restuans	1	1
Culex salinarius	1	1
Culex tarsalis	1	1
Culex spp.	3	1
Psorophora ferox	13	2
TOTALS	1,082	63

TABLE 3

Landing and Biting Collections from Horses in St. Joseph County, Michigan, 1980

Mosquito Species	No. Tested Mosq.	No. Tested Pools	
Aedes canadensis	2	2	
Aedes dorsalis	1	1	
Aedes sticticus	1	1	
Aedes trivittatus	33	9	
Aedes vexans	1,160	20	
Aedes spp.	267	10	
Anopheles punctipennis	18	8	
Anopheles quadrimaculatus	1	1	
Anopheles walkeri	15	7	
Anopheles spp.	12	5	
Coquillettidia perturbans	745	17	1 EEE Isolation
Culex pipiens	3	2	
Culex (Culex) spp.	3	2	
Psorophora ciliata	2	1	
TOTALS	2,263	86	
Tabanidae	76	3	

TABLE 4

Landing and Biting Collections from Humans in St. Joseph County, Michigan, 1980

Mosquito Species	No. Tested	
	Mosq.	Pools
Aedes cinereus	8	2
Aedes sticticus	7	3
Aedes triseriatus-hendersoni	5	3
Aedes trivittatus	20	4
Aedes vexans	220	6
Aedes spp.	19	4
Anopheles punctipennis	2	1
Anopheles quadrimaculatus	1	1
Anopheles walkeri	1	1
Coquillettidia perturbans	42	4
TOTALS	325	29

TABLE 5

Field Studies Conducted during an Epizootic of Eastern Equine Encephalomyelitis in Southwestern Michigan, 1980: Virological and Serological Results from Vertebrates Collected in St. Josephs and Kalamazoo Counties

Type of Animal	No. Tested	No. Virus Isolations[1]				No. EEE[2]	
		EEE	HJ	FLA	CV	Aby. Pos.	% Pos.
Horses	67	0	0	0	1	20	29.9
Small mammals	28	0	0	0	0	0	0.0
Wild birds	401	6	5	1	0	120	29.9
Captive birds:							
Ring-necked pheasant	152	6	0	0	0	17	11.2
Domestic (chickens, etc.)	68	0	0	0	0	8	11.8
TOTALS	716	12	5	1	1	165	23.0

[1]Viruses: EEE = Eastern equine encephalitis, HJ = Highlands J (western encephalitis complex), FLA = Flanders, CV = Cache Valley.

[2]Antibody to EEE virus determined by serum dilution plaque reduction neutralization test in Vero cell culture.

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Diagnosis of Fatal Eastern Equine Encephalitis by Immunofluorescent Staining of Brain Tissue

The diagnosis of EEE in equines is generally made by tests for antibody or by isolation of the virus from blood or brain tissue. The success of virus isolation from blood is limited by the brief duration and low titer of viremia. Reports of isolations of EEE virus from brain have been infrequent, and isolation from cases in which the encephalitic illness has lasted 3 days or longer before death may be difficult.

During an EEE epizootic in Michigan in 1980, we received specimens of brain from five horses with encephalitis and had the opportunity to investigate the usefulness of immunofluorescent staining of brain tissue for rapid diagnosis. We correlated the results of fluorescent antibody tests on impression smears and cryostat sections of brain with quantitative virus titrations in suckling mice.

Twenty percent (w/v) suspensions of brain tissue in medium 199 containing 5% heated fetal bovine serum were prepared by using a TenBroeck grinder. Suspensions were centrifuged and serial tenfold dilutions of the supernate inoculated into groups of suckling mice by the intracerebral (i.c.) route.

A portion of brain tissue equivalent to that used for viral assay was used to prepare impression smears and frozen sections. Indirect FA tests were performed by using MAF against EEE virus and western encephalitis as first antibody and fluorescein-conjugated goat anti-mouse IgG as second antibody. Trypan blue (1:2000) was added to the conjugate as a counterstain. Optimal concentrations of both antibodies were determined by prior tests at varying dilutions against EEE virus-infected Vero cells grown in 8-chamber slides. Controls included infected and uninfected suckling mouse brain sections and Vero cells as well as test sections incubated with normal MAF.

FA test results were scored semi-quantitatively: 0 = no specific fluorescence; 1+ = less than 1% of cells with specific fluorescence; 2+ = 2-25%; 3+ = 26-75%; 4+ = > 75%.

Table 1 presents information available on the equine cases included in the study. Horses 1-3 were confirmed as EEE cases by virus isolation and FA tests. Case 5 was confirmed by demonstration of a rise in neutralizing antibody titer from <10 to 160 in sera obtained 1 and 3 days after onset of encephalitic illness. A presumptive diagnosis was established for horse No. 4, which had a neutralizing antibody titer of 40 two days after onset.

Table 2 compares the results of viral infectivity assays and FA examinations. EEE virus was recovered from tissues from three horses, at titers ranging from $10^{5.7}$ to $10^{10.0}$ suckling mouse i.c. LD_{50}/gram. In one animal, viral titers were compared in seven different areas of the brain. Highest titers were found in the thalamus and pons. FA

examination revealed specific staining in cryostat sections from all tissues which yielded virus isolates, but not in virus-negative brains. The most intense staining and highest proportion of positive cells were present in tissues with highest viral titers. Impression smears were consistently negative. In sections with low titer and few fluorescing cells, careful examination of many microscopic fields was required. Cross-reactive fluorescent staining was detected with the use of WEE MAF in horses No. 1 and 3; however, staining was considerably weaker than for EEE MAF.

Fluorescent antibody tests for EEE viral antigen in brain tissue have not previously been reported. The technique allowed a rapid and specific diagnosis in all three animals from which EEE virus was subsequently isolated. In some brain tissues with virus titers of $10^{5.7}$ - $10^{6.9}$ LD_{50}/ml, antigen was detected in less than 1% of the cells in frozen sections. Thus, we suspect that clinical specimens with lower infectivity titers would be unsuitable for FA diagnosis. Nevertheless, the immunofluorescent techniques appears to have its place as a rapid screening test in the diagnosis of equine encephalitis and has the advantage of being applicable in laboratories without facilities for virus isolations.

Titers were highest in the thalamus, emphasizing the need to obtain samples of the deep, central areas of the brain rather than a random sample of cortical tissue. Virus isolation and demonstration of antigen by FA tests were successful in three horses from which brain samples were obtained within approximately 24 hours of onset, but not from two horses tested at 2 and 3 days.

Impression smears of brain were negative, possibly because of blocking by antibody, and cryostat sections should, therefore, be used in diagnostic studies.

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TABLE 1. Clinical cases of equine encepahlitis from which brain tissues were submitted for testing, Southwestern Michigan, 1980.

Horse No.	Age (Years)	Location	DATE Onset	DATE Death (D) or Sacrifice (S)	Specimen Received
1	1	Three Rivers	Sept. 5	Sept. 5 (D)	Whole brain
2	4	Portage	Sept. 3	Sept. 4 (S)	Unspec.[1] cortex, serum
3	1	Mendon	Sept. 30 or 31	Sept. 1 (D)	Unspec. cortex
4	9	Centreville	Sept. 3	Sept. 5 (S)	1/2 brain, serum
5	4	Three Rivers	Aug. 24	Aug. 27 (S)	Unspec. cortex, paired sera

[1]Unspecified cerebral cortex

TABLE 2. Results of viral infectivity assays and fluorescent antibody tests on brains of horses dying in Michigan, 1980.

Horse	Specimen	Virus Titer[1]	Indirect Fluorescent Antibody Test[3] Cryostat Section	Impression Smear
1	thalamus	10.0	4+	0
	pons	8.4	3+	0
	parietal cortex	7.6	2+	0
	hippocampus	6.9	2+	0
	corona radiata	6.9	1+	0
	frontal cortex	6.6	2+	0
	cerebellar cortex	5.7	1+	0
2	unspec. brain[2]	7.6	2+	0
3	unspec. brain	6.6	2+	0
4	thalamus	2.7	0	NT[4]
5	unspec. brain	2.7	0	NT

[1]$\log_{10}$ intracerebral suckling mouse LD_{50}/gram.

[2]unspecified cerebral cortical tissue.

[3]0 = no specific fluorescence; 1+ = less than 1% of cells with specific fluorescence; 2+ = 2-25%; 3+ = 26-75%; 4+ = 75%.

[4]Not tested.

REPORT FROM THE EPIDEMIOLOGY BRANCH
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We continue to study the distribution and prevalence of ticks and tickborne disease agents of actual or potential public health significance in the NW United States. Recent recognition in Pacific coastal states of human inflammatory disorders of unknown cause, but presumably originating with tickbite, emphasizes the need for a thorough understanding of man-biting ticks of the endemic areas and the microbial pathogens associated with them. Previously we reported on the distribution in Oregon of medically important tick species, the identity and characteristics of tickborne viruses of migratory seabirds, and the recognition of a new rickettsia of the spotted fever group in ticks of western Oregon. Additional findings include a new group of spiroplasmas in Ixodes pacificus and the distribution of spotted fever-group rickettsias. Here we report a serologically unique new virus of Dermacentor occidentalis in SW Oregon (Jackson, Josephine and Douglas Counties).

The new virus, which we call Cascade, was discovered through use of poikilothermic (amphibian and arthropod) cell lines in pathogen-isolation attempts. Cascade virus was isolated in Xenopus laevis cells (XTC-2) incubated at 34°C, from 30 of 186 pools of unfed D. occidentalis adults, and was reisolated from original tick triturates both in XTC-2 cells and in a newly developed line of D. variabilis cells (RML-15) incubated at 27°C. No virus isolations were made when these same pools were inoculated into suckling mice or Vero cells. The virus was not found in numerous Ixodes pacificus ticks collected in the same general areas and at the same times. Cascade virus is unrelated to any of 169 viruses of at least 35 serogroups with which it was serologically compared; it contains RNA and essential lipids and is sensitive to exposure to heat and acid. Diameter of the virion lies between 100 and 220 nm. Although it readily causes CPE and plaques in XTC-2 cells, its growth in other cells (RML-15, PS, BHK-21) is demonstrable only upon secondary assay. The virus grows poorly or not at all in Vero, L and Aedes albopictus cells. It infects suckling Microtus but causes only sporadic illness and death. Suckling white mice and hamsters, and wet chicks inoculated ic are refractory to infection and the virus fails to grow in embryonated hens' eggs. Growth of the virus in tick, but not mosquito cells, as well as its repeated isolation from D. occidentalis (but not sympatric species) implies that it is tickborne and specific for that species. Its occurrence in unfed adults at a relatively high frequency (2.9%) indicates transstadial transfer. Thus, search for a vertebrate reservoir should commence with hosts for the immature stages, of which ground squirrels, deer mice and woodrats predominate. Although no evidence of human infection with the new agent was obtained from serological testing of a small sample of sera selected from Californians with illness following tick exposure, recent reports in southwestern Oregon and in California of cases of erythema chronicum migrans and Lyme disease suggests further study.

(Conrad E. Yunker)

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

Snowshoe hare (SSH) virus (California encephalitis group) was isolated from 7 of 162 pools comprising 7472 unengorged adult female mosquitoes of 3 species which were collected throughout the Yukon Territory between 9 June and 30 July 1980. All isolates were achieved from Aedes communis mosquitoes collected at the following locations.

Location		Infection rate	$\log_{10}$ mouse LD_{50} per mosquito pool
Marsh Lake 61°N 134°W	9 June	1:483	4.0+
	26 June	1:100	4.0+
	3 July	1:196	4.0+
	14 July	1:78	4.0+
Fish Lake Rd 61°N 135°W	18 June	1:183	3.5
Dempster Highway			
Km 197 66°N 138°W	16 July	1:382	3.5
Km 236 66°N 137°W	29 July	1:182	3.0

The minimum infectivity dose for Aedes aegypti mosquitoes after incubation at 23°C and 32°C following intrathoracic injection with the 1978 Yukon topotype of SSH virus 78-Y-133 was less than 0.1 plaque forming units (PFU) and with the 1980 Yukon topotype 80-Y-1 was 0.01 PFU. Stock viruses, in their second and first mouse brain passage respectively, were titrated in monolayer cultures of continuous baby hamster kidney cells. The minimum infectivity dose of the 1978 Yukon topotype of Northway virus, 78-Y-284, for A. aegypti by intrathoracic injection, was 0.01 PFU.

(Donald M. McLean).