ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 28 March 1975

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Roy W. Chamberlain, Sc.D., Editor
Mary Ellen Gist, Secretary

COMMENTS FROM THE EDITOR

Inclusion of the 1973 Annual Catalogue Report in Info-Exchange No. 27, rather than mailing it separately, was so well received that we have decided to make this a standard practice. Dr. Berge's 1974 Annual Report has been completed and is included in the present issue.

It is with considerable regret that we announce the retirement on June 30, 1975, of Dr. T. O. Berge as Chairman of the Subcommittee on Information Exchange. As I am sure you all appreciate, he has done a magnificent job over the past seven years and will be sorely missed. However, he has consented to serve as consultant on special catalogue matters. Dr. Berge's retirement announcement is on page 3.

The majority of you who contributed to this issue of the Information Exchange will recognize your reports as being in much the same form as you submitted them. Your cooperation in observing the standard format of the Information Exchange has largely made this possible. I hope you will continue to extend efforts in this regard as restrictions on clerical services make it increasingly difficult to maintain a prompt distribution schedule for the Information Exchange unless a large proportion of the reports can be used essentially as submitted. However, let me hasten to assure those who have their own secretarial difficulties that their reports are still welcome, and also that I am happy to do any editing that may be needed or helpful.

The deadline for submission of reports for Info-Exchange Issue No. 29 is September 1, 1975. Please mark your calendar.

The address remains the same:

Roy W. Chamberlain, Editor
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ERRATUM

An error has been found in the SEAS listing for Phnom-Penh bat virus, Info-Exchange No. 26, page 24. The entry in the far-right column should read, "ex-bat, Cambodia," rather than "ex-bat, W. Africa."

INTERNATIONAL SYMPOSIUM ON ARBOVIRUSES
HELSINKI, FINLAND

The Medical Research Council, The Academy of Finland, is organizing a four-day symposium on arboviruses to be held in Helsinki, June 3-6, 1975. The scientific program will cover both the ecology and the molecular biology of arboviruses. Under the first heading will come the problems associated with mosquito- and tick-borne viruses in arctic regions of the world. The second will be concerned with the structure and replication of alpha- and bunyaviruses. There is limited time for open communications. Discussions will be arranged on "Arctic arboviruses--the over-wintering problem" and "The translation of arbovirus specified proteins in vitro and in vivo." There will be about 15 invited lecturers from different countries.

Those persons who are interested in participating are requested to contact Prof. N. Oker-Blom, M.D., at the earliest convenience. Address: Department of Virology, University of Helsinki, Haartmaninkatu 3, SF-00290, Helsinki 29, Finland.

OPEN MEETING OF THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES
MADRID, SPAIN

An open meeting of the American Committee on Arthropod-borne Viruses will be held at the International Congress for Virology in Madrid (10-17 September 1975). The meeting is tentatively scheduled for 2000 on 9 September. We hope to have progress reports from each of the standing subcommittees directed toward the international arbovirus community. There will be opportunity to meet with the international advisors to the ACAV to discuss future directions and undertakings, and there will be time for communication concerning present arbovirus disease problems. All are welcome and urged to attend.

Frederick A. Murphy, D.V.M., Ph.D.
Chairman, Executive Committee, ACAV

REPORT FROM CHAIRMAN, SUBCOMMITTEE ON ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Dr. Trygve O. Berge has announced his retirement as chairman of the Subcommittee and as editor of the Arbovirus working Catalogue effective 30 June 1975. During the time of his tenure the total number of viruses registered in the Catalogue has increased from 205 to more than 350 viruses, reflecting the excellent cooperation from arbovirologists in every part of the world. For this, his heartfelt thanks are expressed to all contributors who have provided the materials which have made the registry a useful tool for laboratory workers and educators interested in this branch of virology.

The Infoexchange Subcommittee remains in good hands, and continued financial support appears to be assured for the immediate future. For the past 14 years, operation of the working Catalogue has been made possible by subvention through contracts and grants by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. Beginning on 1 July, 1975, the Center for Disease Control, through the Vector-Borne Diseases Division, Bureau of Laboratories, will provide financial support for these activities. Dr. Roy W. Chamberlain has been appointed Chairman of the Subcommittee on Arthropod-borne Virus Information Exchange as of that date, and will continue to act as editor of the Infoexchange Newsletter.

Dr. Nick Karabatsos will assume the duties of editor of the working Catalogue. After 1 July, all communications regarding the registration of new viruses should be directed to Dr. Karabatsos at the present address:

USPHS, CDC
Vector-Borne Diseases Division
P.O. Box 2087
Fort Collins, Colorado 80522, U.S.A.

Inquiries in regard to participation in the Arbovirus Information Exchange program should be addressed to Dr. Chamberlain:

Roy W. Chamberlain, Sc.D.
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All information to be included in the Newsletter should be sent to Dr. Chamberlain as before.

As stated elsewhere in this issue, the second edition of the published Catalogue (the International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates) is scheduled for publication between March and June, 1975. Free copies will be routinely distributed to all members of the Arbovirus Information Exchange program.

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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1974 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 Research Resources Branch, Collaborative Research, of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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

Catalogues Distributed: Eight complete Catalogue sets and accumulated abstracts were issued to new participants in 1974, and 2 participants were dropped. At the end of the year, 164 mailings of Catalogue material were being made, including 62 within the continental U.S.A. and 102 to foreign addresses. Distribution by continent was: Africa 14 , Asia 19 , Australasia (including Hawaii) 8 , Europe 33 , North America 72 , and South America 18 .

Abstracts and Current Information: 625 abstracts or references were collected, coded by subject matter, collated, and distributed to participants during 1974. Of these, 485 were obtained from Biological Abstracts, 123 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 17 from current journals, personal communications, or other sources. A total of 8,872 such references or units of information have been issued since the start of the program.

Recommended Antigenic Grouping: Two new antigenic groups, Malakal and Sakhalin, were added during the year. The Malakal group consists of two newly registered viruses, the Malakal virus isolated in 1964 from Sudan, and the Puchong virus recovered in 1965 in Malaysia. The Sakhalin group consists of the previously registered Sakhalin virus and the recently registered Avalon virus isolated in 1972 from ticks in Canada, and the 1973 isolate, Clo Mor, from ticks collected in Scotland.

Registration of "New" Viruses: 1974 represented a high point in the number of new virus registrations submitted for inclusion in the working Catalogue, with 45 viruses initially registered during this period, as listed below:

Name of Virus	Year Isolated	Country	Initial Source	Antigenic Group
Aguacate	1969	Panama	Phlebotomine flies	PHL
Avalon	1972	Canada	Ixodid ticks	SAK
Bangoran	1969	Cent.Afr.Rep.	Mosquitoes	
Barur	1962	India	Rodent	
Batken	1970	U.S.S.R.	Ixodid ticks	
Batu Cave	1971	Malaysia	Bat	B
Bimbo	1970	Cent.Afr.Rep.	Bird	
Birao	1969	Cent.Afr.Rep.	Mosquitoes	BUN
Cacao	1970	Panama	Phlebotomine flies	PHL
Caimito	1971	Panama	Phlebotomine flies	PHL
Cape Wrath	1973	Scotland	Ixodid ticks	KEM
Carey Island	1970	Malaysia	Bat	B
Chilibre	1969	Panama	Phlebotomine flies	PHL
Clo Mor	1973	Scotland	Ixodid ticks	SAK
Frijoles	1969	Panama	Phlebotomine flies	PHL
Garba	1970	Cent.Afr.Rep.	Bird	MTY
Gomoka	1970	Cent.Afr.Rep.	Mosquitoes	
Gordil	1971	Cent.Afr.Rep.	Rodent	PHL
Huacho	1967	Peru	Argasid ticks	HUG
Jugra	1969	Malaysia	Mosquitoes	B

Name of Virus	Year Isolated	Country	Initial Source	Antigenic Group
Kadam	1967	Uganda	Ixodid ticks	B
Kaeng Khoi	1969	Thailand	Bat	SBU
Karshi	1972	U.S.S.R.	Argasid ticks	B
Kolongo	1970	Cent.Afr.Rep.	Bird	
Landjia	1970	Cent.Afr.Rep.	Bird	
Malakal	1964	Sudan	Mosquitoes	MAL
Marburg	1967	W.Germany	Man	
Mono Lake	1966	U.S.A.	Argasid ticks	KEM
Nique	1972	Panama	Phlebotomine flies	PHL
Nola	1970	Cent.Afr.Rep.	Mosquitoes	SIM
Ouango	1970	Cent.Afr.Rep.	Bird	
Pata	1968	Cent.Afr.Rep.	Mosquitoes	EUB
Phnom-Penh bat	1969	Cambodia	Bats	B
Pretoria	1973	South Africa	Argasid ticks	DGK
Puchong	1965	Malaysia	Mosquitoes	MAL
Punta Salinas	1967	Peru	Argasid ticks	HUG
Saint-Floris	1971	Cent.Afr.Rep.	Rodent	
Sandjimba	1970	Cent.Afr.Rep.	Bird	
Seletar	1961	Singapore	Ixodid ticks	KEM
Tanjong Rabok	1968	Malaysia	Sent. monkey	
Tettnang	1970	W. Germany	Ixodid ticks	
Yata	1969	Cent.Afr.Rep.	Mosquitoes	
Zinga	1969	Cent.Afr.Rep.	Mosquitoes	
Zingilamo	1970	Cent.Afr.Rep.	Bird	BTK
Zirqa	1969	Persian Gulf	Argasid ticks	HUG

Nineteen viruses were isolated in Africa (Central African Republic, 16; Republic of South Africa, 1; Sudan, 1; and Uganda, 1). Twelve viruses were discovered in Asia (Cambodia, 1; India, 1; Malaysia, 5; the Persian Gulf, 1; Singapore, 1; Thailand, 1; and the Asiatic U.S.S.R., 2). Four viruses were first recovered from Europe (Scotland, 2; West Germany, 2). Eight viruses were reported from North America (Canada, 1; Panama, 6; and the U.S.A., 1). Two viruses from South America (both from Peru) were also registered. All newly registered viruses were isolated between 1961 and 1973.

Twenty-nine viruses were first isolated from arthropods (ticks, 13; mosquitoes, 10; and phlebotomine flies, 6). The remaining 16 were recovered from mammals (birds, 7; bats, 4; rodents, 3; man, 1; and a sentinel monkey, 1).

Catalogue Publication: Arrangements have been completed for publication of the second edition of the Arbovirus Catalogue through the Center for Disease Control under the sponsorship of the Research Resources Branch of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and the Center for Disease Control. The Catalogue has been re-titled the "International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates". Publication is scheduled for March, 1975 and should be completed by June, 1975.

The published edition will include the 359 viruses registered in the working Catalogue through December, 1974. Copies will be distributed to all participants in the Arbovirus Information Exchange program without charge.

Revision of Virus Registration Forms: Virus registration forms employed in the working Catalogue have been revised by an ad hoc subcommittee of the ACAV under the chairmanship of T.H.G. Aitken. The new form is scheduled for printing and distribution in the near future, and it is expected that all registrations submitted after June, 1975 will be completed on the revised registration sheets.

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.

These tables summarize only a small portion of the information which can be extracted from the registration cards. The reader who is interested in the many types of analysis which can be made from information contained in the virus registrations, such as techniques and materials employed for isolation, physico-chemical characteristics of viruses, susceptible experimental vertebrate hosts, etc., should consult the first edition of the Catalogue.

Table 1. Alphabetical listing of registered viruses. Table 1 lists in alphabetical order the 359 viruses registered in the Catalogue as of December, 1974. After each virus name is given a recommended abbreviation which is frequently employed for conservation of space when frequent reference is made to the name, or when many viruses are being listed for special purposes. Antigenic groups to which viruses have been assigned are also shown in this table in condensed form (See Table 2). Where no antigenic group is indicated, the individual virus is unique in the sense that no serological relationship has yet been demonstrated linking it to any other known virus. In later tables, related viruses are compiled by antigenic groups as well as in other ways designed to suggest biological or morphological interrelationships.

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.

Table 2 lists the antigenic groups established thus far for viruses registered in the Catalogue and the number of registered viruses assigned to each group. Largely through the work of the Yale Arbovirus Research Unit (YARU) and the associated WHO Reference Centre for Arboviruses, 47 distinct antigenic groups have been designated and a supergroup conceived. About 21 percent of registered viruses remain in an ungrouped category, i.e., have not been found to be serologically related to any previously described viruses or to each other.

Where only one virus is shown in a group, the registered virus has been found to be related to one or more other viruses which have not been registered. African horsesickness, bluetongue and probably epizootic hemorrhagic disease of deer have been designated as groups because there are a number of antigenically distinct viruses belonging to each, although only one of each is registered. The degree of relationship between individual members within a serogroup may be very close or relatively distant. A Subcommittee of the ACAV, the Subcommittee on Interrelationships among Catalogued Arboviruses (SIRACA), under the chairmanship of Jordi Casals, has been delegated general responsibility for determining whether antigenically related viruses are independent members of a serological group, or if they should be considered as strains of a single virus.

In some instances, a few of the members within one antigenic group of viruses have been found to show small but reproducible cross complement fixation or hemagglutination-inhibition reactions with certain members of other groups. The Bunyamwera Supergroup was created to reflect these intergroup relationships, and contains 10 separate antigenic groups of registered viruses, plus an additional category of unrelated viruses which fall within the supergroup but which cannot be assigned to any of the individual groups. Taken collectively, the 87 viruses placed in the Bunyamwera Supergroup comprise almost one fourth of all registered viruses.

The largest single antigenic group is Group B (57 viruses) followed by Group A and the phlebotomus fever group (20 viruses each). Five other groups contain more than 10 members each (Bunyamwera, C, California, Simbu, and Kemerovo). All other groups consist of less than 10 members each.

Figure 1. Year of initial isolation. Figure 1 depicts the number of registered viruses isolated by year, with the cumulative proportion of isolations shown from 1902 through 1973. Only 35 of the 359 catalogued viruses had been isolated in the first half of this century with 90 percent being initially discovered after 1950. The great proportionate increase in the number of viruses recovered in nature can be seen to begin in 1954 with about 88 percent of all registered viruses first recognized in the past two decades.

As has been observed previously, the time of initial isolation probably bears little relation to the time of first existence of most of these viruses, but rather to the period of establishment and staffing of special arbovirus laboratories, and to the time when extensive general surveys were begun to search for the presence of previously unrecognized viruses in biting arthropods or naturally infected vertebrates.

Table 3. Initial isolations by decade and country of origin. While Figure 1 shows the total number of viruses isolated in each given year beginning with 1902, 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.

Viruses discovered initially in the first three decades of this century, and many of those first recognized in the 1940s, were those associated with diseases of domestic animals or of man. They were isolated as a result of specific searches for etiologic agents of human or veterinary diseases, many occurring in epidemic or epizootic form. Subsequent to 1950, a number of viruses continued to be found because they were capable of inducing disease. A large number, however, were isolated from wild-caught insects, from feral animals or birds, usually without signs and symptoms of illness; or from sentinel animals exposed to bites of flying insects, in the course of systematic searches for the existence of known or previously unrecognized viruses in particular geographic areas. Such areas of search were frequently determined by the location of a functioning laboratory or field station and, more importantly, by the presence of highly motivated and capable workers on the scene.

Table 4. Initial isolation of viruses by continent, country, and chronological period. Data presented in Table 3 are further summarized and shown in slightly different fashion in Table 4. From this it can readily be seen that the past decade 1960-1969 was the most productive period from the standpoint of numbers of new viruses discovered; 50 percent of all registered viruses were initially isolated during this time. Since publication of the first edition of the Catalogue, the continent of Africa has forged ahead in the greatest number of viruses initially isolated (96), followed by North America (72), South America (71), Asia (64), Australasia (35), and Europe (21).

The countries which have yielded the largest number of registered viruses are the United States of America (45), Brazil (40), Australia (29), India (22), the Central African Republic (21), Panama (21), Trinidad (18), the Republic of South Africa (18), Uganda (16), Egypt (15), Malaysia (14), the Union of Soviet Socialist Republics (12), and Senegal (10). The clusters of isolations in the various geographic areas appear to reflect the activities of regional laboratories which are or have been definitely oriented toward the study and isolation of arboviruses, and it is these laboratories that have contributed most of the isolations.

Tables 5 through 27 list registered viruses primarily by antigenic groups. An attempt has also been made, where practicable, to further group viruses according to their actual or suspected principal arthropod vector and by taxonomic status. In each table, information is summarized on isolation from arthropod vectors and vertebrate hosts, broad geographic (continental) areas of virus occurrence, production of disease in man in nature or by laboratory infection, evaluation of arbovirus status, and proved or possible taxonomic status based on morphological or physicochemical characterization of representative viruses of some serogroups.

Table 5. Group A arboviruses. Twenty members of the group A viruses are registered, all but 3 of which are considered to be proved or probable arboviruses. Viruses of group A all appear to be associated primarily with mosquito vectors, although some have also been recovered from naturally infected mites, small biting flies or midges, and reduviids. Representative group A arboviruses have been isolated on every continent and from many classes of vertebrates, including man. More than half have been shown to induce human disease, often in epidemic form (as chikungunya, o'nyong-nyong, EEE, VEE, and WEE.

While group A viruses are related serologically to each other, they do not cross react with members of any other serogroup. Within the group, the SIRACA considers that six immunological sub-sets or complexes exist, some of which consist of single viruses, or types, having no known close relatives. Other complexes contain several viruses which are more closely related to each other than to other members of the group. Some of these viruses can be further subdivided on an antigenic basis into subtypes and even varieties, although more extensive serological evaluation may be required for final determinations. Detailed Subcommittee reports on immunological relationships between viruses in this and other groups will be published in the near future.

In the present international taxonomic schema, group A viruses form a single genus Alphavirus in the 'family' Togaviridae as designated by the International Committee on Taxonomy of Viruses (ICTV) (5,6).

Tables 6, 7, and 8. Group B viruses. The family Togaviridae also includes the large and important set of serologically related group B viruses, all provisionally placed by the ICNV in a single genus, Flavivirus. While bound together in a single group by their antigenic cross reactivity and, so far as has been studied, by their physico-chemical characteristics, group B viruses can be subdivided according to their principal vectors.

The largest subgroup, 27 of the 57 registered viruses assigned to group B, appear to be transmitted in nature by mosquito vectors and have seldom been isolated from other blood-sucking arthropods. These are listed in Table 6; with the exception of several inadequately studied members, they are regarded as true arboviruses. The majority of these mosquito-borne viruses are capable of causing disease in man, and, frequently, large outbreaks. Viruses have been recovered from all categories of vertebrates listed, most often from man and birds. Isolations have been reported from all continents, although only one representative (WN) has been found in Europe.

The second important subgroup of group B viruses includes those which are tick-borne in nature (Table 7). These consist of 14 registered viruses; however, 4 of the 14 may be regarded as strains of a single virus, the Western type of group B tick-borne encephalitis virus. The

Absettarov, Hanzalova, Hypr, and Kumlinge viruses are serologically very closely related or indistinguishable by the usual techniques, but are said to be clearly differentiated on the basis of clinical, epidemiological and ecological markers from RSSE and other members of the same complex. Members of the tick-borne group B complex are found predominantly in Europe and Asia, but two viruses have been found in North America and one in Africa; none has been isolated in Australasia or South America. They have not been recovered from mosquitoes or most arthropods other than ticks, nor have isolations been reported from marsupials or sentinel animals. Human infections have been commonly reported, both in nature and as a result of laboratory exposure. As with the mosquito-borne viruses of group B, all members of the tick-borne complex are regarded as arboviruses except two for which insufficient data are available.

The remaining 16 viruses in antigenic group B (Table 8) have never been isolated from wild-caught arthropods or from sentinel animals and their arbovirus status is, therefore, for the most part in doubt. Six viruses have been isolated only from rodents, 8 others only from bats, and one each from man and domestic birds (turkey). None has been recovered in Australasia, Europe, or South America.

Tables 9, 10, 11, 12, 13, and 14. Bunyamwera Supergroup. In these tables are listed viruses placed in 10 serogroups, plus 7 additional unassigned viruses, all of which have been assembled in the large Bunyamwera supergroup as discussed earlier. The different antigenic groups within the supergroup are tabulated alphabetically. In addition to their immunological interrelatedness, almost one third of the viruses contained in the supergroup have been examined in thin section electron microscopy, and found to be indistinguishable from the Bunyamwera virus and from each other morphologically and morphogenetically (8,9). The family name Bunyaviridae has been proposed with all member viruses of the supergroup classified as the bunyaviruses.

Table 9. Bunyamwera group. The Bunyamwera group is comprised of 18 registered viruses, although one of these, Calovo, is regarded by the SIRACA as serologically indistinguishable from Batai, and thus may be considered to be a strain of the latter virus. Also, Maguari, Tensaw, and Tlacotalpan are considered to be varieties of the Cache Valley virus. Data on 15 of the listed BUN group viruses were examined by SIRACA at a meeting in March 1971. (Anhembi, Birao, and Northway were registered later). Within the group as then constituted, 5 complexes could be discerned, the members of which were more closely related serologically to each other than to viruses falling in other complexes within the group. The 5 sets were given as follows:

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

Members of the BUN group, with the exception of Lokern and Main Drain appear to be associated primarily with mosquito vectors; isolations of the other 2 viruses have been recorded principally from culicoides among the arthropods tested.

Several members have been recovered from rodents, several others from lagomorphs, and 2 from domestic animals. Six viruses have been reported to cause sporadic cases of febrile illness in man. Twelve of the registered viruses are rated as arthropod-borne, with data on the remaining 6 inadequate to make an informed judgement.

Representatives of the group have been encountered most frequently in North America (8), South America (6), and Africa (4). Only one virus has been reported from Asia and Europe, with none yet found in Australasia.

Table 10. Bwamba group and group C viruses. The Bwamba group consists of 2 serologically related, mosquito-associated arboviruses reported only from Africa. Bwamba virus has been recovered on a number of occasions from man, but neither member has yet been isolated from lower vertebrates.

Group C viruses, on the other hand, have been found only in the western hemisphere. All 11 members appear to be transmitted by culicine mosquitoes, and all have been classified as arboviruses. Most have been shown to infect rodents or marsupials in nature, and 9 of 11 (82%) have been associated with human febrile illness.

Following examination of the available immunological data in 1968 and 1970, the SIRACA concluded that group C arboviruses fell into 3 complexes, each containing 2 or more viruses:

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

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

Table 11. California and Capim group viruses. The California group consists of 11 registered members. As with the group C viruses, all members of the California group are associated with mosquito vectors; most of them (9 of 11) have been reported only from the western hemisphere. European representatives include the Inkoo and Tahyna viruses, with the latter also occurring in Africa (Lumbo strain). Two members have been recovered from naturally infected rodents and one from bats. Four members have been implicated in human illness, but this may not give a true picture since the establishment of specific viral etiology of disease induced by closely related viruses could not ordinarily be

accomplished without isolation and detailed serological identification of the causative agent in each case. The majority are considered to be proved or probable arboviruses, with adequate data being lacking in 3 instances to permit full evaluation of their status.

Available data on 9 of the 11 registered California group viruses have been examined by SIRACA; the Bocas and Inkoo viruses had not been registered at the time of the meetings of this group in 1969 and 1970. Three complexes were suggested within the California group: California encephalitis, Trivittatus, and Melao, each complex consisting of a single virus or type. Jamestown Canyon, Keystone, La Crosse, San Angelo, and Tahyna were considered to be subtypes of the California encephalitis virus, all of which were distinguishable from each other. Jerry Slough was regarded as being indistinguishable serologically, or nearly so, from the Jamestown Canyon virus.

The second group listed in Table 11, the Capim group viruses, have been reported only from North and South America. Five of the 6 registered viruses were isolated from culicine mosquitoes, 3 from rodents, one also from a marsupial, and 5 from sentinel animals. None has been implicated in human disease.

Table 12. Guama, Koongol, and Patois group viruses. Guama group viruses consist of 6 members limited to the western hemisphere in distribution. Five of the 6 have been isolated from mosquitoes and 1 also from phlebotomine flies. Five have been recovered from rodents, 5 from sentinel animals, 3 from marsupials, 2 from bats, and 2 from human cases of febrile illness.

The Koongol group consists of 2 viruses reported only from Northern Australia, both isolated from mosquitoes. Neither has been recovered from other arthropods or naturally infected vertebrates and are thus of unknown significance.

The Patois group of 4 viruses, found only in North America, have been isolated from mosquitoes and/or sentinel animals, with 3 recovered from naturally infected cotton rats.

Most of the viruses listed in Table 12, except for those where necessary information is lacking, have been evaluated as proved or probable arboviruses.

Table 13. Simbu group viruses. Thirteen of the 16 members of the Simbu group have been isolated either from culicine mosquitoes or from culicoides; of these recoveries from arthropods, 5 viruses have been isolated from mosquitoes alone; 5 only from culicoides; and 3 from both. Of those not yet recovered from naturally infected arthropods, one came from a presumably healthy, wild caught howler monkey, and 2 from birds. Six members have been isolated from blood of domestic

animals (cattle, goats, sheep, and pigs), one from a monkey, 3 from birds, one from lagomorphs, and one from a sloth. Two viruses have been associated with disease in man. One of these, Oropouche, has been responsible for several epidemics in Pará, Brazil, in 1961, 1967, and 1968. Rodents, bats, and marsupials have not been shown to act as reservoir hosts.

Nine members of the Simbu group have been reported from Africa, 5 from Asia, and 2 each from Australia, North America, and South America, but none from Europe.

Six viruses are considered to be proved or probable arboviruses, and the remaining 10 as possible arboviruses.

Table 14. Tete group and unassigned (SBU) viruses. Little is known of the 4 Tete group viruses which have been isolated only from wild caught birds in Europe (Cyprus, Italy), Africa (Egypt, Nigeria, South Africa), and Asia (Japan). The Bahig and Matruh viruses are indistinguishable by CF tests, but can readily be differentiated by cross HI reactions. All 4 are regarded as possible arboviruses, but definitive information is lacking.

Of the 7 unrelated, unassigned viruses belonging in the Bunyamwera supergroup, 5 have been isolated from culicine mosquitoes but no other arthropods; 1 from a bird, 1 from bats, and 4 from sentinel animals. Two have been rated as proved or probable arboviruses, and 5 as possible arboviruses.

Table 15. Phlebotomus fever group viruses. The PHL group has grown rapidly in number in recent years, now consisting of 20 viruses which have been linked serologically to each other by cross reactions in one or more test systems: complement fixation, hemagglutination inhibition, plaque reduction (tissue culture neutralization test), or agar gel precipitation. Thirteen of the agents have been isolated from phlebotomine flies; one (Icoaraci) has been recovered from both sandflies and mosquitoes in nature. Three others have been isolated from wild caught mosquitoes but never from phlebotomine flies; 2 of these, Arumowot and Itaporanga, have been shown to multiply in Aedes albopictus and Culex fatigans following intrathoracic inoculation, raising the possibility that the PHL group consists of mosquito-borne as well as sandfly-borne agents (Robert B. Tesh, personal communication). Four others have never been isolated from arthropods (1 from a sloth, 2 from rodents, and 1 from man).

Representatives of the serogroup have been reported from all major continents but not from Australasia. However, it may be noted that members isolated in the western hemisphere have not been found in the eastern hemisphere, and vice versa. Five viruses in this group have been associated with disease in man, although only the Naples and Sicilian sandfly fever viruses have been shown to cause large outbreaks.

Three of 20 PHL group viruses are considered to be proved arboviruses, 5 are probably arboviruses, while in regard to the remaining 12, there are insufficient data to assess their arbovirus status.

Several members of this group have been studied electron-microscopically. These have been found to resemble the bunyaviruses morphologically and, therefore, are termed bunyavirus-like, although none of the group has been shown to cross react serologically with accepted members of the Bunyamwera supergroup.

Table 16. Tick-borne groups other than group B viruses. Table 16 lists members of 5 minor antigenic groups which are drawn together because they appear to be predominantly tick-borne in nature, and because representative members of each of these groups have shown morphology characteristic of the bunyaviruses when examined by electron microscopy. With one exception (Silverwater), these viruses have been found only in Africa, Asia and Europe.

The Congo virus, which is known to have caused only a few cases of human disease in Africa, cannot be distinguished antigenically from the agent of Crimean hemorrhagic fever (CHF). In the latter form, the virus has been implicated in hundreds of cases of disease in the U.S.S.R. Little is known of the serologically related Hazara virus from West Pakistan.

The 2 members of the Ganjam group* have been recovered repeatedly from ixodid ticks, and, rarely, from culicine mosquitoes. The Dugbe virus of Africa has been isolated from culicoides, from a rodent, and frequently from the blood of apparently normal cattle. Both viruses have been associated with sporadic cases of febrile illness in man.

The Kaisodi group consists of 3 members, 2 of which have been isolated in Asia from ticks collected in forest undergrowth or from forest rodents, and one in North America from snowshoe hares and their ticks.

The Thogoto group contains only one registered virus, recovered in Africa and possibly in Europe, and found primarily in viremic cattle or ticks from cattle. The THO virus has been shown to cause human disease.

Of the 5 members of the Uukuniemi group, 3 have been discovered in Europe and 2 in Asia. Most were isolated from ticks collected in the vicinity of bird nesting places, while UUK has also been recovered from birds, a rodent, and ticks feeding on domestic cattle.

Table 17. Tick-borne groups other than group B viruses. Table 17 lists the 15 presently registered members of the growing Kemerovo group of tick-borne non-group B viruses. The Kemerovo group members differ

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* It has recently been found that a close relationship exists by the complement fixation test between Ganjam and Nairobi sheep disease viruses (F. G. Davies and J. Casals, personal communication); until additional studies are done, the designation of this antigenic group should be held in abeyance.

morphologically from the bunyavirus-like agents included in Table 16; several of them have been studied and classified taxonomically as orbiviruses on the basis of their relative resistance to lipid solvents, lability at acid pH, and double-stranded RNA genome. All have been isolated from ticks but none from other arthropods. Ten of the viruses in the KEM group were recovered initially from ticks taken from nesting areas of marine birds (8 instances) or land birds (2 instances). Two viruses were isolated from ticks collected from cattle and sheep (1 each), while the remaining 3 viruses were recovered from ticks from forested areas.

Isolations of KEM group viruses from vertebrate hosts have been seldom reported; the Kemerovo virus has been recovered from man and a bird, while the Tribec virus has been isolated from a rodent.

Five of these viruses have been found only in North America, 4 only in Europe, 2 only in Asia, 1 each only in Africa and South America, 2 in more than one continent, but none in Australasia. Of the 15 viruses registered, 2 have been rated as probable arboviruses with 13 classified as possible arboviruses.

Table 18. Tick-borne groups other than group B viruses. Included in Table 18 are tick-borne viruses which have not been classified taxonomically; 5 minor antigenic groups containing 2 to 5 members each are represented.

The DGK group consists of 3 viruses from Asia and 2 from Africa. None has been isolated from any vertebrate host, nor from arthropods other than ticks. Once again, most of the viruses were isolated from ticks collected in the immediate vicinity of bird colonies (4 viruses), while 1 virus was isolated from infected ticks taken from a camel. All are rated as possible arboviruses.

The 4 viruses of the Hughes group are serologically related by the CF test. All have been isolated from naturally infected ticks collected in areas frequented by sea birds; the Hughes virus has also been recovered from the blood of sea birds. Three of the viruses have been found only in the western hemisphere, while the fourth was discovered in the Persian Gulf. One has been evaluated as a probable arbovirus, with 3 regarded as possible arboviruses.

The 2 members of the Qalyub group have been reported only in Africa and from ticks taken from rodent burrows or from rodents themselves. Both are possible arboviruses.

The Quaranfil group also has 2 known members, both isolated from ticks collected in bird nesting areas, Johnston Atoll from nests of terns in the Central Pacific area and Australia (Abal strain), and Quaranfil from trees in an egret rookery and from pigeon houses in Africa. The QRF virus has also been isolated from birds and man; this is the only virus listed in Table 18 which has been shown to cause human infection and is classified as a proved arbovirus.

The newly created Sakhalin group consists of 3 viruses which again have been isolated from ticks taken from nesting areas of sea birds, with one also recovered from a young herring gull. All are considered possible arboviruses.

Table 19, 20, 21, and 22. Minor antigenic groups of viruses. These tables include a number of small antigenic groups of viruses consisting of one to 5 registered viruses per group. The serogroups have been listed in alphabetic order, after first having been arranged according to what their taxon status is known or is thought to be.

In Table 19 are listed 5 minor groups which can not be placed in the Bunyamwera supergroup on serological grounds, but which resemble the bunyaviruses morphologically.

The 3 members of the ANA and 2 members of the ANB groups have all been isolated from naturally infected mosquitoes but not from other arthropods, nor have they been recovered from man or wild caught lower vertebrates. They have been reported only from South America.

The Bakau group contains 2 members found originally in mosquitoes in Malaysia. The Bakau virus has also been isolated from the blood of a naturally infected monkey in Malaya, and from ticks in West Pakistan (Lahore strain).

Three serologically related viruses constitute the Mapputta group; these viruses have been isolated only from wild-caught mosquitoes in Australasia. None has been recovered from man or lower animals.

All 3 members of the Turlock group have been isolated from mosquitoes and 2 also from birds, and one from hares. Individual members of the group are found in different continents, with M'Poko (Yaba 1) in Africa, Turlock in North and South America, and Umbre in Asia.

None of the viruses listed in Table 19 has been implicated in human disease. Six have been rated as proved or probable arboviruses, and 7 as possible arboviruses.

Table 20. Minor antigenic groups of viruses. Table 20 lists members of 8 small antigenic groups having in common the physico-chemical characteristics placing them in the orbivirus taxon.

Several of the groups, represented by one registered virus each, are important in the causation of disease in large animals, namely AHS in horses, mules and donkeys; BLU in both wild and domestic ruminants; and EHD in deer. The first 2 are widespread in geographic occurrence, with bloodsucking gnats involved in their transmission. EHD has been reported only from North America and no arthropod vector has yet been demonstrated. All are considered proved or probable arboviruses.

The Changuinola group consists of 2 members, one of which has been isolated repeatedly from phlebotomine flies and once from man (CGL), while the other has been recovered a single time from a rice rat and is, therefore, of unknown significance. Both are found in the western hemisphere.

Members of other groups listed are associated either with mosquitoes or biting gnats and are reported from Africa, Asia, and Australasia. Little information is available concerning their role in nature.

Table 21. Minor antigenic groups of viruses. In Table 21 are listed 3 small antigenic groups, the members of which show a morphology characteristic of the "bullet shaped" rhabdoviruses.

The Kwatta virus, isolated only once from mosquitoes in Surinam, is related serologically to an unregistered virus recovered from birds in Brazil. Little more is known of the 2 members of the Mossuril group viruses from Africa, although Mossuril has been isolated a number of times from mosquitoes and birds.

The vesicular stomatitis group is comprised of 5 rhabdoviruses, at least 4 of which have been shown to cause infection in man. The Chandipura virus, occurring in Asia and Africa, has been isolated from phlebotomine flies, man, and hedgehogs. The other 4 members of the group have been found only in the New World, all from South America, with the vesicular stomatitis viruses also occurring in North America. VSI and VSNJ have been recovered repeatedly from livestock and on several occasions from man. The Indiana strain has been isolated many times from phlebotomine flies and at least once from mosquitoes, while the New Jersey type has not yet definitely been shown to be associated with arthropod vectors. The Piry virus is related antigenically most closely to Chandipura; it has been isolated from man and from an opossum, but not from arthropods. The Cocal virus is serologically related to VSI and has been recovered from a horse and a rodent as well as from mosquitoes and mites.

Table 22. Minor antigenic groups of viruses. In this table are listed members of 5 small groups of viruses which have not been classified taxonomically. Four of the 10 viruses represented in these groups have been isolated from mosquitoes; one of these has also been recovered from man. One of the 2 viruses in the Boteke group has been isolated only from birds. Members of 2 of the groups have not been found in arthropods; the 3 viruses of the Matariya group represent bird isolates from Egypt and the Central African Republic. Viruses of the Timbo group have been recovered only from reptiles in Brazil.

The unclassified members of these minor antigenic groups have been reported chiefly from Africa (7 of 10), with one from Asia and 2 from South America. Only one is considered a probable arbovirus, with 9 rated as possible arboviruses.

Table 23. Tacaribe group viruses. Table 23 contains a listing of those viruses placed in the Tacaribe antigenic group. It has been recognized for some years that these agents are serologically related to the long known lymphocytic choriomeningitis virus and are morphologically identical as well. All are classified in the genus Arenavirus. Members of the group are associated predominantly with rodent hosts and there is little or no evidence that they are transmitted by arthropod vectors in nature; all are rated as non-arthropod-borne.

Of the 9 registered viruses, 7 are found only in South America, one in North America, and one in Africa. Three members of the group have been shown to cause severe, often fatal, cases of disease in man. These include Junin (Argentine hemorrhagic fever), Machupo (Bolivian hemorrhagic fever), and Lassa (Lassa disease).

In Tables 24 through 27 are listed all the remaining registered viruses which have not yet been found to be related antigenically to any other known virus and, thus, are presently ungrouped.

Table 24. Ungrouped mosquito-associated viruses. Table 24 includes serologically ungrouped viruses which have been placed in 4 different genera plus the "bunyavirus-like" category. Two of the 11 viruses listed here are rated as definite arboviruses and 4 others as probably arboviruses. The remaining 5, if arthropod-borne, are probably transmitted by mosquitoes. However, 2 of the listed viruses are considered by the SEAS to be probably not arboviruses. The Nodamura virus from Japan, a picornavirus, was isolated from a pool of wild-caught mosquitoes and has been shown to multiply in arthropods and to be experimentally transmitted by mosquitoes, but it is regarded as unlikely that this would be its usual mode of transmission in nature. The Cotia virus, a poxvirus from Brazil and French Guiana, has been isolated repeatedly in sentinel mice, a number of times from mosquitoes, at least once from phlebotomine flies and from the blood of a human patient, but is considered not to be an arbovirus.

Among the rhabdoviruses, it will be noted that Flanders and Hart Park are listed as ungrouped viruses although they have been shown to be very closely related serologically to each other. It is still not clear whether these viruses form a group or complex of viruses, or whether they should be regarded as variants of a single virus.

The Rift Valley fever virus is noteworthy from the standpoint of disease production in lambs, sheep, and cattle; herdsmen often become infected, and infections are common among veterinary field officers and laboratory workers where the disease in livestock occurs.

Table 25. Ungrouped mosquito-associated viruses. Here are listed 19 additional antigenically ungrouped viruses which have not been classified taxonomically. They have not been isolated from arthropods other than mosquitoes, and only 3 from vertebrates.

Table 26. Ungrouped tick-, culicoides-, or phlebotomus-associated viruses. In Table 26 are listed ungrouped viruses which appear to be associated predominantly with arthropod vectors other than mosquitoes. The majority (17 of 20) are probably tick-borne in nature, but 2 have been isolated only from biting midges and one from phlebotomine flies. Three tick-borne viruses are considered to be proved arboviruses; the bunyavirus-like Nairobi sheep disease agent and the African swine fever virus, an iridovirus, are important causes of veterinary disease, while the third, an orbivirus, causes Colorado tick fever in man.

Table 27. Ungrouped viruses, no arthropod vector known. Table 27 lists the remaining registered viruses which have been isolated only from man or lower vertebrates. With the possible exception of one virus which was recovered from the blood of a sentinel monkey held on a platform in a forest canopy, no inferences can be made regarding possible arthropod vectors.

Of the 26 viruses in this category, 9 were isolated from birds, 5 from bats, 4 from rodents, 3 from man, 2 from lizards, and one each from shrews, naturally-infected monkeys, and a sentinel monkey. From the standpoint of danger to man, the Marburg virus appears to be the most important virus listed in this table.

Tables 28 through 32 provide a further condensation of data which may be extracted from Tables 5 through 27. They serve to draw attention in a single table to certain aspects of the information contained in multiple tabulations.

Table 28 summarizes distribution of viruses in different antigenic groups by continents as determined by actual isolations of viruses. It can be seen that only a few viruses, 14 or 3.9 percent, show a truly broad distribution, being found on 3 or more continents. While different members of some antigenic groups may be recovered in many parts of the world, individual viruses within the groups tend to show a much more limited spread. About 85 percent of all registered viruses have been found only on a single continent, while 96 percent have been reported from one or two continents only. The largest number of viruses have been isolated in Africa, followed by South America, Asia, North America, Australasia, and Europe, in that order.

Table 29 lists the numbers of viruses by antigenic group which have been isolated from various classes of arthropods. 184 (51%) of all registered viruses have been recovered from mosquitoes, 78 (about 22%) from ticks, and 54 (15%) from all other classes. 71 viruses have never been isolated from any arthropod host, including 16 members of the large group B, and 26 of the ungrouped category. By far the largest number of viruses which have been isolated from any arthropod, 263 of 288 (91.3%) have been reported from one class only.

Table 30 shows that a few viruses have been shown to infect as many as 4 to 6 classes of vertebrates but again, analogous to the situation in arthropod hosts, most of the viruses isolated from vertebrates have been recovered from one class only (150 of 216 or 69.4%). The largest number of viruses have been isolated from man and rodents, followed by birds and other hosts.

In Table 31 are listed the number of viruses in each serogroup which are known to cause disease in man. Of the major groups of viruses, A and B, more than half of the members have caused disease in nature or in the form of laboratory infections. Viruses in group C show a high ratio of human infection, 9 of the 11 members having been associated with human illness. Overall, 25.6 percent of all registered viruses have been implicated in human infections.

The SEAS ratings of registered viruses summarized in Table 32 show that data are considered adequate in 150 registrations (42%) to indicate that the viruses are arthropod-borne or probably arthropod-borne. In 25 additional instances (7%), viruses have been rated with some degree of confidence as not, or probably not, arboviruses. However, it may be noted that in 184 registrations, or 51%, data are lacking which would permit classification other than as possible arboviruses.

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Table 1. Alphabetical Listing of 359 Viruses Registered in Catalogue with Recommended Abbreviations and Antigenic Grouping

Name	Abbr.	Ant. Group
Absettarov	ABS	B
Abu Hammad	AH	DGK
Acado	ACD	COR
Acara	ACA	CAP
African horsesickness	AHS	AHS
African swine fever	ASF	
Aguacate	AGU	PHL
Aino	AINO	SIM
Akabane	AKA	SIM
Alfuy	ALF	B
Almpiwar	ALM	
Amapari	AMA	TCR
Anhanga	ANH	PHL
Anhembi	AMB	BUN
Anopheles A	ANA	ANA
Anopheles B	ANB	ANB
Apeu	APEU	C
Apoi	APOI	B
Arkonam	ARK	
Aruac	ARU	
Arumowot	AMT	PHL
Aura	AURA	A
Avalon	AVA	SAK
Bahig	BAH	TETE
Bakau	BAK	BAK
Baku	BAKU	KEM
Bandia	BDA	QYB
Bangoran	BGN	
Bangui	BGI	
Banzi	BAN	B
Barur	BAR	
Batai	BAT	BUN
Batken	BKN	
Batu Cave	BC	B
Bauline	BAU	KEM
Bebaru	BEB	A
Belmont	BEL	
Bertioga	BER	GMA
Bhanja	BHA	
Bimbo	BBO	
Bimiti	BIM	GMA
Birao	BIR	BUN
Bluetongue	BLU	BLU
Bocas	BOC	CAL
Boracea	BOR	ANB
Botambi	BOT	SBU
Boteke	BTK	BTK
Bouboui	BOU	B
Bujaru	BUJ	PHL
Bunyamwera	BUN	BUN
Burg el Arab	BEA	MTY
Bushbush	BSB	CAP
Bussuquara	BSQ	B
Buttonwillow	BUT	SIM
Bwamba	BWA	BWA
Cacao	CAC	PHL
Cache Valley	CV	BUN
Caimito	CAI	PHL
California encephal.	CE	CAL
Calovo	CVO	BUN
Candiru	CDU	PHL
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	
Chenuda	CNU	KEM
Chikungunya	CHIK	A
Chilibre	CHI	PHL
Chobar Gorge	CG	
Clo Mor	CM	SAK
Cocal	COC	VSV
Colorado tick fever	CTF	
Congo	CON	CON
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	GAN

Name	Abbr.	Ant. Group
Eastern equine enceph.	EEE	A
Edge Hill	EH	B
Entebbe bat	ENT	B
Epizootic hem. dis.	EHD	EHD
Eubenangee	EUB	EUB
Everglades	EVE	A
Flanders	FLA	
Frijoles	FRI	PHL
Gamboa	GAM	SBU
Ganjam	GAN	GAN
Garba	GAR	MTY
Germiston	GER	BUN
Getah	GET	A
Gomoka	GOM	
Gordil	GOR	PHL
Gossas	GOS	
Grand Arbaud	GA	UUK
Great Island	GI	KEM
Guajara	GJA	CAP
Guama	GMA	GMA
Guaratuba	GTB	SBU
Guaroa	GRO	BUN
Gumbo Limbo	GL	C
Hanzalova	HAN	B
Hart Park	HP	
Hazara	HAZ	CON
Huacho	HUA	KEM
Hughes	HUG	HUG
Hypr	HYPR	B
Icoaraci	ICO	PHL
Ieri	IERI	
Ilesha	ILE	BUN
Ilheus	ILH	B
Ingwavuma	ING	SIM
Inkoo	INK	CAL
Irituia	IRI	CGL
Israel turkey meningo.	IT	B
Issyk-Kul	IK	
Itaporanga	ITP	PHL
Itaqui	ITQ	C
Jamestown Canyon	JC	CAL
Japanaut	JAP	
Japanese encephalitis	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
Kairi	KRI	BUN
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
Kokobera	KOK	B
Kolongo	KOL	
Koongol	KOO	KOO
Koutango	KOU	B
Kowanyama	KOW	
Kumlinge	KUM	B
Kunjin	KUN	B
Kwatta	KWA	KWA
Kyasanur Forest dis.	KFD	B
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
Lokern	LOK	BUN
Lone Star	LS	
Louping ill	LI	B
Lukuni	LUK	ANA

* Rabies related

Name	Abbr.	Ant. Group
Machupo	MAC	TCR
Madrid	MAD	C
Maguari	MAG	BUN
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	
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	SBU
Minnal	MIN	
Mirim	MIR	SBU
Mitchell River	MR	WAR
Modoc	MOD	B
Moju	MOJU	GMA
Mono Lake	ML	KEM
Montana myotis leuko.	MML	B
Moriche	MOR	CAP
Mossuril	MOS	MOS
Mount Elgon bat	MEB	
M'Poko	MPO	TUR
Mucambo	MUC	A
Murray Valley enceph.	MVE	B
Murutucu	MUR	C
Nairobi sheep disease	NSD	
Nariva	NAR	
Navarro	NAV	
Ndumu	NDU	A
Negishi	NEG	B
Nepuyo	NEP	C
Ngaingan	NGA	
Nique	NIQ	PHL
Nkolbisson	NKO	
Nodamura	NOD	
Nola	NOLA	SIM
Northway	NOR	BUN
Ntaya	NTA	B
Nyamanini	NYM	
Nyando	NDO	NDO
Okhotskiy	OKH	KEM
Okola	OKO	
Omsk hemorrhagic fev.	OMSK	B
O'nyong nyong	ONN	A
Oriboca	ORI	C
Oropouche	ORO	SIM
Ossa	OSSA	C
Ouango	OUA	
Pacora	PCA	
Pacui	PAC	PHL
Pahayokee	PAH	PAT
Palyam	PAL	PAL
Parana	PAR	TCR
Pata	PATA	EUB
Pathum Thani	PTH	DGK
Patois	PAT	PAT
Phnom-Penh bat	PPB	B
Pichinde	PIC	TCR
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
Restan	RES	C
Rift Valley fever	RVF	
Rio Bravo	RB	B
Ross River	RR	A
Royal Farm	RF	B
Russian spr.sum.enc.	RSSE	B
Sabo	SABO	SIM
Saboya	SAB	B
Sagiyama	SAG	A
Saint-Floris	SAF	
Sakhalin	SAK	SAK
Salehabad	SAL	PHL
Samford	SAM	SIM

Name	Abbr.	Ant. Group
San Angelo	SA	CAL
Sandfly fever (Naples)	SFN	PHL
Sandfly fev.(Sicilian)	SFS	PHL
Sandjimba	SJA	
Sango	SAN	SIM
Sathuperi	SAT	SIM
Sawgrass	SAW	
Seletar	SEL	KEM
Sembalam	SEM	
Semliki Forest	SF	A
Sepik	SEP	B
Shamonda	SHA	SIM
Shark River	SR	PAT
Shuni	SHU	SIM
Silverwater	SIL	KSO
Simbu	SIM	SIM
Simian hemorrhagic fev.	SHF	
Sindbis	SIN	A
Sixgun City	SC	KEM
Sokoluk	SOK	B
Soldado	SOL	HUG
Sororoca	SOR	BUN
Spondweni	SPO	B
St.Louis encephalitis	SLE	B
Stratford	STR	B
Tacaiuma	TCM	ANA
Tacaribe	TCR	TCR
Tahyna	TAH	CAL
Tamiami	TAM	TCR
Tanga	TAN	
Tanjong Rabok	TR	
Tataguine	TAT	
Tembe	TME	
Tembusu	TMU	B
Tensaw	TEN	BUN
Tete	TETE	TETE
Tettnang	TET	
Thimiri	THI	SIM
Thogoto	THO	THO
Thottapalayam	TPM	
Timbo	TIM	TIM
Tlacotalpan	TLA	BUN
Toure	TOU	
Tribec	TRB	KEM
Triniti	TNT	
Trivittatus	TVT	CAL
Trubanaman	TRU	MAP
Tsuruse	TSU	
Turlock	TUR	TUR
Tyuleniy	TYU	B
Uganda S	UGS	B
Umatilla	UMA	
Umbre	UMB	TUR
Una	UNA	A
Upolu	UPO	
Usutu	USU	B
Uukuniemi	UUK	UUK
Vellore	VEL	PAL
Venezuelan equine enc	VEE	A
Venkatapuram	VKT	
VSV-Indiana	VSI	VSV
VSV-New Jersey	VSNJ	VSV
Wad Medani	WM	
Wallal	WAL	
Wanowrie	WAN	
Warrego	WAR	WAR
Wesselsbron	WSL	B
West Nile	WN	B
West.equine enceph.	WEE	A
Whataroa	WHA	A
Witwatersrand	WIT	
Wongal	WON	KOO
Wongorr	WGR	
Wyeomyia	WYO	BUN
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

FIGURE 1

YEAR OF ISOLATION OF 359 VIRUSES REGISTERED IN THE CATALOGUE WITH CUMULATIVE PERCENTAGE OF ISOLATIONS BY YEAR

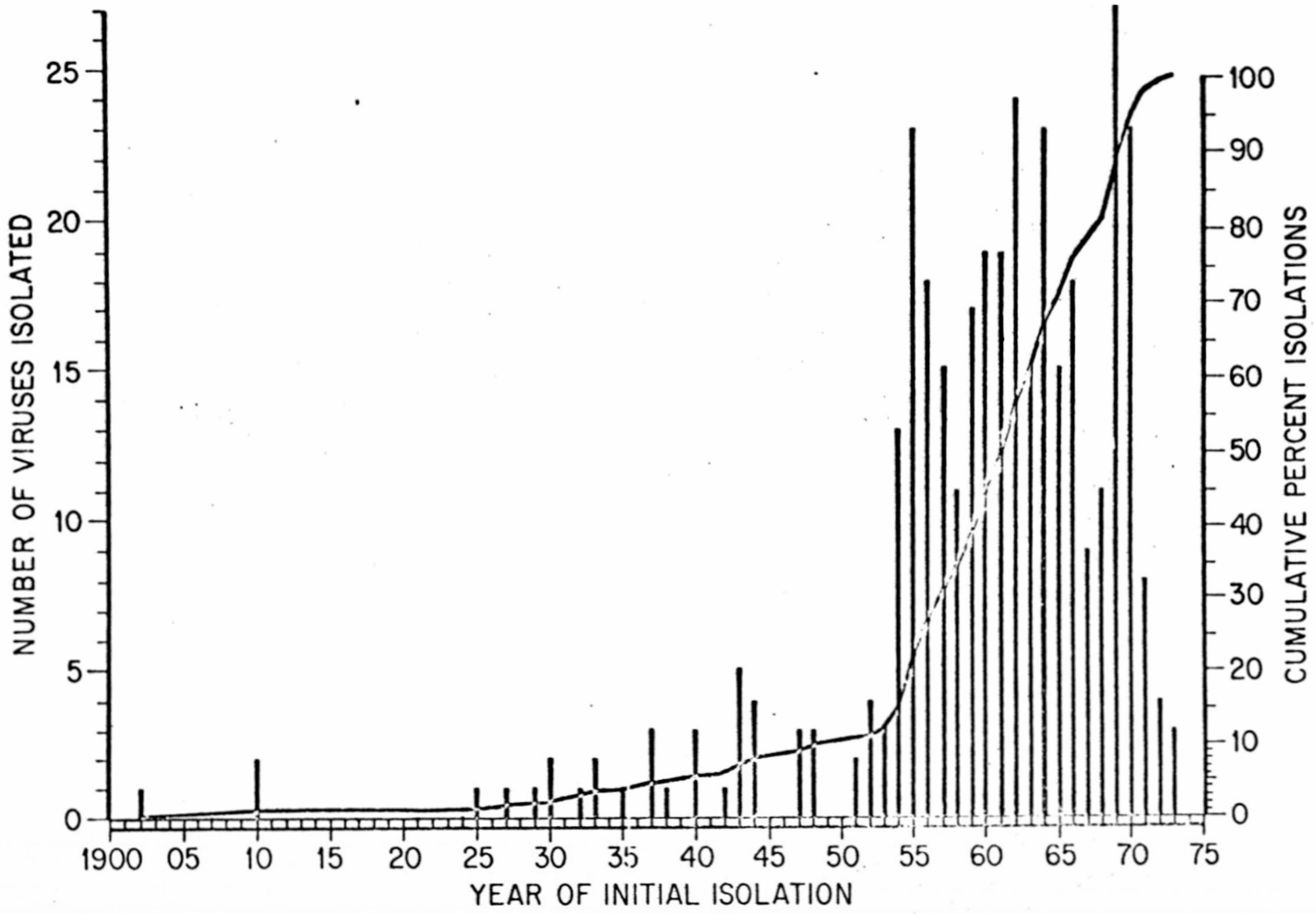


Table 2. Antigenic Groups of 359 Viruses Registered in Catalogue

Antigenic Group	Abbreviation	No. Registered Viruses in Group		%
A	A		20	5.6
African horsesickness	AHS		1	0.3
Anopheles A	ANA		3	0.8
Anopheles B	ANB		2	0.6
B	B		57	15.9
Bakau	BAK		2	0.6
Bluetongue	BLU		1	0.3
Boteke	BTK		2	0.6
Bunyamwera Supergroup			87	24.2
Bunyamwera	BUN	18		
Bwamba	BWA	2		
C	C	11		
California	CAL	11		
Capim	CAP	6		
Guama	GMA	6		
Koongol	KOO	2		
Patois	PAT	4		
Simbu	SIM	16		
Tete	TETE	4		
Unassigned	SBU	7		
Changuinola	CGL		2	0.6
Congo	CON		2	0.6
Corriparta	COR		2	0.6
Dera Ghazi Khan	DGK		5	1.4
Epizootic hemorrhagic disease	EHD		1	0.3
Eubenangee	EUB		2	0.6
Ganjam	GAN		2	0.6
Hughes	HUG		4	1.1
Kaisodi	KSO		3	0.8
Kemerovo	KEM		15	4.2
Kwatta	KWA		1	0.3
Malakal	MAL		2	0.6
Mapputta	MAP		3	0.8
Matariya	MTY		3	0.8
Mossuril	MOS		2	0.6
Nyando	NDO		1	0.3
Palyam	PAL		4	1.1
Phlebotomus fever	PHL		20	5.6
Qalyub	QYB		2	0.6
Quaranfil	QRF		2	0.6
Sakhalin	SAK		3	0.8
Tacaribe	TCR		9	2.5
Thogoto	THO		1	0.3
Timbo	TIM		2	0.6
Turlock	TUR		3	0.8
Uukuniemi	UUK		5	1.4
Vesicular stomatitis	VSV		5	1.4
Warrego	WAR		2	0.6
Ungrouped viruses			76	21.2
	Total		359	

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

Decade	Continent	Country	Virus
1900-09	Africa	South Africa	BLU
1910-19	Africa	Kenya	ASF, NSD
1920-29	Africa	Nigeria	YF
	Europe	Scotland	LI
	North America	U.S.A.	VSI
1930-39	Africa	Kenya	RVF
		S. Africa	AHS
		Uganda	BWA, WN
	Asia	Japan	JE
		U.S.S.R.	RSSE
	N. America	U.S.A.	EEE, SLE, WEE
	S. America	Venezuela	VEE
1940-49	Africa	Uganda	BUN, NTA, SF, UGS, ZIKA
	Asia	Japan	NEG
		U.S.S.R.	OMSK
	Australasia	Hawaii	DEN-1*
		New Guinea	DEN-2*
	Europe	Czechoslovakia	HAN
		Italy	SFN*, SFS*
	N. America	U.S.A.	CE, CTF, TVT
	S. America	Brazil	ILH
		Colombia	ANA, ANB, WYO
1950-59	Africa	Egypt	CNU, QRF, QYB, SIN, WM
		Nigeria	ILE, LB
		South Africa	BAN,GER,ING,LEB,MID,MOS,NDU,NYM, PGA,SIM,SPO,TETE,USU,WIT,WSL.
		Uganda	CHIK,CON,ENT,NDO,ONN
	Asia	India	ARK,BHA,GAN,KAS,KSO,KFD,MIN,PAL, SAT,VKT,UMB,WAN.
		Israel	IT
		Japan	AKA,APOI,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,TUR,VSNJ.
	S. America	Argentina	JUN
		Brazil	APEU, AURA, BSQ, CAP,CAR,CATU, GJA,GMA,ITQ,MAG,MIR,MOJU,MTB,MUC, MUR,ORI,TCM,UNA.
		Colombia	GRO,NAV
		Trinidad	ARU,BIM,BSB,IERI,KRI,LUK,MAN,MAY, MEL,NEP,ORO,TCR,TNT.

* Isolated in U.S.A. Laboratory

Table 3. (Continued)

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

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

Table 3. (Continued)

Decade	Continent	Country	Virus
1970-73	Africa	Cent.Afr.Rep.	BBO,BGI,GAR,GOM,GOR,KOL,LJA,NOLA,OUA SAF,SJA,ZGO
		Egypt	AH,KS,PTH
		S. Africa	PRE***
	Asia	India	CG
		Malaysia	BC,CI
		U.S.S.R.	BKN,IK,KSI,SOK
	Australasia	Australia	NGA,WAL,WGR
	Europe	Germany	TET
		Scotland	CM,CW
		U.S.S.R.	BAKU
	N. America	Canada	AVA,BAU,GI
		Panama	CAC,CAI,NIQ
		U.S.A.	NOR,YH

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

Table 4. Initial Isolation of 359 Registered Viruses by Continent, Country, and Chronological Period

Continent	Country or Area	Before 1930	1930 -39	1940 -49	1950 -59	1960 -69	1970	Totals
AFRICA	Cameroon					2		2
	Cent.Afr.Rep.					9	12	21
	Egypt				5	7	3	15
	Kenya	2	1			1		4
	Nigeria	1			2	6		9
	Senegal					10		10
	S.Africa	1	1		15		1	18
	Sudan					1		1
	Uganda		2	5	5	4		16
	Totals	4	4	5	27	40	16	96
ASIA	Cambodia					1		1
	India				12	9	1	22
	Iran					2		2
	Israel				1			1
	Japan		1	1	5	1		8
	Malaysia				7	5	2	14
	W.Pakistan					3		3
	Persian Gulf					1		1
	Singapore					1		1
	Thailand					1		1
	U.S.S.R.(East)		1	1		4	4	10
	Totals		2	2	25	28	7	64
AUSTRAL-	Australia				1	25	3	29
ASIA	Hawaii			1				1
and	Johnston Island					1		1
PACIFIC	New Guinea			1				1
ISLANDS	New Zealand					1		1
	Philippines				2			2
	Totals			2	3	27	3	35
EUROPE	Czechoslovakia			1	2	4		7
	Finland				1	2		3
	France					2		2
	West Germany					1	1	2
	Italy			2				2
	Scotland	1					2	3
	U.S.S.R.(West)				1		1	2
	Totals	1		3	4	9	4	21
NORTH	Canada				1	1	3	5
AMERICA	Guatemala					1		1
	Mexico					2		2
	Panama				3	15	3	21
	U.S.A.	1	3	3	9	25	2	43
	Totals	1	3	3	13	44	8	72
SOUTH	Argentina				1			1
AMERICA	Bolivia			1		1		2
	Brazil				18	22		40
	Colombia			3	2	1		6
	Peru					2		2
	Surinam					1		1
	Trinidad				13	5		18
	Venezuela		1					1
	Totals	0	1	4	34	32	0	71
	Grand Totals	6	10	19	106	180	38	359

TABLE 5. GROUP A ARBOVIRUSES

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

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

TABLE 6. GROUP B ARBOVIRUSES, MOSQUITO-BORNE

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	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		
Alfuy	+										+				+	+		+						20	Flavivirus
Banzi	+							+							+	+						+		20	"
Bouboui	+	+														+								22	"
Bussuquara	+							+		+					+	+				+	+	+		20	"
Dengue-1	+							+								+	+	+			+	+	+	20	"
Dengue-2	+							+	+							+	+	+			+	+	+	20	"
Dengue-3	+							+									+	+			+	+	+	20	"
Dengue-4	+							+									+	+				+		20	"
Edge Hill	+	+															+	+						20	"
Ilheus	+							+			+				+					+	+	+	+	20	"
Japanese encephalitis	+	+						+			+			+			+					+	+	20	"
Jugra	+																+							22	"
Kokobera	+																	+						21	"
Kunjin	+							+			+							+					+	20	"
Murray Valley enceph.	+							+										+				+		20	"
Ntaya	+															+								21	"
Sepik	+																	+				+		21	"
St. Louis encephalitis	+	+					+	+			+			+						+	+	+	+	20	"
Spondweni	+							+								+						+	+	20	"
Stratford	+																	+						22	"
Tembusu	+	+															+	+						21	"
Uganda S	+										+				+	+								20	"
Usutu	+									+	+					+								22	"
Wesselsbron	+	+						+		+				+		+	+					+	+	20	"
West Nile	+	+	+	+				+		+	+			+	+	+	+		+			+	+	20	"
Yellow fever	+							+	+				+		+	+					+	+	+	20	"
Zika	+							+							+	+						+	+	20	"

* See footnote Table 5

TABLE 7. GROUP B ARBOVIRUSES, TICK-BORNE

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	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	"
Tyuleniy			+														+			+				21	"

* See footnote Table 5

TABLE 8. GROUP B VIRUSES, NO ARTHROPOD VECTOR DEMONSTRATED

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

* See footnote Table 5

TABLE 9. BUNYAMWERA SUPERGROUP: BUNYAMWERA GROUP VIRUSES

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks				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	Phlebotomine	Culicoides																			
Anhembi	+									+											+			22	Bunyavirus
Batai	+	+															+		+					22	"
Birao		+														+								22	"
Bunyamwera	+							+								+						+	+	20	"
Cache Valley	+	+																		+				20	"
**Calovo		+																	+			+		22	"
Germiston	+							+		+					+	+							+	20	"
Guaroa		+						+												+	+	+		20	"
Ilesha		+						+								+						+		21	"
Kairi	+								+	+					+						+			20	"
Lokern	+					+								+						+				20	"
Maguari	+	+												+	+					+				20	"
Main Drain						+								+						+				20	"
Northway	+														+					+				21	"
Sororoca	+																				+			22	"
Tensaw	+	+												+	+					+		+		20	"
Tlacotalpan	+	+																		+				22	"
Wyeomyia	+	+						+												+	+	+		21	"

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

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

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

* See footnote Table 5

TABLE 11. BUNYAMWERA SUPERGROUP: CALIFORNIA AND CAPIM GROUP VIRUSES

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

* See footnote Table 5

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

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

* See footnote Table 5

TABLE 13. BUNYAMWERA SUPERGROUP: SIMBU GROUP VIRUSES

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Rats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
Aino	+																+							22	Bunyavirus
Akabane	+					+											+	+						21	"
Buttonwillow						+								+						+				20	"
Ingwavuma	+										+			+		+	+							20	"
Manzanilla									+												+			22	"
Mermet											+									+				22	"
Nola	+															+					+			20	"
Oropouche	+							+						+		+					+	+	+	21	"
Sabo						+								+		+								22	"
Samford						+								+				+						22	"
Sango	+					+								+		+								22	"
Sathuperi	+					+								+		+	+							22	"
Shamonda						+								+		+								22	"
Shuni						+		+						+		+						+		22	"
Simbu	+															+								21	"
Thimiri											+					+	+							22	"

* See footnote Table 5

TABLE 14. 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											+					+			+					22	Bunyavirus
Matruh											+ +					+ +			+					22	"
Tete											+ +					+ + +								22	"
Tsuruse											+						+							22	"
UNASSIGNED - "SBU"																									
Botambi	+															+								22	Bunyavirus
Gamboa	+																			+				22	"
Guaratuba	+										+				+						+			21	"
Jurona	+																				+			22	"
Kaeng Khoi												+			+		+							22	"
Minatitlan															+ +					+				22	"
Mirim	+														+ +						+			20	"

* See footnote Table 5

TABLE 15. PHLEBOTOMUS FEVER GROUP VIRUSES

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		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																					
Aguacate					+									+						+				21	Bunyavirus-like
Anhanga														+							+			22	=
Arumowot	+									+				+		+					+			22	=
Bujaru										+											+			22	=
Cacao					+															+					21	=
Caimito					+															+					22	=
Candiru								+	+												+	+		22	=
Chagres	+							+												+		+		22	=
Chilibre					+															+					21	=
Frijoles					+															+					22	=
Gordil										+						+									22	=
Icoaraci	+	+			+					+	+				+						+				21	=
Itaporanga	+										+		+		+						+				20	=
Karimabad					+												+								22	=
Nique					+															+					22	=
Pacui					+					+											+				21	=
Punta Toro					+			+												+		+			22	=
Salehabad					+												+								22	=
SF-Naples					+			+								+	+					+			20	=
SF-Sicilian					+			+								+			+			+			20	=

* See footnote Table 5

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

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	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	Bunyavirus-like
Hazara			+														+							22	"
GANJAM GROUP																									
Dugbe	+		+			+		+		+				+		+						+		22	Bunyavirus-like
Ganjam	+		+					+									+					+	+	22	"
KAISODI GROUP																									
Kaisodi			+								+						+							22	Bunyavirus-like
Lanjan			+														+							22	"
Silverwater			+					-						+						+				21	"
THOGOTO GROUP																									
Thogoto			+					+						+		+			+			+		22	Bunyavirus-like
UUKUNIEMI GROUP																									
Grand Arbaud				+															+					20	Bunyavirus-like
Manawa			+	+													+							22	"
Ponteves				+															+					22	"
Uukuniemi			+							+	+								+					21	"
Zaliv Terpeniya			+														+							22	"

* See footnote Table 5

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

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

See footnote Table 5

TABLE 18. TICK-BORNE GROUPS OTHER THAN GROUP B VIRUSES

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

*See footnote Table 5

TABLE 19. 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 A GROUP																									
Anopheles A		+																			+			21	Bunyavirus-like
Lukuni	+	+																			+			22	"
Tacaiuma	+	+													+						+			21	"
ANOPHELES B GROUP																									
Anopheles B		+																			+			22	Bunyavirus-like
Boracea	+	+																			+			22	"
BAKAU GROUP																									
Bakau	+			+					+								+							22	Bunyavirus-like
Ketapang	+																+							21	"
MAPPUTTA GROUP																									
Mapputta		+																+						22	Bunyavirus-like
Maprik	+	+																+						21	"
Trubanaman		+																+						22	"
TURLOCK GROUP																									
M'Poko (=Yaba-1)	+															+								22	Bunyavirus-like
Turlock	+										+			+	+					+	+			20	"
Umbre	+										+						+							21	"

* 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																						
	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																									
Changuinola					+			+												+		+		21	Orbivirus
Irituia										+											+			22	"
CORRIPARTA GROUP																									
Acado	+															+								22	Orbivirus
Corriparta	+										+							+						22	"
EHD GROUP																									
Epizootic hem. dis.														+						+				21	Orbivirus
EUBENANGEE GROUP																									
Eubenangee	+?	+?																+						22	Orbivirus
Pata	+															+								22	"
PALYAM GROUP																									
D'Aguilar						+												+						22	Orbivirus
Kasba	+																+							22	"
Palyam	+																+							22	"
Vellore	+																+							22	"
WARREGO GROUP																									
Mitchell River						+												+						22	Orbivirus
Warrego						+												+						22	"

* See footnote Table 5

TABLE 21. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
KWATTA GROUP																									
Kwatta	+																				+			22	Rhabdovirus
MOSSURIL GROUP																									
Kamese	+															+								22	Rhabdovirus
Mossuril	+										+					+								22	"
VESICULAR STOMATITIS GR.																									
Chandipura					+			+						+		+						+		21	Rhabdovirus
Cocal	+						+	+		+				+	+		+				+			20	"
Piry								+					+	+							+		+	22	"
VSV-Indiana	+				+			+						+						+	+	+	+	20	"
VSV-New Jersey							+	+						+						+	+	+	+	22	"

* See footnote Table 5

TABLE 22. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	Mosq.		Ticks		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	"
MATARIYA GROUP																								
Burg el Arab											+					+							22	Unclassified
Garba											+					+							22	"
Matariya											+					+							22	"
NYANDO GROUP																								
Nyando		+						+								+						+	21	Unclassified
TIMBO GROUP																								
Chaco														+							+		22	Unclassified
Timbo														+							+		22	"

* See footnote Table 5

TABLE 23. TACARIBE (LCM) GROUP VIRUSES

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

* See footnote Table 5

TABLE 24. UNGROUPED MOSQUITO-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anopheline	Ticks Ixodid	Ticks Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: Africa	Asia	Australasia	Europe	North America	South America	HUMAN DISEASE: Natural Infection	Lab Infection	SEAS RATING *	TAXONOMIC STATUS
Rift Valley fever	+							+						+		+						+	+	20	Bunyavirus-like
Tataguine	+	+						+								+						+		22	"
Witwatersrand	+									+					+	+								21	"
Japanaut	+											+						+						21	Orbivirus
Lebombo	+						+	+		+						+								21	"
Umatilla	+										+									+				20	"
Nodamura	+																+							23	Picornavirus
Cotia	+				+			+							+						+	+		24	Poxvirus
Flanders	+										+									+				22	Rhabdovirus
Hart Park	+										+									+				21	"
Joinjakaka	+																	+						22	"

* See footnote Table 5

TABLE 25. UNGROUPED MOSQUITO-ASSOCIATED VIRUSES

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

* See footnote Table 5

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

	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
Lone Star			+																	+				22	"
Nairobi sheep disease			+					+						+		+						+	+	20	"
African swine fever				+										+		+			+		+**			20	Iridovirus
Colorado tick fever			+	+				+		+										+		+	+	20	Orbivirus
Barur			+							+							+							22	Rhabdovirus
Batken	+		+														+							22	Unclassified
Charleville					+									+				+						22	"
Chobar Gorge				+													+							22	"
Dhori			+													+	+							22	"
Issyk-Kul				+								+					+							22	"
Keterah				+								+					+							21	"
Matucare				+																	+			22	"
Ngaingan						+												+						22	"
Nyamanini				+							+						+							21	"
Sawgrass			+																	+				22	"
Tettnang			+																+					22	"
Upolu				+														+						22	"
Wallal						+												+						22	"
Wanowrie	+		+					+								+	+							22	"

* See footnote Table 5

** Cuba

TABLE 27. UNGROUPED VIRUSES: NO ARTHROPOD VECTOR KNOWN

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anopheline	Ticks Ixodid	Ticks Argasid	Phlebotomine	Culicoides	Other	ISOLATED FROM: VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: Africa	Asia	Australasia	Europe	North America	South America	HUMAN DISEASE: Natural Infection	Lab Infection	SEAS RATING *	TAXONOMIC STATUS
Nariva										+											+			23	Paramyxovirus
Kern Canyon												+								+				23	Rhabdovirus
Lagos Bat												+				+								24	"
Mount Elgon Bat												+				+								23	"
Navarro											+										+			22	"
Almpiwar														+				+						21	Unclassified
Bangui								+								+						+		22	"
Bimbo											+					+								22	"
Gossas												+				+								23	"
Kammavanpettai											+						+							22	"
Kannamangalam											+						+							22	"
Keuraliba										+						+								22	"
Kolongo											+					+								22	"
Landjia											+					+								22	"
Le Dantec								+								+								22	"
Marburg								+											+			+	+	23	"
Marco														+							+			22	"
Ouango											+					+								22	"
Saint-Floris										+						+								22	"
Sandjimba											+					+								22	"
Sembalam											+						+							22	"
Simian Hemorrh. fever									+								?			+				24	"
Tanjong Rabok															+		+							22	"
Thotlapalayam														+			+							22	"
Toure										+						+								22	"
Yogue												+				+								22	"

* See footnote Table 5.

TABLE 28. CONTINENTAL DISTRIBUTION OF GROUPED AND UNGROUPED VIRUSES

Antigenic Group	Total in Group	Africa	Asia	Australasia	Europe	North America	South America	No. of Continents involved 1	2	3	4	5
A	20	6	6	5	1	5	8	13	5	1	0	1
AHS	1	1	1	0	1	0	0	0	0	1	0	0
ANA	3	0	0	0	0	0	3	3	0	0	0	0
ANB	2	0	0	0	0	0	2	2	0	0	0	0
B	57	17	24	12	7	10	6	43	10	3	1	0
BAK	2	0	2	0	0	0	0	2	0	0	0	0
BLU	1	1	1	0	1	1	0	0	0	0	1	0
BTK	2	2	0	0	0	0	0	2	0	0	0	0
Bunyamwera Supergroup BUN	18	4	1	0	2	8	6	15	3	0	0	0
Bunyamwera Supergroup BWA	2	2	0	0	0	0	0	2	0	0	0	0
Bunyamwera Supergroup C	11	0	0	0	0	5	8	9	2	0	0	0
Bunyamwera Supergroup CAL	11	1	0	0	2	8	2	9	2	0	0	0
Bunyamwera Supergroup CAP	6	0	0	0	0	3	5	4	2	0	0	0
Bunyamwera Supergroup GMA	6	0	0	0	0	2	5	5	1	0	0	0
Bunyamwera Supergroup KOO	2	0	0	2	0	0	0	2	0	0	0	0
Bunyamwera Supergroup PAT	4	0	0	0	0	4	0	4	0	0	0	0
Bunyamwera Supergroup SIM	16	9	5	2	0	2	2	12	4	0	0	0
Bunyamwera Supergroup TETE	4	3	1	0	2	0	0	2	2	0	0	0
Bunyamwera Supergroup SBU	7	1	1	0	0	2	3	7	0	0	0	0
CGL	2	0	0	0	0	1	1	2	0	0	0	0
CON	2	1	2	0	1	0	0	1	0	1	0	0
COR	2	1	0	1	0	0	0	2	0	0	0	0
DGK	5	2	3	0	0	0	0	5	0	0	0	0
EHD	1	0	0	0	0	1	0	1	0	0	0	0
EUB	2	1	0	1	0	0	0	2	0	0	0	0
GAN	2	1	1	0	0	0	0	2	0	0	0	0
HUG	4	0	1	0	0	1	3	3	1	0	0	0
KSO	3	0	2	0	0	1	0	3	0	0	0	0
KEM	15	3	4	0	4	6	1	13	1	1	0	0
KWA	1	0	0	0	0	0	1	1	0	0	0	0
MAL	2	1	1	0	0	0	0	2	0	0	0	0
MAP	3	0	0	3	0	0	0	3	0	0	0	0
MTY	3	3	0	0	0	0	0	3	0	0	0	0
MOS	2	2	0	0	0	0	0	2	0	0	0	0
NDO	1	1	0	0	0	0	0	1	0	0	0	0
PAL	4	0	3	1	0	0	0	4	0	0	0	0
PHL	20	4	4	0	2	8	6	18	0	2	0	0
QYB	2	2	0	0	0	0	0	2	0	0	0	0
QRF	2	1	0	1	0	0	0	2	0	0	0	0
SAK	3	0	1	0	1	1	0	3	0	0	0	0
TCR	9	1	0	0	0	1	7	9	0	0	0	0
THO	1	1	0	0	1	0	0	0	1	0	0	0
TIM	2	0	0	0	0	0	2	2	0	0	0	0
TUR	3	1	1	0	0	1	1	2	1	0	0	0
UUK	5	0	2	0	3	0	0	5	0	0	0	0
VSV	5	1	1	0	0	2	4	2	3	0	0	0
WAR	2	0	0	2	0	0	0	2	0	0	0	0
Ungrouped	76	31	17	10	4	10	10	72	2	2	0	0
Totals	359	105	85	40	32	83	86	305	40	11	2	1

TABLE 29. NUMBER OF VIRUSES ISOLATED FROM WILD CAUGHT ARTHROPODS

Antigenic Group	Total in Group	Isolated From						No. of Classes involved		
		Mosq.	Ticks	Phlebotomine Flies	Culicoides	Mites	Other	1	2	3
A	20	20	0	0	1	4	2	16	3	1
AHS	1	0	0	0	1	0	0	1	0	0
ANA	3	3	0	0	0	0	0	3	0	0
ANB	2	2	0	0	0	0	0	2	0	0
B	57	27	15	0	0	1	1	38	3	0
BAK	2	2	1	0	0	0	0	1	1	0
BLU	1	0	0	0	1	0	0	1	0	0
BTK	2	1	0	0	0	0	0	1	0	0
Bunyamwera Supergroup: BUN	18	17	0	0	2	0	0	17	1	0
Bunyamwera Supergroup: BWA	2	2	0	0	0	0	0	2	0	0
Bunyamwera Supergroup: C	11	11	0	0	0	0	0	11	0	0
Bunyamwera Supergroup: CAL	11	11	0	0	0	0	1	10	1	0
Bunyamwera Supergroup: CAP	6	5	0	0	0	0	0	5	0	0
Bunyamwera Supergroup: GMA	6	5	0	1	0	0	0	4	1	0
Bunyamwera Supergroup: KOO	2	2	0	0	0	0	0	2	0	0
Bunyamwera Supergroup: PAT	4	3	0	0	0	0	0	3	0	0
Bunyamwera Supergroup: SIM	16	8	0	0	8	0	0	10	3	0
Bunyamwera Supergroup: TETE	4	0	0	0	0	0	0	0	0	0
Bunyamwera Supergroup: SBU	7	5	0	0	0	0	0	5	0	0
CGL	2	0	0	1	0	0	0	1	0	0
CHF-CON	2	0	2	0	1	0	0	1	1	0
COR	2	2	0	0	0	0	0	2	0	0
DGK	5	0	5	0	0	0	0	5	0	0
EHD	1	0	0	0	0	0	0	0	0	0
EUB	2	2	0	0	0	0	0	2	0	0
GAN	2	2	2	0	1	0	0	0	1	1
HUG	4	0	4	0	0	0	0	4	0	0
KSO	3	0	3	0	0	0	0	3	0	0
KEM	15	0	15	0	0	0	0	15	0	0
KWA	1	1	0	0	0	0	0	1	0	0
MAL	2	2	0	0	0	0	0	2	0	0
MAP	3	3	0	0	0	0	0	3	0	0
MTY	3	0	0	0	0	0	0	0	0	0
MOS	2	2	0	0	0	0	0	2	0	0
NDO	1	1	0	0	0	0	0	1	0	0
PAL	4	3	0	0	1	0	0	4	0	0
PHL	20	4	0	13	0	0	0	15	1	0
QYB	2	0	2	0	0	0	0	2	0	0
QRF	2	0	2	0	0	0	0	2	0	0
SAK	3	0	3	0	0	0	0	3	0	0
TCR	9	1	1	0	0	3	0	3	1	0
THO	1	0	1	0	0	0	0	1	0	0
TIM	2	0	0	0	0	0	0	0	0	0
TUR	3	3	0	0	0	0	0	3	0	0
UUK	5	0	5	0	0	0	0	5	0	0
VSV	5	2	0	2	0	1	1	2	2	0
WAR	2	0	0	0	2	0	0	2	0	0
Ungrouped	76	32	17	2	2	0	1	46	4	0
Totals	359	184	78	19	20	9	6	263	23	2

TABLE 30. NUMBER OF VIRUSES ISOLATED FROM NATURALLY INFECTED VERTEBRATES

Antigenic Group	Total in Group	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Livestock	All Others	Number of Classes Involved					
										1	2	3	4	5	6
A	20	8	2	6	7	2	5	5	3	6	2	1	3	1	1
AHS	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
AHA	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	57	26	3	17	14	14	1	5	6	27	6	5	4	2	1
BAK	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0
BLU	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
BTK	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Bunyamwera Supergroup BUN	18	5	1	3	0	0	0	1	3	9	2	0	0	0	0
Bunyamwera Supergroup BWA	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Bunyamwera Supergroup C	11	9	0	8	0	1	5	0	1	2	6	2	1	0	0
Bunyamwera Supergroup CAL	11	2	0	2	0	1	0	0	0	5	0	0	0	0	0
Bunyamwera Supergroup CAP	6	0	0	3	0	0	1	0	0	2	1	0	0	0	0
Bunyamwera Supergroup GMA	6	2	0	5	0	2	3	0	0	2	1	0	2	0	0
Bunyamwera Supergroup KOO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bunyamwera Supergroup PAT	4	0	0	3	0	0	0	0	0	3	0	0	0	0	0
Bunyamwera Supergroup SIM	16	2	1	0	3	0	0	6	2	8	3	0	0	0	0
Bunyamwera Supergroup TETE	4	0	0	0	4	0	0	0	0	4	0	0	0	0	0
Bunyamwera Supergroup SBU	7	0	0	0	1	1	0	0	0	2	0	0	0	0	0
CGL	2	1	0	1	0	0	0	0	0	2	0	0	0	0	0
CUN	2	1	0	0	0	0	0	1	1	0	0	1	0	0	0
COR	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
DGK	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EHD	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
EUB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GAN	2	2	0	1	0	0	0	1	0	1	0	1	0	0	0
HUG	4	0	0	0	1	0	0	0	0	1	0	0	0	0	0
KSO	3	0	0	0	1	0	0	0	1	2	0	0	0	0	0
KEM	15	1	0	1	1	0	0	0	0	1	1	0	0	0	0
KWA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAL	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAP	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MTY	3	0	0	0	3	0	0	0	0	3	0	0	0	0	0
MOS	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
NDO	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0
PAL	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHL	20	5	0	5	2	0	1	0	2	9	3	0	0	0	0
QYB	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0
QRF	2	1	0	0	1	0	0	0	0	0	1	0	0	0	0
SAK	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
TCR	9	3	0	8	0	1	0	0	1	6	2	1	0	0	0
THO	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0
TIM	2	0	0	0	0	0	0	0	2	2	0	0	0	0	0
TUR	3	0	0	0	2	0	0	0	1	1	1	0	0	0	0
UUK	5	0	0	1	1	0	0	0	0	0	1	0	0	0	0
VSV	5	4	0	1	0	0	1	3	1	1	4	0	0	0	0
WAR	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped	76	11	1	10	13	8	0	4	5	39	5	0	0	0	0
Totals	359	86	9	76	58	30	17	29	30	150	40	11	10	3	2

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

Antigenic Group		Total in Group	In Nature	Lab Infection	Either or Both Number	Either or Both Percent
Group A		20	10	7	11	55.0
Afr.horsesickness		1	0	0	0	
Anopheles A		3	0	0	0	
Anopheles B		2	0	0	0	
Group B		57	27	22	29	50.9
Bakau		2	0	0	0	
Bluetongue		1	0	0	0	
Boteke		2	0	0	0	
Bunyamwera Supergroup	Bunyamwera	18	4	2	5	27.8
	Bwamba	2	1	0	1	50.0
	C	11	9	2	9	81.8
	California	11	4	0	4	36.4
	Capim	6	0	0	0	
	Guama	6	2	0	2	33.3
	Koongol	2	0	0	0	
	Patois	4	0	0	0	
	Simbu	16	2	1	2	12.5
	Tete	4	0	0	0	
	SBU	7	0	0	0	
Changuinola		2	1	0	1	50.0
CHF-Congo		2	1	1	1	50.0
Corriparta		2	0	0	0	
Dera Ghazi Khan		5	0	0	0	
Epizoot.hem.dis.		1	0	0	0	
Eubenangee		2	0	0	0	
Ganjam		2	2	1	2	100.0
Hughes		4	0	0	0	
Kaisodi		3	0	0	0	
Kemerovo		15	1	1	1	6.7
Kwatta		1	0	0	0	
Malakal		2	0	0	0	
Mapputta		3	0	0	0	
Matariya		3	0	0	0	
Mossuril		2	0	0	0	
Nyando		1	1	0	1	100.0
Palyam		4	0	0	0	
Phlebot. fev.		20	5	0	5	25.0
Qalyub		2	0	0	0	
Quaranfil		2	1	0	1	50.0
Sakhalin		3	0	0	0	
Tacaribe		9	3	3	3	33.3
Thogoto		1	1	0	1	100.0
Timbo		2	0	0	0	
Turlock		3	0	0	0	
Uukuniemi		5	0	0	0	
Vesic. stom.		5	3	3	4	80.0
Warrego		2	0	0	0	
Ungrouped		76	8	5	9	11.8
Totals		359	86	48	92	25.6

TABLE 32. EVALUATION OF ARTHROPOD-BORNE STATUS OF 359 REGISTERED VIRUSES (SEAS)

Antigenic Group	Total in Group	Arbovirus	Probably an Arbovirus	Possible Arbovirus	Probably not Arbovirus	Not an Arbovirus	Arbo or Probably Arbo		Not or Probably Not Arbo	
							No.	%	No.	%
A	20	14	3	3	0	0	17	85.0	0	
AHS	1	1	0	0	0	0	1	100.0	0	
ANA	3	0	2	1	0	0	2	66.7	0	
ANB	2	0	0	2	0	0	0		0	
B	57	29	7	14	2	5	36	63.2	7	13.0
BAK	2	0	1	1	0	0	1	50.0	0	
BLU	1	1	0	0	0	0	1	100.0	0	
BTK	2	0	0	2	0	0	0		0	
Bunyamwera Supergroup BUN	18	9	3	6	0	0	12	66.7	0	
Bunyamwera Supergroup BWA	2	1	1	0	0	0	2	100.0	0	
Bunyamwera Supergroup C	11	10	1	0	0	0	11	100.0	0	
Bunyamwera Supergroup CAL	11	6	2	3	0	0	8	72.7	0	
Bunyamwera Supergroup CAP	6	3	1	2	0	0	4	66.7	0	
Bunyamwera Supergroup GMA	6	4	0	2	0	0	4	66.7	0	
Bunyamwera Supergroup KOO	2	0	2	0	0	0	2	100.0	0	
Bunyamwera Supergroup PAT	4	1	1	2	0	0	2	50.0	0	
Bunyamwera Supergroup SIM	16	3	3	10	0	0	6	37.5	0	
Bunyamwera Supergroup TETE	4	0	0	4	0	0	0		0	
Bunyamwera Supergroup SBU	7	1	1	5	0	0	2	28.6	0	
CGL	2	0	1	1	0	0	1	50.0	0	
CON	2	1	0	1	0	0	1	50.0	0	
COR	2	0	0	2	0	0	0		0	
DGK	5	0	0	5	0	0	0		0	
EHD	1	0	1	0	0	0	1	100.0	0	
EUB	2	0	0	2	0	0	0		0	
GAN	2	0	0	2	0	0	0		0	
HUG	4	0	1	3	0	0	1	25.0	0	
KSO	3	0	1	2	0	0	1	33.3	0	
KEM	15	0	2	13	0	0	2	14.3	0	
KWA	1	0	0	1	0	0	0		0	
MAL	2	0	0	2	0	0	0		0	
MAP	3	0	1	2	0	0	1	33.3	0	
MTY	3	0	0	3	0	0	0		0	
MOS	2	0	0	2	0	0	0		0	
NDO	1	0	1	0	0	0	1	100.0	0	
PAL	4	0	0	4	0	0	0		0	
PHL	20	3	5	12	0	0	8	40.0	0	
QYB	2	0	0	2	0	0	0		0	
QRF	2	1	0	1	0	0	1	50.0	0	
SAK	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	
TUR	3	1	1	1	0	0	2	66.7	0	
UUK	5	1	1	3	0	0	2	40.0	0	
VSV	5	2	1	2	0	0	3	60.0	0	
WAR	2	0	0	2	0	0	0		0	
Ungrouped	76	5	9	53	6	3	14	18.4	9	11.8
Totals	359	97	53	184	8	17	150	41.8	25	7.0

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

During the past four months (Sept.-Dec.'74) the SEAS Subcommittee has evaluated 10 newly registered viruses. For want of sufficient information, all have been classified as "possible arboviruses". Six of the viruses are from the Far East (Malaysia and Singapore), two are from Scotland and one each from Canada and the Sudan.

Four viruses are presumably tick-transmitted agents, three may involve mosquitoes and the mechanism of transmission for the remaining three is unknown. It is conceivable that the two group B bat agents (Batu Cave and Carey Island)have a mosquito cycle as postulated for the related B bat agent Jugra (same bats); on the other hand, their isolation from salivary glands is sufficiently troublesome to suggest they probably are not arboviruses.

The evidence for Jugra and Avalon is a bit stronger than for the other viruses. Jugra was isolated from the blood of a bat in addition to the mosquito (Aedes) isolate from the same locality as well as from Uranotaenia mosquitoes from another locality. Avalon virus was recovered from 3 pools of ticks, nymphs, adult females and adult males; the latter is significant as the male Ixodes uriae does not take blood so its infection must stem either from transovarian transmission of virus or else infection acquired as a feeding larva or nymph. Avalon virus was also recovered from the blood of a naturally infected gull chick. Clo Mor virus (also from Ixodes uriae ticks) produced a viremia in baby mice when inoculated intraperitoneally as well as subcutaneously.

(Thomas H.G. Aitken)

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

Virus Evaluations, September through December, 1974

Name of Virus	Abbreviation	Group Complex	Suspected Vector	Isol. from nat. infect. arth.			Biological arth. transmission demonstrated				Arth. inf. by inj.		passage	Vertebrate viremia demonstrated			Rel	Epi	Negative information				Rating				Original Source and Host Source
	a	b	c	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
				Once only	2-9 times	≥10 times	By arth. infected by feeding	"Isolated" sentinel animal infected	By wild naturally infected arth.	By arth. infected by injection	Isolation from salivary glands	Isolation from whole arthropod	Transtadial or transovarial	Experimentally infected animal or man	In "isolated" sentinel (col. 5)	In feral animal or man by other than lab infection	Antigenically related to proven arbovirus	Strong epidemiologic associations	Unusual physical, chemical or biologic characteristics	No replication in suspect arthropod	Virus found in vertebrate salivary glands, urine, or feces	Neg. transtadial passage of tick isolate	Arbovirus	Probable arbovirus	Possible arbovirus	Probably not arbovirus	Not arbovirus	
Avalon		SAK	T		X											X									X			Canada Tick
Batu Cave		B															X				X				X			Malaysia Bat
Cape Wrath		KEM	T	X																					X			Scotland Tick
Carey Island		B															X				X				X			Malaysia Bat
Clo Mor		SAK	T	X										X											X			Scotland Tick
Jugra		B	M		X											X	X								X			Malaysia Mosquito

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

Virus Evaluations, September through December, 1974

Name of Virus	a	b	c	Isol. from nat. infect. arth. 1	2	3	Biological arth. transmission demonstrated 4	5	6	7	Arth. inf. by inj. 8	9	Passage 10	Vertebrate viremia demonstrated 11	12	13	Rel. 14	Epi 15	Negative information 16	17	18	19	Rating 20	21	22	23	24	Remarks
Malakal		MAL	M		X															X					X			Sudan Mosquito
Puchong			M	X																					X			Malaysia Mosquito
Seletar		KEM	T		X																				X			Singapore Tick
Tanjang Rabok								X						X	X										X			Malaysia Monkey

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY,
UNIVERSITY OF MELBOURNE,
VICTORIA, AUSTRALIA.

1. Laboratory Studies with Mapputta Group Viruses

By electron microscopy of thin sections of infected mouse brain or Vero cell cultures, it was shown that Mapputta (MAP), Trubanaman (TRU) and Maprik (MPK) were typically like Bunyamwera virus in morphology and morphogenesis. Since they do not serologically belong in the Bunyamwera Supergroup, they must be described as Bunyavirus-like. Studies were undertaken to evaluate their suitability as laboratory models for biochemical studies of this type of virus.

In Vero cell cultures, growth curves showed that MAP virus grew to the highest titre ($0.5\text{-}1 \times 10^7$ pfu/ml. released at 45 hr.) but the plaques were small (0.5-1 mm.) and difficult to count even after 4-5 days incubation. TRU virus produced easily countable plaques, mostly 2-6 mm. diameter, at 4 days. Despite this fact, in cultures infected at multiplicities between 0.2 and 1.0 pfu/cell maximum yields were about 1×10^6 pfu/ml. at 48 hrs., and yields decreased with increase in multiplicity of infection.

Growth of TRU virus was not inhibited in the presence of 0.1 μg/ml. actinomycin D or of 10^{-4} M 5-bromodeoxyuridine, showing that the viral genome is RNA. A purification procedure involving centrifugation through a zone of 30% w/w sucrose on to a cushion of 60% w/w sucrose was worked out, but yields were too low for chemical studies. At pH 7.6 the heat stability was moderate (half life about 5 hr. at 37°) but infectivity was extremely labile below pH 7. The sedimentation coefficient of TRU virus, measured in a 5-20% w/w sucrose gradient, was 570S.
(Lisbeth Ann Bowles and Ian H. Holmes.)

2. Morphological and Chemical Studies on Orbiviruses

Two relatively new orbiviruses isolated by Dr I. D. Marshall, Canberra, were studied by electron microscopy. They were Japanaut virus, from Papua New Guinea and Tilligerry virus, from Nelson Bay, New South Wales, Australia (not yet catalogued). Intracytoplasmic inclusions produced by Tilligerry virus contained striated structures apparently composed of parallel sheets. These resembled, but may not be identical to those observed in cells infected with Kemerovo group viruses.

D'Aguilar (DAG) and Eubenangee (EUB) viruses were purified and analysed by poly acrylamide gel electrophoresis. Because of the yields and behaviour during purification, DAG was much easier to handle than EUB virus. Nevertheless it was possible to show that each contained 10 segments of double stranded RNA, with molecular weight distribution quite distinct from that of reovirus. The gel patterns obtained with DAG and EUB virus RNAs were similar overall, but diverging in the low molecular weight region.

Purified DAG virus yielded 8 polypeptide bands, of which two were enhanced in preparations from sucrose gradients, but almost disappeared after an equilibrium centrifugation (density 1.36) in caesium chloride. By analogy with Bluetongue virus, this suggests that the variable two are outer shell polypeptides, and the other 6 form a more stable virus core.
(Roger D. Schnagl and Ian H. Holmes.)

REPORT FROM THE VIRUS LABORATORY, DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE, UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA

Bovine ephemeral fever virus.

1. Production of experimental vaccines.

Two types of attenuated vaccines have been used to vaccinate housed cattle, which are then experimentally challenged with virulent virus, and to vaccinate cattle exposed in the field to natural challenge. The first vaccine was made from the brains of suckling mice infected with the 525 strain of bovine ephemeral fever virus and the second from cultures of Vero cells infected with the 919 strain. Both vaccines can be lyophilised, and both are mixed with aluminium hydroxide adjuvant immediately before inoculation. After two vaccinations neutralising antibodies have persisted for at least 8 months, and in some animals for longer than 15 months. Vaccinated animals developed no clinical disease when inoculated intravenously with virulent virus.

Cattle in two herds were exposed to natural infection 7 months after vaccination with mouse-brain virus. Eight of 11 control animals developed clinical disease, as did 3 of 26 vaccinated animals. Clinical bovine ephemeral fever has not yet occurred in any of the herds receiving cell culture vaccine.

Pathogenesis of bovine ephemeral fever.

Six pregnant heifers lacking neutralising antibody to bovine ephemeral fever virus were inoculated with virulent bovine ephemeral fever virus. All heifers developed clinical disease, and all subsequently produced normal calves. Foetuses of immune cows were inoculated with bovine ephemeral fever virus. There was no apparent effect on the foetuses, and one calf, inoculated after 160 days of gestation, had neutralising antibody to bovine ephemeral fever virus in precolostral serum.

Newborn colostrum-deprived calves developed clinical ephemeral fever when inoculated with avirulent bovine ephemeral fever virus. Calves that had received colostrum resisted infection, even when the colostrum contained no neutralising antibody to ephemeral fever virus. The 525 strain produced fatal encephalitis in calves

that were inoculated by the intracerebral route. Calves 2 months of age resisted intravenous challenge with virulent virus, but calves 4 months of age were fully susceptible.

Field strains of low pathogenicity.

During vaccine trials, it appeared that field strains of ephemeral fever virus of low pathogenicity were circulating. Some control cattle were developing neutralising antibody in the absence of obvious clinical disease. Two strains of virus, L73/1 and 461 were isolated from the blood of cattle with very mild clinical signs of ephemeral fever. The L73/1 strain was used to inoculate 14 susceptible calves. Four calves developed mild clinical signs of ephemeral fever after 7 to 10 days, and 10 remained clinically normal. Only 7 of the calves developed neutralising antibody. One of 2 calves inoculated with the 461 virus developed mild ephemeral fever, but both developed antibodies.

(P. Spradbrow and S. Tzipori)

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

Serological conversion for dengue and chikungunya infectiom in the boarders of two hostels in Calcutta.

To know the dengue and chikungunya infection rates, 160 boarders of two hostels in Calcutta were bled in 1964, 135 in 1965, 118 in 1968 and 77 in 1969. The number of boarders common in 1964-65 and in 1968-69 were 47 and 28 respectively. On the basis of serological conversion in the latter groups , chikungunya infection rate was 21.3% in 1964-65 and 14.2% in 1968-69. The 1964-65 sera were not available for testing for Gr. B antibodies and the dengue infection rate in 1968-69 sera was 21.4%. Of the 14 subjects, showing serological conversion to chikungunya, 5 did not give any history of fever, indicating the possibility of inapparent infection. Similarly, out of 6 subjects with conversion of Gr. B antibodies, one did not give history of fever. Thus it seems that, based on the history alone, (which is not always reliable) inapparent infection by dengue and chikungunya is possible.

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

REPORT FROM THE NATIONAL HEALTH LABORATORY
RANGOON, BURMA

Japanese encephalitis in Burma.

In July 1974, an outbreak of encephalitis occurred in Tachileik Township in the southeastern part of the Union of Burma. During the period between July 11 and August 7, five cases were hospitalized in Tachileik. The patients developed high fever, severe headache, neck rigidity, disorientation and sensorial changes, twitching of muscles, aphasia, coma and death. Most of the cases had CSF under pressure. Four of the cases were children under 11 years of age; the other patient was 30 years old. Three were males and two were females. Four came from rural villages and one from the town. Four died before their blood samples could be collected. Convalescent serum from the only survivor (a male of 7 years) showed an HI titer of 1/640 for JE. N antibody tests (done in the Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu, USA by Dr. S.B. Halstead) showed a titer of 1/80 on this same serum. Eight sera collected from contact cases had no detectable antibodies to either JE or dengue. Investigations confirmed the presence of Culex tritaeniorhynchus in the affected township.

Clinical findings, together with the epidemiological situation (an outbreak of JE reported in Chiang Mai Valley of Thailand, bordering the affected township) and positive JE antibodies of the sole surviver, suggests the occurrence of JE in Burma. This is the first recognition of JE in Burma.

Dengue haemorrhagic fever in Burma, 1974.

In January 1974, an epidemic of febrile diseases commenced, presenting with one or a mixture of manifestations like rash, bone and joint pains, cough, vomiting and lymphadenopathy. A considerable number of cases also had haemorrhagic manifestations and some went into dengue shock syndrome. The peak of the epidemic was in August and September, and the epidemic lasted until December 1974. The majority of the cases were from Rangoon, and very few were from districts. Most of the cases were from the 1 to 10 year age group.

From Rangoon, 1576 paired sera and 86 convalescent sera were examined by the HI test for dengue and chikungunya antibodies. From the paired sera, 318 (20.1%) were positive for dengue, 107 (6.7%) were positive for chikungunya and 57 (3.6%) were positive for both dengue and chikungunya. Single sera were tested only with dengue and 30 (35.2%) were found to be positive.

From the districts, 23 paired and 13 unpaired sera were tested for infection with the above-mentioned viruses. From the paired sera, 9 (39.1%) were positive for dengue, 1 positive for chikungunya and 1 positive for dengue and chikungunya mixed infection. From unpaired sera only one was positive for dengue.

In 1971, we had an outbreak of febrile diseases with some haemorrhagic manifestations which were serologically proved to be caused by influenza A. This has prompted us to test the negative sera for dengue and chikungunya viruses with influenza viruses. From 507 paired sera tested, 138 (27.2%) were positive with influenza A and 86 (16.9%) with influenza B viruses.

From the above it may be concluded that the epidemic was caused by dengue viruses.

(Than Swe and Soe Thein)

REPORT FROM THE SEATO MEDICAL RESEARCH LABORATORY
BANGKOK, THAILAND

I. Dengue hemorrhagic fever (DHF) and shock syndromes (DSS): immunological evidence for primary dengue infections in younger children

Serum IgM antibody generally appears after the first infection with dengue virus but not after a second dengue infection. Thus in dengue fever outbreaks, IgM antibody to dengue has been detected in serum of persons having uncomplicated fever and serological patterns diagnostic of primary, or first dengue infections. By contrast, IgM anti-dengue antibody has not been detected in persons who develop DHF; these persons mostly have HAI serological patterns diagnostic of secondary dengue infections. We studied 4 patients admitted to Bangkok Children's Hospital in 1971 with clinically confirmed DHF or DSS. These 4 children were 4 to 6 months old and, unlike most DHF patients, had HAI serological patterns consistent with primary dengue infections. Dengue-2 virus was isolated from one child who developed severe DSS. A description of this patient (No. 26) was published in the Bulletin of the WHO, vol. 48, 117, 1973. Serum complement (C3) levels were measured in this child and in one patient with DHF, and the levels were abnormally low. All 4 patients developed detectable IgM antibody to dengue, 4 to 14 days after the onset of illness. This antibody was heterospecific and cross-reacted by HAI with one or more of the 4 dengue serotypes and with Japanese encephalitis virus antigens. Two of 5 primary dengue fever patients from Koh Samui, Thailand also developed heterospecific IgM antibody. There is no clear explanation for the occurrence of heterospecific IgM dengue antibody in Thailand. Human IgM antibody found in other group B arbovirus infections and in New World dengue fever has found to be monospecific for the infecting virus serotype. Nevertheless, we believe the appearance of IgM antibody to dengue, irrespective of its specificity, confirms the routine serological diagnosis of a first dengue infection, and thus supports the contention that Asian patients under the age of one year can develop DHF or DSS after a primary dengue infection.

(Robert Edelman, Anong Pariyanonda)

II. Evaluation of the plasma kinin system in dengue hemorrhagic fever

Clinical, laboratory and postmortem observations suggest that humoral mediators may play a role in causing the hypovolemia and shock that occurs in severe dengue hemorrhagic fever (DHF). There is circumstantial evidence that the plasma kinin system, in addition to the complement system, may play a role in DHF. We studied the plasma kinin system in patients with DHF by simultaneously measuring factor XII (Hageman Factor), prekallikrein, Kallikrein inhibitors, bradykinin, and complement in the blood of Thai children with DHF and other acute febrile illnesses. The activities of Factor XII, prekallikrein and Kallikrein inhibitors were measured using functional enzymatic assays. Bradykinin concentrations were measured by

radioimmunoassay. Prekallikrein, factor XII and C3 levels were significantly lower in DHF patients compared to other febrile patients; the mean lowest levels were found in dengue patients with shock. However, bradykinin concentrations were not elevated and the mean activity levels of Kallikrein inhibitors were not depressed in dengue patients. Two DHF patients were studied 3 and 5 days before they developed shock; both patients had low prekallikrein and factor XII levels on hospital admission which were rising to normal levels when they went into shock. By contrast, their C3 levels were normal on admission, but began to fall just before the onset of shock. Taken together, these results fail to provide convincing evidence for activation of the plasma kinin system leading to free bradykinin or a significant role for bradykinin in the immune-pathogenesis of DHF. The results do refocus attention on complement as a potentially important humoral mediator of the dengue shock syndrome.

(Robert Edelman and Franklin H. Top, Jr., SEATO Med Lab; Suchitra Nimmannitya, Bangkok Children's Hospital; Robert W. Colman and Richard Talamo, Harvard Medical School.)

III. Evidence of dengue virus cross-protection against Japanese encephalitis in Thai patients

Epidemiological studies in Guam, Thailand and Florida suggest that dengue virus infections partially protect against the development of symptoms of encephalitis in persons subsequently infected by Japanese encephalitis or St. Louis encephalitis viruses. In order to determine if prior dengue virus infection reduces the severity of Japanese encephalitis (JE), we examined 127 patients hospitalized during the 1970 JE epidemic in the Chiangmai and Lampang Valleys of northern Thailand. Patients were studied during the first 30 days after onset of JE; 120 of these patients were examined one year later for residual sequelae. About 21% of patients had serological evidence of a prior dengue virus infection. Morbidity and mortality in patients with and without prior dengue virus experience were compared. These comparisons were made within two age groups ($\leq$ 10 years and $\geq$ 11 years) to exclude differences due to age alone. Death occurred in 7% of dengue-positive and in 19% of dengue-negative patients. During the first 30 days significantly fewer dengue-positive survivors had convulsions or abnormal muscle tone, and they also tended to have less coma, motor paralysis, tremor, ataxia and abnormal electroencephalograms compared to dengue-negative patients; the mean numbers of abnormal neurological signs observed in dengue-positive and - negative patients were 1.8 and 2.5, respectively ($p<0.005$). By one year both groups of patients had shown considerable recovery, but dengue-positive again had significantly fewer neurological residua. These results suggest that prior dengue infection moderately reduces the morbidity, and possibly the mortality, of persons hospitalized with Japanese encephalitis.

(Robert Edelman and Robert J. Schneider, SEATO Med Lab; Pien Chieowanich and Rungsri Pornpibul, Chiangmai University; Prathan Voodhikul, McCormick Hospital.)

IV. Long-term persistence of JEV IgM antibody after Japanese encephalitis

Increased amounts of virus-specific IgM antibody have been reported in human serum during the acute phase of many virus diseases, with a rapid return of IgM to normal levels within 60 to 90 days after onset in most patients. In a few patients the virus-specific IgM antibody levels were still raised after 3 months to 2 years; many of these patients suffered late clinical sequelae, consistent with the current immunological theory that persistent virus-specific IgM antibody is the result of a continuing virus stimulus. Japanese investigators report that anti-JEV HAI antibody has been found in the cerebral-spinal fluid of JE patients having long-term clinical sequelae following their acute illness, and "continuing active inflammation" of the brain has been reported at autopsy of some Japanese patients many years after their acute illness (Professors Goto, Shiraki, and Ishii).

We have searched for evidence of a chronic JE infection in Thai patients convalescing from acute JE. These patients, hospitalized in Chiangmai and Lampang Valleys of northern Thailand in 1970, were evaluated for the presence or absence of 7 abnormal neurological signs and bled at intervals for 1 to 2 years. Sixty-five of 100 JE patients were tested for JEV-specific IgM antibody; 41 were IgM positive. In 6 patients, specific IgM was detected in sera obtained more than 3 months after the onset of illness, on days 116, 121, 155, 218, 320, and 420. Neurological sequelae were compared between the 6 persons with persisting IgM and 94 other (control) patients. Despite the small number of long-term IgM patients, the composition of the 2 groups closely resembled each-other in terms of age, sex, and prior dengue infections.

The 6 patients seemed to have an unusually severe acute clinical course. They developed significantly more abnormal neurological signs than the control group of patients during the first 30 days; the mean number ($\pm$ SD) of abnormal signs per patient for the IgM and control groups was 3.8 ± 0.8 and 2.3 ± 1.3, respectively ($P<.01$). After one year both groups showed considerable recovery, but the long-term IgM patients still had significantly more abnormal neurological signs than controls (1.3 ± 0.8 versus 0.6 ± 1.1 ($P<.01$)); neurological sequelae were detected in 80% of IgM patients but in only 29% of control patients ($P<.025$). However, the rate of recovery (loss of abnormal neurological signs) in the IgM patients was equal to or slightly greater than, recovery in controls over the first convalescent year. The long term IgM group was reexamined at 2 years and they showed no residual neurological impairment. Lumbar punctures were not performed at 1 year, but they were done at the 2 year follow-up exam in the 6 IgM patients. The cerebral spinal fluids contained normal cellular, protein, and immunoglobulin concentrations. No IgM immunoglobulin was detected in the CSF using the sensitive electro-immunodiffusion technique. HAI antibody to dengue or JEV was found in several CSF specimens from IgM patients, but the titers were low ($\leq$ 1:8) and did not differ from 12 Thai patients who had other neurological diseases. We have thus been unable to find evidence of a continuing clinical

or subclinical JEV infection two years after the onset of Japanese encephalitis in 6 patients with persistent IgM antibody. The severe acute illness and the relatively rapid rate of recovery in these patients suggests that IgM persistence may be related to the virulence of the acute infection rather than to any possible chronicity of the infectious process.

(Robert Edelman, Robert J. Schneider, Athasit Vejjajiva, Rungrsi Pornpibul, Prathan Voodhikul)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Interferon Induction by Rice Dwarf Virus RNA and Its Antiviral Activity against Certain Arbovirus Infections

Double-stranded RNA extracted from purified rice dwarf virus (RDV) has been shown to be highly active in inducing interferon (IF) production. In addition, IF inducing capacity of RDV-RNA was more resistant to some hydrolytic enzymes or physical agents such as heat and UV-irradiation, as compared to polyriboinosinic-polyribocytidylic acid (Poly I:C). Higher host-resistance to CHIK virus infection was obtained in chick embryo (CE) or rabbit kidney (RK 13) cells exposed to RDV-RNA compared to Poly I:C. Antiviral activity of the IF induced by RDV-RNA was approximately equivalent to that Poly I:C in RK cells. The highest activities by both RNA inducers were shown in CE and RK cells while no IF production could be induced in BHK-21 and Aedes albopictus cells. On the other hand, a significant reduction in mortality was observed in mice infected intraperitoneally with about 10^3 x LD_{50} of WEE virus when a single dose of RDV-RNA (2.5 to 10 mg/kg) was given 3 to 24 hours prior to the virus challenge. The protective effects by RDV-RNA treatment against WEE infection in mice were significantly higher than those by Poly I:C of the same dose administered intraperitoneally.

References :

1. Takehara, M. et al. : Studies on interferon production and antiviral activity by rice dwarf virus RNA. Abstracts, 22nd Annual Meeting, Society of Japanese Virologists, pp. 3025, 1974.
2. Takehara, M. : Interferon inducing capacity and antiviral action of double-stranded RNA from rice dwarf virus in vitro. Kobe J. Med. Sci. Vol. 21, No. 1, 1975 (in press).

Interferon Production and Antiviral Activities Induced by Chikungunya and Dengue Virus Infections

Interferon (IF) inducing ability of Chikungunya (CHIK) and dengue (DEN) virus infections was comparatively studied using several mammalian and mosquito cell lines. IF production by CHIK was higher than those by DEN in RK 13, mouse L and LLC-MK_2 cells. Highest antiviral activity of the IF was shown in RK cells, whereas no IF production was induced either by CHIK or DEN in BHK-21 and *Aedes albopictus* cells. The IF inducing capacity of both viruses heated at 56°C for 15 minutes or irradiated with UV for 3 minutes was markedly reduced. The IF inducing capacity of CHIK-RNA extracted from the infected cells was generally higher than that of DEN-RNA, but its activity was significantly lower than that by infection of active CHIK virus. Cellular RNA and protein syntheses in RK cells were markedly inhibited by infection with CHIK virus, while DEN virus infection did not result in a clear suppression of macromolecular syntheses in the host cells.

Reference :

Takehara, M. : Studies on interferon induction and antiviral activity by certain arboviruses. Kobe J. Med. Sci. Vol. 21, No. 1, 1975 (in press).

(M. Takahara)

Further experiments on the microculture plaque formation techniques for group B arboviruses.

The techniques described previously for DEN-1 virus (N. Fujita, et al., Arbovirus Info-Exchange, No. 27, September 1974, p. 145; Proc. Soc. Exp. Biol. & Med. 148, 472, 1975) have been successfully applied to yellow fever virus. Easily countable plaques (about 1 mm in diameter) can be produced 5-6 days after inoculation of 17D strain virus. Virus neutralization tests by use of "piggy back" transfer-plates are also feasible.

By using a microtiter plate sealer (Cooke Engineering Co.), instead of a plastic lid, the cultures can be inocubated in an ordinary incubator without any CO_2 aeration device. Substitution of 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) buffer for bicarbonate is applicable, but plaque sizes produced under the HEPES medium appear to be smaller than those produced in the medium without HEPES.

The microculture plaque method is simple and easy to perform and can be applied to certain representative group B arboviruses for titration and neutralization.

(N. Fujita and M. Yamamoto)

Production of Incomplete Chikungunya Virus in BHK-21 Cells Cultivated with Low Ionic Strength Media

Further aspects of the mechanism underlying the phenomenon that the production of Chikungunya(CHIK) virus in BHK-21 cells was inhibited by lowering the sodium chloride concentrations of the culture media, were investigated. Thin-sectioned cells directly fixed with glutaraldehyde either in low(L) or normal(N) ionic strength phosphate buffer solutions were examined electron microscopically. Viral particles concentrated from L or N-culture fluids were observed by the negative staining method. Morphological features noted in the L-cultures were: (1) Inhibition of the virus budding process and the aggregation of the viral cores beneath the cell membrane; (2) precipitation of apparently immature viral particles attached to the surface of the membrane; and (3) release of less infectious, irregular particles which could not be seen in control non-infected samples. The incompleteness of the virus produced in the L-cultures was confirmed in experiments in which it was shown that the ratio of specific activity of the ^{3}H-uridine labeled fraction to infectivity was higher in the L-medium samples than in the N-medium samples. These data suggest that the inhibitory effect of low ionic strength upon virus production is due to an abortion of the envelopment of the viral cores that results in the release of less infectious, incomplete viral particles.

Reference:

Matsumura, T., and Hotta, S. : Production of incomplete chikungunya virus from cells cultivated with low ionic strength media. Proc. Ist. International Congress of Intersectional Association of Microbiological Societies. University of Tokyo Press, Vol. II, 1975.

(Matsumura, T., Shiraki, K. and Hotta, S.)

(Susumu Hotta)

REPORT FROM DEPARTMENT OF VIRUS ECOLOGY, THE D.I. IVANOVSKY INSTITUTE OF VIROLOGY, ACADEMY OF MEDICAL SCIENCE, MOSCOW, USSR

Some results of the work carried out in 1973 are communicated. Attention was largely focused on examination of ticks (Table 1) as in previous years. We examined 63802 ticks of 10 genera and 29 species collected in the European part of the country, the Caucasus, Central Asia, Siberia and the Far East. 102 isolates are under study to date. Virus strains isolated during previous years are being studied and identified.

In 1972 Ornithodoros papillipes ticks collected in the vicinity of Karshi (Uzbekistan) yielded 3 identical strains (prototype LEIV-2247 Uz) antigenically related to group B. LEIV 2247 Uz is most closely related antigenically to West Nile virus but possesses unilateral relations. The virus which was given a name "Karshi" appears to be a new group B virus. Karshi virus has been forwarded to YARU (USA) for confirmation of identification.

In Turkmenistan Hyalomma asiaticum ticks yielded 5 strains of virus antigenically similar to Wad Medani virus of the Kemerovo group. Confirmatory identification of the prototype strain (LEIV-2534 Tur) is being carried out in YARU (USA).

In the north of the European part of the USSR, Murmansk province, 16 strains of Okhotskiy virus (Kemerovo group) were isolated from Ixodes putus ticks; Okhotskiy virus was first isolated in 1971 from this same tick species in the Far East.

Studies on the ecology of Baku virus (Kemerovo group) isolated from Ornithodoros capensis ticks collected in a colony of Larus argentatus in Azerbaijan in 1971 were continued. Two strains of this virus have been isolated from O. coniceps ticks from the nests of wild doves (Columbia livia) in Uzbekistan as well as from the same tick species collected at the nesting grounds of Larus argentatus (2 strains) and Sterna hirundo (2 strains) in Turkmenistan on the eastern littoral of the Caspian sea.

In Turkmenistan we isolated a strain of West Nile virus from Hyalomma detritum ticks as well as a strain of CHF virus from H. asiaticum.

In Azerbaijan, foci of CHF-Congo (isolated from H. plumbeum and Rhipicephalus bursa ticks) were discovered for the first time and found to be associated with cattle.

In Armenia, strains of Bhanja virus were isolated from Dermacentor marginatus and also in Kirghizia from H. plumbeum.

Tahyna virus was isolated from Culex pipiens mosquitoes collected in Tajikistan. This is the second time this virus has been isolated in the USSR (the first isolation was in Azerbaijan).

The laboratory now has reference mouse ascitic fluid preparations for 80 arboviruses, mainly tick-borne, as shown in Table 2.

(D.K. Lvov)

TABLE 1. EXAMINATION OF TICKS IN 1973

(The numerator is the number of ticks examined; the denominator is the number of isolated ticks.)

EXAMINATION OF TICKS IN 1973

(the numerator is the number of ticks examined, the denominator is the number of isolated strains)

TABLE 1

Regions	Species of ticks	Ixodes				Hyalomma							Boophilus	Haemaphysalis			Dermacentor			Rhipicephalus				Argas			Ornithodoros				Haemaphysalis	Total (by regions)
		persulcatus	ricinus	putus	signatus	plumbeum	anatolicum	asiaticum	dromedarii	aegyptium	detritum	in sp.	calcaratus	neumanni	concinna	numidiana	sulcatus	marginatus	silvarum	nuttalli	turanicus	bursa	rossicus	in sp.	persicus	vulgaris	vespertilionis	capensis	coniceps	papillipes	lahorensis	nidi	
European part	Ukrainian SSR					899/0																	378/1									I277/I
	Murmansk region			I294/27																												I294/27
Caucasus	Azerbaijan SSR		750/0			I229/6						63/0	584/0								I45/0	28I/I						I550/27				4602/34
	Armenian SSR					3I/0		66/0		I3/0							26/0	3478/3				II/0									600/0	4225/3
Central Asia	Turkmen SSR					I279/I	I0I8/I	6681/9	I726/0		38/I		I34/0			38/0					2095/0	92/0		500/0	3I7/0				I475/4		56/0		I54II/I6
	Tadzhik SSR																	428/I														428/I
	Kirghiz SSR					227/0		I2/0				300/0									I05/0						I40/0						784/0
	Uzbek SSR					5/0	I9I6/0	685/0				II500/0	I78/0			40/0									7/0	I00/0			222/2	49/0	42/0		I4744/2
Siberia	Altai Territory																			I665/0												I665/0
Far East	Primorye Territory	I286/3												I4300/0																		I5586/3
	Khabarovsk Territory	I620/0													293/0				I3/0													I926/0
	Commodore Islands			I450/I5	4I0/0																											I860/I5
	Total (by species)	2906/3	750/0	2744/42	4I0/0	3670/7	2934/I	7444/9	I726/0	I3/0	38/I	II863/0	896/0	4300/0	293/0	78/0	26/0	3906/4	I3/0	I665/0	2345/0	384/I	378/I	500/0	324/0	I00/0	I40/0	I550/27	I697/6	49/0	98/0	600/0	63802/I02

Table 2

A LIST OF IMMUNE SERA AND IAFs PREPARED IN THE LABORATORY OF THE ECOLOGY OF ARBOVIRUSES

Name of the group:	Serum
Ungrouped	Colorado tick fever, Lone Star, Matucare, Togoto, Nyamanini, Bhanja, DGK. Dhori, Wanowrie, Hpolu, A-279 (Uukuniemi), A-63 (Caspiy), Batken, Issyk-Kul (K-315, K-424), P-776, P-113, Sakhalin (71c), Uz 858, Sawgrass, Uz 2247, C 2268, Uz 1308, Uz 1577, Oita, K-760, Uz 1311, A 2373
Turlock	Turlock, Umbre
Group A	Sindbis, Getah
Simbu	Aino, Akabane, Sabo, Sango, Satuperi, Simbu, Jaba-7, Samford
Bunyamwera	Batai, Bunyamwera, Shokwe
Hughes	Hughes
Congo	Congo, Xazara
Group B mosquito-borne	Japanese encephalitis, Saint Louis, West Nile (Africa), West Nile (India)
Kemerovo	Kemerovo, Chenuda
Kemerovo	Tribec, Punta-Salinas, Wad Medani, (A-46) Baku, (Ka-287) Akhotskiy
Bakau	Bakau
Ganjam	Dugbe
Kaisodi	Kaisodi, Lanjan, Silverwater
Uukuniemi	Uukuniemi, Grand Arbaud, (Ka 271) Zaliv Terpeniya (1470-M)
Qalyub	Qalyub, Bandia
Quaranfil	C-5502
Group B (tick-borne)	Powassan, Lonping ill, tick-borne encephalitis, KFD, Langat, Kadam, (K-400) Sokuluk, (6c) Tyuleniy

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

Molecular virology of the Uukuniemi virus.

Two glycosylated envelope proteins could be separated from themselves in a 15% polyacrylamidegel-electrophoresis. Internal ribonucleoprotein was released with Triton X 100 and analyzed on sucrose gradients. Three species of RNP sedimenting at 140-150 S, 105-120 S and 85-90 S could be separated. All of them contained the same ratio of core polypeptide (m.w. 25 ooo daltons) to RNA. Electron microscopy using rotatory shadowing showed that all three species were circular. Free ends were rarely seen. Measurements of the strands revealed three distinct length classes of about 2.8 um, 1.4 um and 0.7 um. In polyacrylamidegel-electrophoresis the largest RNP contained the L RNA, the medium-sized RNP the M RNA and the smallest RNP the S RNA.

(Ralf Filip Pettersson)

Tick-borne viruses in seabird colonies.

A field team made a three-day visit in July,1974, in the bird islands of Röst, Lofoten, Norway. 1812 samples of Ixodes uriae (=putus) were collected from rock crevices, most of them engorged nymphs and larvae. After moult, ticks were divided in pools and processed for isolation. The 196 pools yielded some 60 isolates, which are under typing with the aid of immune ascites fluids kindly sent by Dr. A. Main, Y.A.R.U. So far twelve orbiviruses of the Kemerovo group have been identified.

(P. Saikku,M. Brummer, I.Ulmanen)

References:

Pettersson RF, vonBonsdorff C-H. The ribonucleoproteins of Uukuniemi virus are circular. J. virology (in press)

REPORT FROM THE ARBOVIRUS UNIT, LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

Virus isolation studies are continuing on material collected by the Medical Research Council Project, Kisumu, Kenya. Five strains of a virus provisionally identified as Pongola have been isolated from pools of Mansonia uniformis collected in CDC-type light traps in and around houses on the Kano Plain and on the Ahero Ricefields. A single strain of Sindbis virus has been isolated from a pool of Anopheles gambiae. Six strains of a virus closely resembling Ilesha virus were isolated from three pools of M. uniformis, two pools of A. gambiae and a single pool of Anopheles funestus. A strain of Ilesha virus has also been isolated from a liver/spleen suspension of a rodent (Aethomys kaiseri). Two further isolates obtained from pools of A. funestus and Culex antennatus respectively have not yet been identified.

Two strains of a rhabdovirus, Barur, were isolated from a pool of M. uniformis and from a mixed pool of unidentified fleas taken off several shrews (crocidura spp). 11 additional isolates, none of which has yet been identified, have been made from tissues taken from a bird (Lanius collaris) and from a variety of small mammals including Aethomys kaiseri, Arvicanthis abyssinicus, Pelomys fallax and Otomys species. No human or tick isolates have yet been obtained.

Work is under way to identify two virus strains isolated from ticks identified as Ornithodoros maritimus which were collected on Puffin Island near Anglesey, North Wales. The ticks were found in or around nests of herring gulls, Larus argentatus. No virus was isolated from blood samples taken from 81 juvenile herring gulls and shags Phalacrocorax aristotelis.

A third virus strain was isolated from ticks found in a kittiwake, Rissa trydactyla, colony at St Abb's head, Berwick.

D I H Simpson and
B K Johnson

REPORT FROM THE PRINCE LEOPOLD INSTITUTE OF TROPICAL MEDICINE
ANTWERP, BELGIUM

Replication of arboviruses in mouse organ cultures.

With the purpose of obtaining information on the pathogenesis of experimental arbovirus infection, studies were performed on mouse organ cultures. Small fragments of mouse organs, dipped in a virus suspension and rinsed several times, were placed in contact with tissue culture medium and examined at different time intervals for the appearance of virus.

Middelburg, Semliki Forest (virulent and avirulent strains) and WEE viruses were used, and compared with EMC virus.

The most important results can be summarized as follows:

1. Functional organ cultures can be initiated from animals aged 0-3 weeks, and will remain viable, by the method used, for 7-8 days.

2. Skeletal muscle tissue is the most important multiplication site for arboviruses; it can be taken from mice aged 0-3 weeks.

3. Viruses whose lethal effect is limited to young mice multiply in organs cultured in vitro from somewhat older animals; for example, MBL (large plaque variant of Middelburg virus) does not kill mice older than 4-5 days but will multiply in muscle and brain taken from mice up to the age of 10-16 days. Viruses whose lethal effect extends to adult age will multiply in organ tissue cultures taken from animals up to 21 days old. This indicates that in the intact animal the age dependent susceptibility for arboviruses is determined by intrinsic organ specificity and host defense mechanisms.

4. The parallelism in host-parasite relationship between the intact animal and in vitro cultured organs is further illustrated by the comparison between MBL and S (small) plaque virus: the latter multiplies after a longer lag phase in vitro; in vivo, peak viremia titers also appear 24 hours later than after inoculation of the MBL variant.

5. Avirulence of the avirulent SF virus strain used is due to a significantly lower neurotropism; its ability to multiply in organ-cultured brain was much less than that of the virulent strain. In muscle, however, the degree of multiplication of both strains was comparable.

6. Virus multiplication was not observed in fragments of mouse peritoneal wall tissue, whereas it was seen to occur in organ-cultured kidney tissue. Virus was not recovered from urine in intact animals.

7. The arboviruses studied did not multiply in non-stimulated mouse peritoneal macrophage cultures, but Mengo (an enterovirus) did.

8. Some arbovirus strains do multiply, however, in cultures of (peptone) stimulated mouse peritoneal macrophages. The difference observed between these two populations of macrophages deserves further study.

(S.R. Pattyn)

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

Isolation of TBE virus strains from Ixodes ricinus ticks in the Federal Republic of Germany.

During May and June 1974 a total of 6998 ticks of the species Ixodes ricinus (L.) has been collected in Swabia and Bavaria in localities where central European encephalitis could be identified as a human disease. From the ticks, adults and nymphs, collected in these sites, 10 strains of TBE virus were isolated. After four IC passages in suckling mice the LD_{50} titer obtained was of the order of 8.4 to 9.5 log 10. All the strains showed a neutralizing reaction with a human convalescent serum. The minimum-infection index (MII) in these three localities was for Muhringen, 0.23; Obernzell, 2.05; and Jochenstein, 5.71.

Country and Region	No.of ticks	strain	no.of ticks/pool	NI
Mühringen, district Freudenstadt, Baden-Würtemberg	4177	N 98	40 N	⩾3.1
Obernzell, district Passau, Bavaria	1946	B 50	12 ♂♂	⩾3.4
		B 52	10 ♀♀	4.3
		B 70	7 ♀♀ + 6 ♂♂	4.6
		B 74	5 ♀♀ + 13 ♂♂	4.2
Jochenstein, district Passau, Bavaria	875	B 109	40 N	⩾4.0
		B 115	50 N	3.9
		B 116	3 ♀♀ + 7 ♂♂	3.8
		B 117	3 ♀♀ + 7 ♂♂	3.6
		B 118	40 N	3.5

(R. Ackerman)

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

In the period of 1971-1973 106 greylag geese (Anser anser), 35 mallard ducks (Anas platyrhynchos) and 3 garganeys (Anas querquedula) were examined. Birds were usually netted; however, some of the ducks were shot.

Birds for isolation purposes were bled into 1% heparin. Blood for antibody assay of live birds was taken from vena alea and of shot birds by cardial puncture. Isolation attempts were performed in 2-to-3-day-old suckling mice inoculated intracerebrally (ic) with 0.01 ml of blood diluted in Earle's solution containing 10% inactivated calf serum.

Bird sera were tested for the presence of virus neutralizing (VN) antibodies against 100 CPD_{50} of Sindbis virus on 1-day-old tube cultures of chick embryo cells and against 100 CPD_{50} of tick-borne encephalitis (TBE) virus, West Nile, Calovo and Tahyna viruses in cloned PS porcine epithelial cells, in which culture they exert a cytopathic effect.

All isolation attempts from the samples of 106 greylag geese, 35 mallards, and 3 garganeys were negative.

Results of serological surveys are given in Table 1. From the 106 geese, VN antibodies against Sindbis were found in 15 trapped in Nesyt, Nova Ves and Bila Hurka localities, against Calovo in 5 from Nesyt and Bila Hurka localities, and against Tahyna in 6 from Nesyt, Nova Ves and Bila Hurka localities. None had antibodies against TBE and West Nile viruses.

From the 38 ducks, antibodies against Sindbis virus were detected in 6 mallards and 1 garganey trapped in the locality of Rakaren-Malacky. Sera of some of the mallards also contained antibodies against other viruses as follows: 6, Calovo; 4, Tahyna; 2, TBE; and 1, West Nile. Among garganeys one serum reacted with Tahyna virus, another with TBE virus.

Summarizing all these data from the bird order Anseriformes, VN antibodies were found in 15.2% against Sindbis virus, in 2.0% against TBE virus, in 0.7% against West Nile virus, in 11.1% against Calovo virus and in 7.6% against Tahyna virus (Table 1). The VN antibody titers varied from 4 to 64.

In migration, the Czechoslovak population of the greylag goose passes over the Czechoslovak territory directed to the north, and penetrates various distances into Scandinavia. Besides passing over Czechoslovakia or occasional wintering in our territory, two flyway directions of wild geese are recognized, south and southwest, with main wintering areas being in the Coto Donana region (at the mouth of Quadalquivir) or in North Africa in a limited area from East Algeria up to Tunisia.

Migrating routes of the Czechoslovak population of mallard ducks are quite different. Areas to which they fly or from which they pass over Czechoslovakia are directed to the northeast. Mallards winter mainly at the

seaboard of the northeast Atlantic, in the western Mediterranean from Marseille through north Italy to Istria, only rarely to North Africa. Part of their population winters in Czechoslovak territory, in Austria, Hungary and Switzerland.

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

Virus neutralizing Antibodies to Arboviruses in Birds of Order Anseriformes in Western Slovakia, South Moravia and South Bohemia

Bird species	Number	Number of positive					Locality
		Sindbis	TBE	WN	Čalovo	Ťahyňa	
Anser anser	22	3	.	.	3	2	Nesyt 8.6.1971 /Moravia/
Anser anser	19	2	.	.	2	2	Bílá Hůrka 11.6.1971 /Bohemia/
Anser anser	12	2	.	.	.	1	Nová Ves 5.6.1972 /Moravia/
Anser anser	53	8	.	.	.	1	Nesyt 8.6.1973
Totally I	106	15	.	.	5	6	
Anas platyrhynchos	17	3	2	.	5	2	Malacky W.Slovakia 25.8.1972
Anas querquedula	3	1	1	.	.	1	Malacky W.Slovakia 25.8.1972
Anas platyrhynchos	18	3	.	1	6	2	Malacky 17.8.1973
Totally II	38	7	3	1	11	5	
Anseriformes I-II	144	22	3	1	16	11	

REPORT FROM THE NATIONAL INSTITUTE OF HYGIENE

BUDAPEST, HUNGARY

Arbovirus surveys in the Transdanubian hilly-land of Hungary were continued during 1971 and 1972 in collaboration with the Institute of Virology SAS, Bratislava. Small mammals were trapped and ticks were collected in order to isolate virus in suckling mice. A TBE and a WN strain were isolated from the brain of two vole mice, trapped in different places. Two Uukuniemi and three yet unidentified arbovirus strains /different from TBE, WN, UUK, TRB/ were isolated from Ixodes ricinus ticks collected in five different places.

Sera of 70 residents and those of 102 wild-living small mammals were tested for antibodies against different arboviruses. When TBE antigen was used HI antibodies were found in 12.8% of the examined human sera and in 1.0% of the small mammals' sera. Of the human sera 1.4% proved to be positive when WN antigen was applied. Of the small mammals' sera 2.0% contained antibodies to UUK antigen.

/E. Molnár/

REPORT FROM THE ANDRIJA STAMPAR SCHOOL OF PUBLIC HEALTH, MEDICAL FACULTY
UNIVERSITY OF ZAGREB, ZAGREB, YUGOSLAVIA

Isolation of Bhanja Virus

HI antibodies to WN and TBE viruses have been found in certain regions of Croatia, especially in the Adriatic. We therefore decided to repeat our attempts to isolate these or possibly some other arboviruses at the time of the peak activity of the tick Ixodes ricinus in the Adriatic, i.e. in December to February. Between 22 and 25 February 1974, 685 ticks were collected on the island of Brač, mostly from sheep, some from vegetation, and one from a man. The ticks collected belonged to the following species: Dermacentor marginatus, I. gibbosus, and Haemaphysalis punctata.

From female H. punctata collected on 24 and 25 February from sheep (pool 7, 16 ticks + pool 14, 13 ticks), 2 viruses were isolated in suckling albino mice. The mice succumbed with the symptoms of CNS disorders 10-11 days after inoculation. Further passages shortened this period to 5 days. One mouse inoculated with strain 14, survived 25 days after inoculation. Dissection revealed:

> CNS normal, spleen markedly enlarged and tense, thymus with enlarged lobes.
>
> Histological finding: The cerebrum, in the base of the frontal lobe, round large blood vessels, showed some weak infiltrates consisting of mononuclear cells, lymphocytes, and the proliferation of endothelial cells. In the lateral ventricle there was a proliferation of subependymal cells. In the cerebellum we found scattered Purkinje cells, markedly homogenized, while in the molecular layer there are multiple astroglial cells. In the spinal cord, homogenization affected large motor cells, and neuronophagia was seen around some of these cells.

These findings suggested a recent infection of the central nervous system by a small dose of weakly virulent, neurotropic virus.

Spleen - hyperplasia of follicles and the red pulp. Marked megakaryocytosis.

Thymus-marked hyperplasia of cortical zone.

The findings of the spleen and thymus are not specific and may indicate only a recent virus infection.

The passage of the virus in primary Pekin duck embryo and Vero cells produced CPE or plaques on the fourth day after inoculation.

Complement fixation tests gave the following results:

SERUM / ANTIGEN	Bhanja (POOL #7)	Bhanja * IbAr 2709	Bhanja * IG 690	Normal	
Bhanja (POOL #7)	128	64	32	<8	
Bhanja * IbAr 2709	64	64	32	<8	
Bhanja * IG 690	128	64	64	<8	
Normal	<8	<8	<8	<8	

* Kindly provided by the Yale Arbovirus Research Unit, New Haven, Conn.

A portion of these results have been confirmed by neutralization tests. For a description of a human laboratory infection with this virus, see the report of the Vector-Borne Diseases Division, CDC, this issue.

Serological testing of humans and animals on Brač with the newly isolated virus is under way.

This is the second isolation of Bhanja virus (the first was in Italy in 1967) outside tropical areas.

(J. Vesenjak-Hirjan, C.H. Calisher (CDC, Ft. Collins, Colorado, U.S.A.), Z. Brudnjak, D. Tovornik)

REPORT FROM ARBOVIRUS UNIT, VIRUS DEPARTMENT,

ISTITUTO SUPERIORE DI SANITA', ROME, ITALY

During field studies on arboviruses carried out in 1973 in four Italian provinces in northern and central Italy (Gorizia, Siena, Rome, Latina), 4,774 ticks were collected between March and December 1973 and processed for virus isolation (Table 1).

In Gorizia province ticks were collected only on vegetation and, as previously observed, all of them were Ixodes ricinus. In the other three provinces the ticks collected were among the most common species present in the respective regions. Latina province is the only area where a periodical tick collection was regularly performed by Dr.E. Stella from the Parasitology Department of this Institute.

Only one virus strain was isolated from a pool of females of Haemaphysalis punctata ticks and it was identified as Bhanja virus (Table 2). This is the second isolation of Bhanja virus in the same area, from the same vector and in the same season, since the first isolation occurred in 1967. This should support the hypothesis of the presence of a permanent natural "focus" of Bhanja virus in Fondi area (Latina province).

Collaborative study

In 1974 Dr.Retno Iswari, from Microbiology Institute of University of Indonesia, Jakarta, joined us for a six months period. Some Ticks collected in Java (Indonesia) were processed for virus isolation. From two pools of Argas robertsi collected in Dua Island (West Java) two virus strains were isolated and successfully reisolated.

The prototype strain (ISS.IR.802) was lethal for suckling mice by i.c. inoculation with an incubation period of 6-8 days, but failed to kill weanling mice inoculated by the same route. The ISS.IR.802 strain was shown to be sensitive both to ether and to SDC. No hemagglutinin was produced after sucrose aceton extraction of infected suckling mouse brains.

The serological identification of this virus strain is in progress.

(M.Balducci, P.Verani, M.C.Lopes)

Table 1 – Species and number of the ticks collected in northern and central Italian provinces from May to December 1973.

Collection site	Species	Total ticks
Gorizia province	Ixodes ricinus	1,090
Siena province	Ixodes ricinus	83
	Rhipicephalus sanguineus	5
	Rhipicephalus bursa	17
Rome province	Haemaphysalis punctata	305
	Rhipicephalus bursa	26
Latina province	Ixodes ricinus	269
	Haemaphysalis punctata	1,636+
	Haemaphysalis otophila	5
	Dermacentor marginatus	14
	Rhipicephalus sanguineus	54
	Rhipicephalus bursa	1,196
	Hyalomma marginatum	74
Total		4,774

+ Isolation of Bhanja virus

Table 2 – Number and stage of ticks collected for virus isolation.

Species	Stage	Number of Specimen	Number of isolations
Ixodes ricinus	Nymphs	973	0
	Males	159	0
	Females	310	0
Haemaphysalis punctata	Nymphs	914	0
	Males	137	0
	Females	890	1 +
Haemaphysalis otophila	Nymphs	–	0
	Males	–	0
	Females	5	0
Dermacentor marginatus	Nymphs	–	0
	Males	8	0
	Females	6	0
Rhipicephalus sanguineus	Nymphs	–	0
	Males	31	0
	Females	28	0
Rhipicephalus bursa	Nymphs	10	0
	Males	780	0
	Females	449	0
Hyalomma marginatum	Nymphs	–	0
	Males	40	0
	Females	34	0
Total		4,774	1 +

+ Bhanja virus

REPORT FROM THE VIROLOGY DEPARTMENT, GIRLS COLLEGE,
AL-AZHAR UNIVERSITY, CAIRO, U.A.R.

Isolation of a Tete group virus from Argas ticks

Argas species ticks were collected on 20 January 1970 by hand from the bark of Eucalyptus trees in a rookery of buff-backed herons, Nile Barrage Park, 18 miles north of Cairo, Qalyubiya province, ARE. An isolate, ART 28, was recovered from a pool of five Argas ticks. The isolate was filterable, lost 0.7 logs of infectivity by treatment with SDC and did not produce a hemagglutinin. By complement fixation tests ART 28 sucrose-acetone extracted antigen was not related to group A, B, C, Bunyamwera, sandfly fever, Quaranfil, Qalyub, Nyamanini, Chenuda or Wad Medani viruses. ART 28 virus was sent in 1971 to the Virology Department, NAMRU-3, for further typing by complement fixation and was found to be related to Matruh and ANB-12502 viral antigens as shown in Table 1.

Table 1. ART 28 Antigenic Relationships by Complement Fixation Test.

	Matruh AS	ANB 12502 AS
ART 28	64/256	2/8
Matruh	64/512	16/256
ANB 12502	8/8	64/128

Previous isolates of Tete group viruses have been from birds. This is the first evidence that ticks may be involved in the epidemiology.

(Kouka S.E. Abdel-Wahab)

REPORT FROM THE EGYPTIAN ORGANIZATION FOR BIOLOGICAL AND VACCINE PRODUCTION, AGOUZA, CAIRO, UAR

Introduction

In the last ten years arboviruses have become an increasingly recognisable cause of diseases in humans and animals. Many arboviruses were isolated in Egypt and other parts of the world.

Information is clearly needed about the epidemiologic interaction of those new viruses in various segments of wildlife in Africa.

In addition, two viruses (Obodhiang and Kotankan), repeatedly isolated from mosquitoes, have the unusual potential (for rabies-related viruses) of being arthropod-borne.

Attempts were made to isolate arboviruses from ectoparasites, rodents and humans. In addition, sera from humans, domestic animals and different species of rodents collected from different governorates in Egypt were screened by serological tests for evidence of natural infection. The following results cover the period from January 1974 to November 1974.

Attempted isolation

1. From ticks. Ticks were collected from different species of animals in different localities in Egypt, were identified, and were inoculated in suckling mice.
   A new viral isolate was under study (Matruh virus), from Hyalomma marginatum collected from migratory birds in Matruh governorate.

2. From blood-sucking flies. 200 pools were tested with negative results up till now.

3. From humans. CSF and sera from the Imababa Fever Hospital were tested by inoculating suckling mice. No isolates up till now.

4. From rodents. Brain tissues of wild rodents from different localities in Egypt were inoculated into suckling mice in attempts to isolate arboviruses or rabies-related viruses. Results thus far have been negative.

Survey for antibodies to some arbovirus in humans, domestic animals and rodents.

Sera

a. 236 human sera were collected and tested by HI against Semliki Forest, Chikungunya, Sindbis, Zika, West Nile, Langat and Bunyamwera viruses. Antigens were prepared by sucrose acetone extraction of infected mouse brain. HI tests were done according to the method of Clarke and Casals (1958). The sera were treated by kaolin, using goose erythrocytes.

b. 1200 blood samples were collected from different species of domestic animals; the sera were separated and stored at -20°C. 200 rodents were collected from different localities in Egypt and identified according to species. Blood samples were taken and sera separated and stored at -20°C. These sera were tested by CF against Wanowrie and Dhori viruses. Also 898 animal sera, rodents, and pigeons were tested by CF test against Quaranfil, Chenuda, Nyamanini, Qalyub and Wad Medani viruses.

Sera were inactivated at 56°C for 30 minutes. A preliminary CF test was conducted on each serum at a dilution of 1/4. Sera showing positive reactions were further tested using wider serial dilution ranging from 1/4 to 1/128.

The CF test (modified Kolmer microtechnique) was carried out in accordance with the procedure described in Diagnostic Procedures for Viral and Rickettsial Diseases, 4th edition, 1969.

Results

1. Out of 236 human sera tested by HI test 107 (45.3%) were positive against West Nile virus in dilutions of 1/16 up to 1/320, 18 (7.6%) were positive to Sindbis virus in dilutions of 1/10 up to 1/80, 7 (2.9%) were positive to Semliki Forest virus in dilutions of 1/10 up to 1/40, and 1 (0.4%) was positive to Langat virus in a 1/10 dilution, CF tests with Chikungunya, Zika and Bunyamwera viruses were negative. See Table 1.

2. Out of 1400 animal and rodent sera tested by CF test against Wanowrie viruses, 46 (23%) sheep sera were positive, 34 (17%) buffalo sera were positive and 20 (10%) rodent sera were positive in titers of 1/4 to 1/16. Camel, dog, cow and pig sera were negative by CF test. See Table 2.

3. Out of 1400 animal sera tested by CF test against Dhori virus, 40 (20%) sheep sera were positive, 30 (15%) buffalo sera and 25 (12.5%) rodent sera were positive in titers of 1/4 to 1/16. Dog, camel, cow and pig sera showed negative CF tests. See Table 3.

4. Out of 898 animals, rodent and pigeon sera tested by CF test:

a. Against Quaranfil virus (Table 4). 24 (22.2%) buffalo sera, 12 (8.7%) camel sera, 7 (6.9%) dog sera, 15 (8%) donkey sera, and 12 (11.9%) pig sera were positive in titers of 1/4 to 1/32.

b. Against Chenuda virus (Table 5). Only 1 (0.9%) buffalo serum, 4 (3.9%) dog sera, 5 (3.6%) camel sera, 3 (1.6%) donkey sera, 2 (1.9%) pig sera and 1 (1%) rodent sera were positive in titers of 1/4 up to 1/32.

c. Against Nyamanini virus (Table 6). The percentage of positives was small and the titers reached only to 1/8; 3 (2.8%) buffalo sera, 1 (0.7%) camel serum, 1 (0.9%) dog serum were positive.

d. Against Qalyub virus (Table 7). 12 (11.9%) pig sera, 6 (4.3%) camel sera, 8 (2.4%) donkey sera, 2 (1.3%) buffalo sera, 1 (1.4%) pigeon serum, 1 (1%) rodent serum were positive by CF test in titers of 1/4 up to 1/32.

e. Against Wad Medani virus (Table 8). 3 (2.7%) buffalo sera, 1 (0.9%) pig serum, 1 (0.7%) camel serum and 1 (1%) rodent serum were positive by CF test.

These results indicate a high West Nile virus antibody rate in humans, with 45.3% of sera positive in titers of 1/10 up to 1/320. In animals, sheep showed higher percentages positive against Wanowrie virus (23%) and Dhori virus (20%). Buffalos showed higher percentages positive against Quaranfil virus (22.2%), Nyamanini virus (2.8%) and Wad Medani virus (2.7%). Dogs showed a higher percentage positive (3.9%) to Chenuda virus. Pigs were higher against Qalyub virus (11.9%).

(Imam Zaghloul Imam)

T a b l e (1)

HI Antibodies to some Arbovirus in Human Sera.

V i r u s	No of tested sera	P o s i t i v e		
		Total +ve	% +ve	Titre range
Chickungunya	236	0	0	0
Semliki Forst	236	7	2.9%	1/10 up to 1/40
Sindbis	236	18	7.6%	1/10 " " 1/80
Zika	236	0	0	
West Nile	236	107	45.3%	1/10 " " 1/320
Langat	236	1	0.4%	1/10
Bunyammora	236	0	0	

TABLE 2. Wanowrie Virus
CF antibodies in animal and rodent sera to Wanowrie virus

Animal Species	No of tested sera	Positive				
		Total +ve	% +ve	1/4	1/8	1/16
Sheep	200	46	23%	18	20	8
Buffaloes	200	34	17%	13	17	4
Rodents	200	20	10%	10	8	2
Camels	200	0	0	0	0	0
Dogs	200	0	0	0	0	0
Cows	200	0	0	0	0	0
Pigs	200	0	0	0	0	0
T o t a l	1400	100	50%	41	45	14

TABLE 3. Dhori Virus
CF antibodies to Dhori virus in animal and rodent sera

Animal Species	No of tested sera	Positives					
		Total +ve	% +ve	1/4	1/8	1/16	1/32
Sheep	200	40	20%	20	12	8	0
Buffaloes	200	30	15%	15	9	6	0
Rodents	200	25	12½%	17	7	3	0
Dogs	200	0	0	0	0	0	0
Camels	200	0	0	0	0	0	0
Cows	200	0	0	0	0	0	0
Pigs	200	0	0	0	0	0	0
T o t a l	1400	95	47.5%	52	28	17	0

TABLE 4. Quaranfil Virus
CF antibodies to Quaranfil virus in animal and rodent sera

Animal Sp.	No of tested sera	Positives					
		Total +ve	% +ve	1/4	1/8	1/16	1/32
Buffalow	108	24	22.2%	9	8	3	4
Camel	137	12	8.7%	3	3	3	3
Dog	101	7	6.9%	-	5	1	1
Donkey	187	15	8 %	5	4	2	4
Pig	101	12	11.9%	7	5	-	-
Pigeon	70	-	-	-	-	-	-
Rodents	94	-	-	-	-	-	-
Sheep	100	-	-	-	-	-	-
T o t a l	898	70	7.8%	24	25	9	12

TABLE 5. Chenuda Virus
CF antibodies to Chenuda virus in animal and rodent sera

Animal Sp.	No of tested sera	Positive					
		Total +ve	% +ve	1/4	1/8	1/16	1/32
Buffalow	108	1	0.9%	1	-	-	-
Camel	137	5	3.6%	1	1	3	-
Dog	101	4	3.9%	1	-	2	1
Donkey	187	3	1.6%	-	1	2	-
Pig	101	2	1.9%	2	-	-	-
Pigeon	70	-	-	-	-	-	-
Rodent	94	1	1 %	1	-	-	-
Sheep	100	-	-	-	-	-	-
T o t a l	898	16	1.7%	6	2	7	1

TABLE 6. Nyamanini Virus
CF antibodies to Nyamanimi virus in animal and rodent sera

Animal Sp.	No of tested sera	Positive					
		Total +ve	% +ve	1/4	1/8	1/16	1/32
Buffalow	108	3	2.8%	1	2	-	-
Camel	137	1	0.7%	-	1	-	-
Dog	101	1	0.9%	-	1	-	-
Donkey	187	-	-	-	-	-	-
Pig	101	-	-	-	-	-	-
Pigeon	70	-	-	-	-	-	-
Rodent	94	-	-	-	-	-	-
Sheep	100	-	-	-	-	-	-
T o t a l	898	5	5.5%	1	4	-	-

TABLE 7. Qalyub Virus
CF antibodies to Qalyub virus in animal and rodent sera

Animal Sp.	No of tested sera	Positive					
		Total +ve	% +ve	1/4	1/8	1/16	1/32
Buffalow	108	2	1.8%	2	-	-	-
Camel	137	6	4.3%	2	2	1	1
Dog	101	4	3.9%	-	2	1	1
Donkey	187	8	4.2%	4	3	1	-
Pig	101	12	11.9%	8	4	-	-
Pigeon	70	1	1.4%	1	-	-	-
Rodent	94	1	1 %	1	-	-	-
Sheep	100	-	-	-	-	-	-
T o t a l	898	34	3.7	18	11	3	2

Table (8)

CF Antibodies to Wad Medani Virus in animals & Rodent Sera

Animal Sp.	No of tested sera	Positive					
		Total +ve	% +ve	1/4	1/8	1/16	1/32
Buffalow	108	3	2.7%	-	3	-	-
Camel	137	1	0.7%	-	-	1	-
Dog	101	-	-	-	-	-	-
Donkey	187	-	-	-	-	-	-
Pig	101	1	0.9%	1	-	-	-
Pigeon	70	-	-	-	-	-	-
Rodent	94	1	1 %	1	-	-	-
Sheep	100	-	-	-	-	-	-
Total	898	6	0.6%	2	3	1	-

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

This short paper reviews the results of field investigations for arbovirus activity in Senegal from July to December 1974.

Virological Studies

1.1 Human Blood Samples

161 blood specimens, collected from 158 febrile children in Bandia and 3 febrile adults in Dakar, were processed for virus isolation. One strain of Zika virus was isolated from the blood of a 4 year old boy, in Bandia. Blood films taken from this boy revealed heavy malaria infection (Plasmodium falciparum).

1.2 Wild Vertebrates Samples

42 specimens were processed for virus isolation without success.

1.3 Arthropods

13518 mosquitoes were processed in 604 pools. 3 strains of Zika virus were isolated : 2 from pools of Aedes furcifer taylori and 1 from Aedes luteocephalus . All these mosquitoes were captured in Kedougou using human baits.

1.4 Mosquitoes processing experiments

During transmission experiments it has been observed that the processing of mosquitoes for virus isolation was very important :factors such as freezing, thawing, centrifugation may impair the success of isolation. This may explain the lack of yellow fever virus isolation from wild mosquitoes caught in Kedougou where there is evidence that the virus is active.

With the purpose of clearing up this question experimentally, yellow fever infected mosquitoes were processed according to various methods and inoculated into suckling mice. Work is in progress.

Serological Studies

2.1 Human Sera

2.1.1. Senegal - 89 sera were collected from febrile children in Bandia village and tested for HI and CF antobodies. Chikungunya antobody was found in 10% of the sera. 84%

had group B antibodies and 75% were positive with yellow fever but CF test was negative.

14 specimens from patients suspected of arbovirus infection were examined. No definitive diagnosis can be made.

2.1.2 Other West African Countries - 285 sera collected in the Southern part of the Niger republic, near Niamey in the Southern part of the Mali republic and the Northern part of Upper-Volta, were examined for HI and CF antibodies. More than 80% of these sera showed broad group B response but some sera reacted with yellow fever antigen only.

Plaque reduction neutralization tests using PS cells have been performed for neutralizing antibodies and are still in progress.

2.2 Wild Vertebrate Sera

2.2.1. Kedougou (Senegal) - 73 sera from monkeys caught in the area of Kedougou have been studied for HI and CF arbovirus antibodies. 60% were chikungunya positive in HI, 50% were yellow fever and Saboya positive in HI, and 2% had CF antibodies for yellow fever antigen.

2.2.2. Upper-Volta - 59 sera were collected from monkeys shot in the Upper-Volta republic and tested for HI and CF arbovirus antibodies. Over 80% showed yellow fever HI antibodies associated with one or more other group B HI antibodies : W.N, U.G.S, D.B, Zika, N.T.A, S.A.B, W.S.L, 20% of the sera were positive for group B-CF antibodies and 5% were positive for yellow fever virus only.

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

REPORT FROM THE ARBOVIRUS LABORATORY, INSTITUT PASTEUR AND O.R.S.T.O.M. YAOUNDE, UNITED REPUBLIC OF CAMEROON

From July 1 to December 31, 1974, ecological studies on arboviruses with special reference to yellow fever were followed up in our N'Delle field stations near the town of Ayos, where cases of yellow fever have been confirmed by histopathology in the past four years.

1. Virological studies.

1.1. Human blood samples.

Nine human blood specimens have been collected from febrile patients with a rash. Two virus strains have been isolated, H Y 71 which is a Bwamba strain and H Y 132, not yet identified.

Four blood specimens have been collected from men who had died with a hepato-nephritis syndrome, but no virus has been isolated from these materials.

1.2. Wild vertebrate samples.

480 specimens were processed for virus isolation. Three virus strains were isolated from these materials:

An y 1307 from Crocidura sp.
An y 1437 from Lophuromys sikapusi
An y 1444 from Mus musculoides

These three strains are being identified at the Regional Reference Laboratory in Dakar.

In an attempt to facilitate the isolation of yellow fever virus from mosquitoes, we are planning to inoculate our wild mosquito samples intrathoracically into laboratory reared Aedes aegypti; then, after an extrinsic incubation period of 7 days, the inoculated mosquitoes will be ground and inoculated into suckling mice. This will be perhaps a more sensitive yellow fever isolation system.

2. Serological studies.

Five hundred human sera were collected from Yagoua schoolchildren. Yagoua is a typical sahelian savannah vegetation zone. Serological test results showed a high rate of arbovirus positive reactions in young children, as has always been found in this vegetation zone. This is in contrast to results from the equatorial rain forest where a serological survey in the pygmy population has shown a much lower arbovirus activity among children.

(G. Le Gonidec, Institut Pasteur, Yaounde, and A. Rickenbach, O.R.S.T.O.M., Yaounde)

REPORT FROM THE INSTITUT PASTEUR and O.R.S.T.O.M.
BANGUI, CENTRAL AFRICAN REPUBLIC

This report summarizes the results of our arbovirus laboratory for the year 1974. During this year, most of the activities, entomological and virological, have been devoted to the study of the epidemiology of sylvatic yellow fever at the field station established at Bozo (110 km N. of Bangui) by the end of year 1973.

Yellow fever virus has been isolated from 5 pools of Aedes africanus and 1 pool of Aedes opok, caught at Bozo from September to November 1974. If and when the identification of these isolates is confirmed by the WHO Reference Center in Dakar, this will be the first isolation of yellow fever virus from mosquitoes in the Central African Republic (CAR); first confirmation of the role of Aedes africanus in the life cycle of sylvatic yellow fever in C.A.R.; and first indication of the role of Aedes opok as a potential vector.

From other mosquitoes caught in Bozo in 1974 have been isolated also: 1 isolate of Kamese virus from Culex tigripes, 2 isolates of Yaba 1 virus from Culex perfuscus and C. decens, 2 isolates of Pongola virus from Aedes tarsalis and Culex perfuscus, and 1 isolate of Sindbis virus from Culex c.sp.

Investigation on tick-borne viruses has been continued. From ticks collected on cattle in 1973 and inoculated in 1974 have been isolated: 31 isolates of Dugbe virus (30 from Amblyoma variegatum and 1 from Boophilus decoloratus); 2 isolates of Jos virus (from Amblyoma variegatum) and 1 isolate of Congo/CHF virus from Hyaloma nitidum. This is the first isolation of Congo virus in C.A.R. since the beginning of investigations on tick-borne viruses in 1973.

During the study of possible reservoirs, isolates have been obtained from birds and rodents: Ingwavuma virus has been isolated from blood and organs of an Hyphanturgus and another isolate (yet to be identified) has been obtained from blood and organs of an Andropadus virens; from rodents, 3 isolates have been obtained: 1 from a Praomys, 1 from a Lastomys and 1 from a mangoose (Mango mango). All three isolates yet to be determined.

From human cases of acute febrile illnesses (with or without rash and/or arthralgia) we have isolated: 1 isolate of Koutango virus (probably laboratory contamination), 1 Zinga virus and 1 Sindbis virus. Another isolate is yet to be identified.

Note: Modifications to our previous report (see Info-Exchange n° 26).

Arboviruses isolated from mosquitoes: the two isolates reported as "yet to be determined" have been identified in Dakar as Yaba 1 (from Culex c.sp.) and Wesselsbron (from Aedes g.domesticus). Three out of four isolates reported as West Nile and one isolate reported as Bagaza have eventuelly been identified in Dakar as Usutu sub-type Y 276.

(P.Sureau and M.Germain).

REPORT FROM THE INSTITUTO EVANDRO CHAGAS, FOC,
BRAZILIAN MINISTRY OF HEALTH, BELEM, BRAZIL.

1. Arboviruses Research Along the Trans Amazon Highway

Since 1971 ecological studies on arboviruses have been undertaken along forested areas intersected by the newly openned highways in the Amazon region of Brazil. Up to the end of 1974, 10 different areas have been surveyed, 8 of them located along the Trans Amazon highway, and two along the Santarém-Cuiabá highway. Nine areas are in Pará State and one in the Amazonas State (Fig 1). Initially, 3-4 weeks were spent in each area but from the end of 1972 up to 1974, it was expanded to 8-12 weeks. Also a permanent surveillance on arboviruses was started in the Altamira area in November 1972.

In the 10 different areas wild mammals, birds, reptiles, amphibia were trapped or shot, and hematophagous insects were collected. In addition to this, residents of the cities, villages or small settlements located along or nearby the highways were bled, as well as road workers and colonists from outside of these areas. Most of these colonists come from other states. Blood is taken from both sick people and from normal persons. Samples for attempted viral isolation are preserved in liquid nitrogen, whereas serum samples for antibody determination are kept at +4ºC, then frozen later at -20ºC. Specimens are sent by air to the Institute Evandro Chagas where the tests are performed.

This report covers the results obtained with material collected during two trips which were done in 1973. Part of the results obtained up to December of 1972 have already been published (Bulletin of the Pan American Health Organization, Vol. VIII, nº 2, p. 111-122,1974).

The two areas of ecological studies in 1973 were located as follows: 1) Km 212 of the Santarém-Cuiabá highway (April-July). 2) Also at Km 212, but of the section Itaituba-Jacareacanga of the Trans Amazon highway (October-December). Both areas belong to the Pará State. (Fig 1).

Humans. 744 persons were bled in different localities of the Santarém-Cuiabá highway. 56 of these were febrile cases, but no virus could be isolated from their blood, after inoculation in newborne mice. But 3 out of 29 patients had malarial infection, 2 of them being P. falciparum and one had a mixed infection (P. falciparum and P. vivax).

In the Itaituba-Jacareacanga area, 88 persons were bled including 58 febrile from which blood no virus could be isolated. But malarial parasites were demonstrated in 17/49 individuals, 16 of them being P. vivax and only one P. falciparum.

Results of the HI test performed against 18 arboviruses and the laboratory strain of YF virus (17D) with the sera of 744 persons bled in the Santarém-Cuiabá area, are shown in Table 1. It can be seen in this table that the highest number of positive reactions was towards the group B viruses. Thus, in the group with more than six months of residence in the area, 198 (29,0%) persons showed a broad reaction, whereas 52 (7.6%) to the 17D antigen, 27 (4.0%) to the H 111 wild strain of YF virus, 18 (2.6%) to Ilhéus virus, and only 2 (0.3%) to SLE virus. Some of the broad reactions, as well as the ones to 17D antigen, may result fromYF vaccine.

In the group A, 44 (6.4%) had antibodies to Mayaro virus, 28 (4.1%) reacted with 2 or more viruses of the group, 14 (2.0%) inhibited Mucambo virus, and less than 1% of the persons had antibodies to EEE and WEE viruses. Reactions to the other viruses tested were not higher than in 3.6% of the persons tested, or negative. As only few people were included in the other groups shown in Table 1, it is difficult to make an assessment of the data.

The HI antibody rates among the 88 persons bled in the Itaituba-Jacareacanga area were as follows: 1) Group A = Mayaro (15.9%), WEE (2.2%), Mucambo (1.1%) and cross (4.5%). 2) Group B = YF (3.4%), Ilhéus (5.6%) and cross (22.7%). 3) Group C = Caraparu (1.1%). 4)Group Guamá = Catu (3.4%). 5) Group Bunyamwere = Guaroa (5.6%). 6) Group Phlebotomus = Itaporanga (3.4%) and Candiru (1.1%). 7) Group Anopheles A = Tacaiuma (1.1%). No reactions were observed to EEE, Bussuquara, Utinga, Be An 141106 and Be An 174214 viruses.

Wild animals. Table 2 shows the number of wild animals collected during each trip. 14 animals captured in both areas yield 15 virus strains, as shown in Table 3. It is interesting to notice that SLE virus was isolated from the blood of two monkeys. The virus was also recovered from the viscera of one of these monkeys. Reisolation of the agent was obtained from the two blood, but not from the viscera. These two monkeys were killed by shot gun in the Itaituba-Jacareacanga area, where a total of 32 monkeys were shot. In the HI test, 8 of these monkeys showed a specific reaction to YF, two of them showed cross reactions, but nothing else. In the mouse ip neutralization test, using mouse brain as virus source, 19 animals neutralized specifically the H 111 strain of YF virus ($N.I \geq 2.3 \log_{10}$). However, two animals showed a broad type of reaction to YF, SLE and Ilhéus viruses. No specific reactions were observed to SLE and Ilhéus viruses. Excluding one isolation obtained from the blood of a sentinel monkey (confirmed by serology) stationed at the APEG florest, Belém, in the first half of 1973 (see Arthropod Borne Virus Info Exchange, p. 65, nº 26), this is the first time that SLE virus has been isolated from naturally infected monkeys. Plans are made to return to the same area during the next March-April period, for more detailed studies. SLE virus was also isolated from the blood of 1 Didelphis marsupialis (confirmed with reisolation), from the blood of 2 birds (viscera of birds are not collected) and from 1 pool of 24 Culex (Culex) sp., all of these specimens being collected in the Itaituba-Jacareacanga area. In addition, 2 group B viruses were also isolated from birds caught in the same area, but unfortunately these strains were lost due to an accident, before they were typed. One strain of SLE virus was also isolated from

Culex (Culex) declarator collected in the Santarém-Cuiabá area.

Other virus isolations from animals consisted of WEE (1), Turlock (2), Kwata (2) and two of Ungrouped viruses. One of these, Be An 238209 was obtained from the blood of one frog, and the other one, Be An 238758, from the blood of one Oryzomys rodent. These two agents are unrelated by CF test to each other, and also to the other arboviruses found in the Amazon region. No HA antigen was obtained yet from them. Thus, they represent the second and third apparently new types of viruses isolated in areas located along the newly openned highways in the Amazon region. Previously, it has been shown that the strain Be An 213452 isolated from the blood of a Didelphis marsupialis captured 25 km east of Itaituba (Trans Amazon area), in December of 1971, is related only to Candiru virus. The two viruses are indistinguishable in the CF test, but clearly separable by the neutralization test.

The results of HI tests done with the sera of the wild animals against 18 arbovirus antigens can be summarized as follows: 1) Birds. Specific reactions to WEE and SLE viruses were found in 3.7% to 6.8% of the specimens, and cross reactions to the group B viruses varied from 4.8% to 10.6% in the two areas. Rates for Turlock virus were 2.4% and 8.7%, for Oropouche virus they were 0.96% and 11.4% in the two areas respectively, whereas rates varying from 0.2% up to 3.3% were detected for Mayaro, Caraparu, Apeú, Be An 109303, Tacaiuma, Itaporanga, Guaroa and Be An 141106 viruses. 2) Small mammals. Very low rates were found to the viruses tested, with the exception to the members of the Phlebotomus group tested. Antibodies to Bujaru and Candiru of this group were commonly found among the Proechimys rodents (also cross reactions). In general, it can be stated that the majority of antibodies to the other viruses (groups C and Guama) are found predominantly among the Proechimys. It is worthwhile to mention that 4 out of the 5 Dasyprocta rodent tested had HI antibodies to Pixuna virus, with confirmation by the neutralization test. On the other hand, only one animal showed HI antibody to Mucambo, this being the marsupial Philander. 3) Primates. HI antibody rates greater than 15% were found to Mayaro (60% and 53+) and to YF (16% and 25%) and to Tacaiuma (0 and 18%). HI antibodies were also found to Catu, Guaroa, Oropouche, Utinga, Itaporanga, Candiru, but at lower rates.

Hematophagous insects. A total of 22.690 insects, 8920 from the Santarém-Cuiabá area and 13770 from the Itaituba-Jacareacanga, were inoculated for virus isolation attempts. Seven virus strains were isolated, as seen in Table 3. Two of them were SLE virus as already discussed, one strain of WEE virus from Culex (melanoconium) taeniopus, one of Una from Aedes Serratus one of Mirim from Culex (m) taeniopus, one of Turlock from Culex (culex) declarator and one of Irituia from phlebotomine flies.

In both areas the majority of the mosquitoes belonged to the genus Culex. The tree trunk collection by suction & net, and human bait were the most productive methods of insect collection. The Trinidad 17 trap (baited with mouse or chicken) and the CDC light trap were the least productive.

2. Oropouche virus epidemic

On February 3 of the present year we were informed that an outbreak of human febrile illness was occurring in Mojuí dos Campos, a small settlement located 40 km south of Santarém, near the confluence of the Tapajós river with the Amazonas river. Two days later, a field team flew to the area, and stayed there during 4 days collecting specimens from patients, and clinical history of cases. Main symptoms observed were as follows: fever (100%), headache (94%), myalgias (48%), chills (43%). About 10% of the patients had vomiting and diarrhoea. Suden onset was commonly observed and in many patients there was reoccurrence of symptoms a few days or more than one week after the first episode.

Search for malarial parasites in the blood of several acutely ill patients was negative. Blood cultures were also negative, and no pathogenic enterobacteria could be isolated from the stool of a few selected cases. However, 122 (94%) out of the 130 persons tested had HI antibodies to Oropouche virus. 37 (74%) out of 50 cases had CF antibodies to the same agent. Several of these positive reactors had been bled about 20 months ago, a time during which they had no antibodies to Oropouche virus.

23 blood were collected from febrile cases for attempted viral isolations in mice and in Vero cells. The inoculations just started.

The population in the area was said to be around 4,000 people, most of them living in Mojuí dos Campos, and the rest in nearby smaller settlements. Most cases appeared in January and February.

Studies will continue, with more effort to reveal the vector(s). Special attention will be given to the biting midges and Culicini. Wild and domestic animals will also be studied.

This is the fifth epidemic caused by this arbovirus in the Amazon region. Two of them occurred in Belém (1961 and 1968) one in the Bragança area (1967) and one in Baião (1972) all in Pará State. The vector(s) remain unindentified however.

3. Outbreak of yellow fever

Following the isolation of yellow fever virus from the blood of a non-fatal case hospitalized in Belém, investigations were undertaken in the area of residence of the patient, and as result of this an outbreak of yellow fever was uncovered.

The patient (A.P.G., H 251878 AMA 634) was originated from Gurupá island, north of the Marajó island, in the mouth of the Amazon river.

The virus was isolated from a blood sample collected on the March 6, the patient being on the 4th day of illness. About five weeks before, his brother was admitted to the same

hospital in Belém ; he had jaundice and black vomiting, and died on February 9, but no specimens were submitted for the laboratory diagnostic of yellow fever.

A field team moved to the Gurupá at the end of March, where they collected blood from humans, being 12 for attempted viral isolation and 380 for serology. Of these, 305 were obtained at the Jaburu river, 34 at the Tauari river and 41 at the Mulato river. No virus could be isolated from the 12 blood. Eleven per cent of the 380 persons had both HI and CF antibodies to YF virus. All of them dennied previous YF vaccination. In addition, it was gathered that approximately 20 persons living in the Jaburu & Tauari rivers experienced a disease compatible with yellow fever, with 6 deaths, plus about 30 cases with 9-11 deaths at the Mulato and Ajuruxi rivers. These cases occurred during December 1973 and January-February 1974. A few hundred persons live in these rivers. Vaccination against YF was started in April.

It was also learned that moribund monkeys were observed in the forests in December 1973 and January 1974. No virus could be isolated from the blood of 8 monkeys shot in the local forests. However, 2 of them had specific HI antibody to YF virus and another 2 showed a broad group B type of reaction.

155 mosquitoes were collected in the canopy. Of these, 132 belonged to the genus Haemagogus, and they were inoculated into baby mice as 7 pools, yielding one strain of YF virus, from a pool of 25 Haemagogus. The other mosquitoes were Sabethes chioropterus (1 pool of 13), Sabethes cyaneus (1 pool of 3) and Ae. leucocelaeneus (1 pool of 7).

Yellow fever has also been recorded in other areas of Pará State during 1974. One case each occurred in Itupiranga, Marabá and in Tomé-Açu, all south of the Amazonas river. The diagnosis was made by histopathology.

(Francisco P. Pinheiro, Gilberta Bensabath and Amélia P.A.Travassos da Rosa)

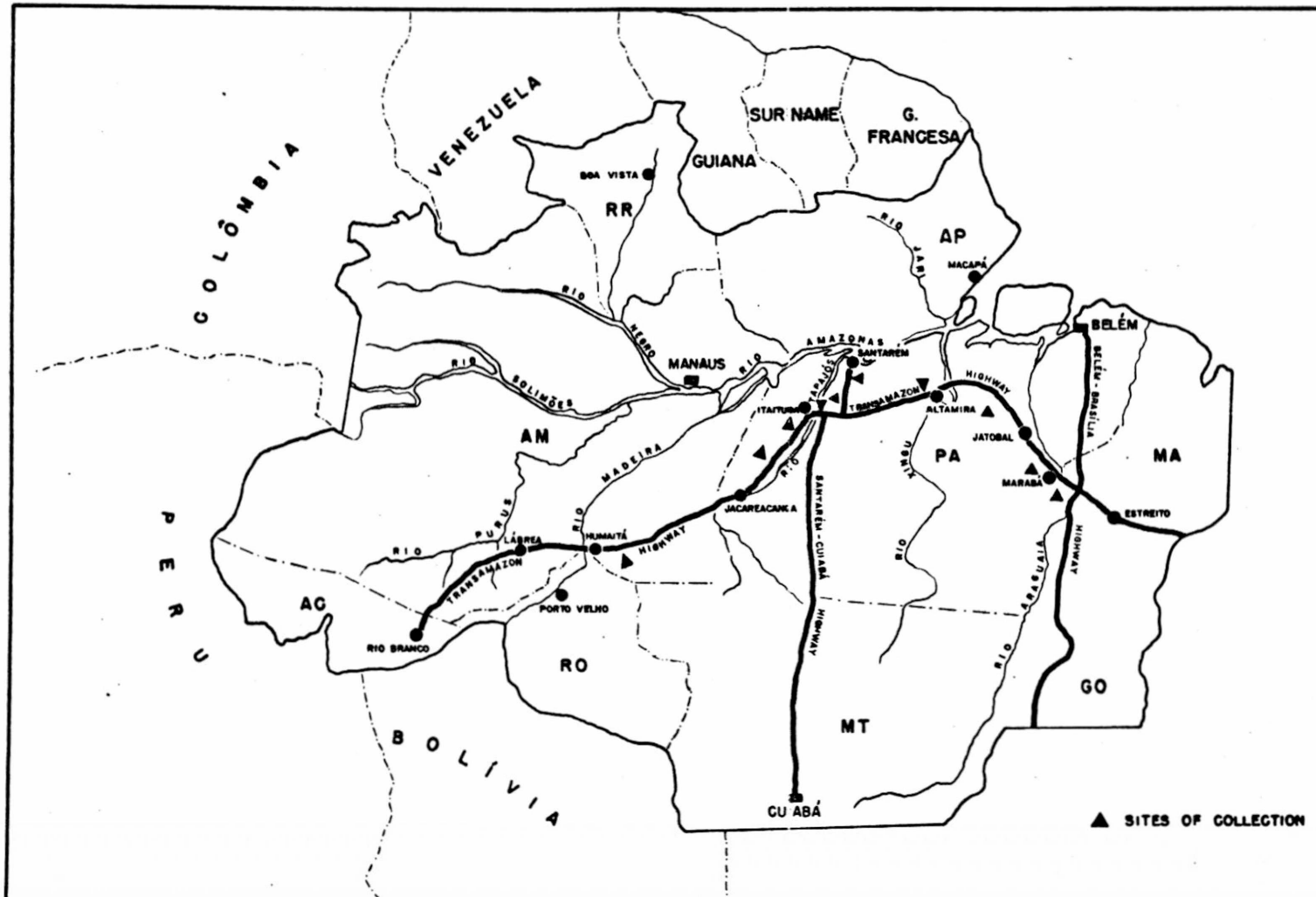


Fig. 1 - AREAS OF ECOLOGICAL STUDIES ON ARBOVIRUSES LOCATED ALONG THE TRANSAMAZON AND SANTARÉM-CUIABÁ HIGHWAYS FROM 1971-1974.

Table 1. HI antibodies in humans according to the time residence in the municipality of Santarém (April-July), 1973

Time of residence in months	ANTIGENS *																					Nº Positives/ Nº Tested	%
	EEE	WEE	Mayaro	Mucambo	CrossA	YF	17D	Ilhéus	S. Louis	CrossB	Caraparu	Catu	Guaroa	Oropouche	Utinga	Itaporanga	Candiru	Cross Phleb	Tacaiuma	An 141106			
< 1						1				1												2/2	100
1 - 6		1		1	1		6	1		17	3	1	2				1	1				25/38	65,7
> 6	1	4	44	14	28	27	52	18	2	198	19	16	20	9	1	25	1	1	1	3		341/682	50
Unknown			1					1		5	2	1	1		1				1	1		9/22	40,9

* All sera negative to Bussuquara and An 174214 viruses.

Areas surveyed : Santarém, Colônia Guaraná, Ruropolis Km 350, Aripapera, Mojuí dos Campos, Colônia Curupira and Colônia Barro Branco

Table 2. Vertebrates captured at two areas of the newly openned highways of the Amazon region

1973

Animals	Km 212 of Santarém-Cuiabá highway (April-July)	Km 212 of Itaituba-Jacareacanga section of Trans Amazon highway (October-December)
Marsupials		
Didelphis m. marsupialis	18	19
Philander o. opossum	23	-
Metachirus n. nudicaudatus	1	2
Marmosa c. cinerea	17	12
Marmosa murina	28	3
Monodelphis	51	1
Rodents		
Proechimys	102	35
Oryzomys capito (?)	136	24
Oryzomys macconnelli	35	-
Oryzomys oecomys	18	1
Neacomys	12	-
Nectomys	27	-
Rat unindentified	8	-
Echimys	1	-
Dasyprocta	3	3
Cuniculus paca	3	-
Agouti paca	-	7
Sciurus	-	2
Primata		
Alouatta	21	4
Cebus a. apella	13	9
Callicebus	5	1
Callithrix	4	-
Chiropotes albinasus (?)	1	4
Saimiri s. sciureus	1	-
Ateles	7	3
Lagothrix	-	4
Phitecia	-	7
Other animals		
Bradypus tridactylus	1	-
Deer (Mazama?)	2	2
Wild pig	4	2
Eira barbara	1	-
Dasypus	-	2
Felis	-	1
Bats	6	13
Land turtle	10	5
Frogs	1	1
Toads	-	3
Lizzards	5	3
Snakes	2	3
Alligator	-	1
Birds	1.283	561

Table 3. Virus isolated from animals and arthropods

Santarém-Cuiabá, km 212 (April-July 1973)

Itaituba-Jacareacanga, km 212 (October-December 1973)

Nº Order	Date	Virus	Number	Species	Specimes or Pool size	Reisolation	Method of capture
1	30.05.73	New type (?)	An 238209	Frog	Blood	Negative	By hand
2	04-12.06.73	Irituia	Ar 243090	Phlebotomus spp ♀	119	Negative	Human bait ground
3	05.06.73	New type (?)	An 238758	Oryzomys	Blood	Positive	Trapping
4	12-30.06.73	SLE	Ar 242587	Culex (culex) declarator	25	Negative	(suction &net) Tree trunk
5	29.06.73	Una	Ar 242256	Aedes serratus	17	Negative	Human bait ground
6	31.10.73	SLE	An 246407	Hylophylax p. poecilonota	Blood	Positive	Mist net
7	01.11.73	SLE	An 246262	Didelphis m. marsupialis	Blood	Positive	Trapping
8	01-15.11.73	WEE	Ar 249748	Culex (melanoconion) taeniopus	24	Positive	Trinidad nº 17
9	08.11.73	Gr. B	An 247244	Hypocnemis cantator	Blood	Inconclusive	Mist net
10	10.11.73	Gr B(SLE?)	An 247287	Myrmoborus myotherinus	Blood	Negative	Mist net
11	14.11.73	Kwatta	An 247304	Phlegopsis nigromaculata	Blood	Not done	Mist net
12	14.11.73	WEE	An 247319	Conopophaga aurita	Blood	Negative	Mist net
13	15.11.73	Turlock	An 247334	Myrmotherula longipennis	Blood	Positive	Mist net
14	15-16.11.73	Turlock	Ar 249443	Culex (culex) declarator	26	Positive	Human bait ground
15	16.11.73	Kwatta	An 247344	Myrmoborus myotherinus	Blood	Positive	Mist net
16	17.11.73	SLE	An 247377	Hylophylax p. poecilonota	Blood	Positive	Mist net
17	19.11.73	Turlock	An 247387	Hylophylax p. poecilonota	Blood	Positive	Mist net
18	21.11.73	Mirim	Ar 249709	Culex (melanoconion) taeniopus	21	Negative	Human bait ground
19	28.11.73	SLE	An 248376	Alouatta	Blood	Positive	Shot gun
20	04.12.73	SLE	An 249398	Ateles	Blood	Positive	Shot gun
21	04.12.73	SLE	An 248450	Ateles	Viscera	Negative	Shot gun
22	04.12.73	SLE	Ar 249670	Culex (culex) sp.	24	Negative	Human bait ground

Nº 1-5 - from Santarém-Cuiabá section

Nº 6-22 - from Itaituba-Jacareacanga section

REPORT FROM THE ARBOVIRUS LABORATORY, INSTITUTE PASTEUR AND O.R.S.T.O.M., CAYENNE, FRENCH GUYANE

1. Virologic studies.

1.1 Isolations from humans.

268 sera taken from man in Guyane have been inoculated in attempts to isolate arboviruses. A total of 9 strains have been isolated: 5 of Mucambo, 1 of Ilheus, 1 of the VEE group other than Mucambo and Pixuna, 1 of a virus similar to Murutucu, and 1 of an apparently new virus (CA Ar 16468).

Mucambo virus. The five strains of Mucambo virus were all isolated from laboratory workers who had handled the virus.

Ilheus virus. Ilheus virus strain was isolated from a patient with falciparum malaria; this isolation was confirmed by detection of specific antibody in the convalescent serum. It was also proved by intrathoracic inoculation of infected serum into Aedes aegypti mosquitoes.

VEE-related virus (strain CA H 73-379). This virus, apparently neither Mucambo or Pixuna, was also from a patient with falciparum malaria, and was confirmed by detection of IHA and CF antibodies in the convalescent serum. The convalescent serum fixed complement at a 1:64 dilution in the presence of the strain isolated from the acute serum and of a strain isolated earlier from birds; but only at 1:8 in the presence of Mucambo, Pixuna and Ca AR 508. The cross reactions are shown in the following table:

Ascites immunes

Antigènes	CA An 410 d	CA H 73-379	Mucambo	Pixuna	CA Ar 508
CA An 410 d	64/16	128/16	8/16	< 8/8	< 8/16
CA H 73-379	32/32	64/32	< 8/8	< 8/8	< 8/8
Mucambo	16/4	16/4	32/8	8/16	< 8/8
Pixuna	< 8/8	8/8	< 8/8	128/128	< 8/8
CA Ar 508	8/16	8/16	8/8	8/8	128/16

Murutucu-like virus. This strain was isolated from the blood of a patient with a fever and myalgia, followed by asthenia. The isolation was confirmed by inoculation of serum intrathoracically into the mosquito, Aedes aegypti.

CA Ar 16468. This virus is different from all the strains in our possession. Serological confirmation was not possible, as the patient died before a convalescent serum could be obtained.

1.2 Isolations from wild vertebrates.

A total of 2,125 birds were captured; selected organs (liver, heart, brain) and blood (diluted 1:5) were inoculated separately. Sixteen virus strains were isolated, as listed below.

CA An 128 d (5 strains). This virus is different from any we possess. It passes with difficulty through a 0.22 milipore filter and does not give a hemagglutinating antigen.

Guama group (1 strain)

CA An 410 d (1 strain). This is a virus of group A different from Mucambo, Pixuna and CA An 508 a. This strain is certainly authentic because it is the first of its type in our laboratory; the strains from man and mosquitoes were all isolated after this strain was isolated. Cross reactions in the CF test with various viruses of the VEE complex have been highest with strain CA H 73-379, described in paragraph 1.1. In seroneutralization tests, results are as follows:

		Sérum non immun	Ascites immunes	
			CA An 410 d	Mucambo
CA An 410 d	Titre du virus	9	<2	7,5
	Indice de neutralisation		>7	1,5
Mucambo	Titre du virus	8,5	5,5	3,8
	Indice de neutralisation		3	4,7

Mucambo (5 strains)

Ilheus (3 strains)

CA An 1093 a (1 strain). This virus is different from any others we possess.

No isolations were obtained from the organs and blood of 100 rodents tested.

1.3 Arthropods.

A total of 338,439 mosquitoes were tested for virus in 4,116 lots. The following viruses were isolated:

Mucambo (1 strain), from Lutzomyia sp.
CA An 410 d (6 strains), from Culex portesi.
Una (2 strains), from Psorophora ferox and C. venezuelensis.
Murutucu (6 strains), 5 from C. portesi and 1 from Anopheles peryassui.
Guama group (10 strains), all from C. portesi.
CA Ar 16102 (1 strain), from 100 Culex albicosta. This strain is different from any in our possession.
CA Ar 16468 (1 strain), from Aedes serratus, different from any in our possession.
CA Ar 16551 (1 strain), from Mansonia titillans, different from any in our possession.
CA Ar 16652 (1 strain), from Anopheles peryassui, different from all the reference strains and all previously isolated viruses.

2. Immunologic studies.

2.1 Human sera.

2.1.1. Sera from military personnel of metropolitan France in Martinique, Guadeloupe and Guyane. A total of 1,424 sera were taken from personnel at arrival and departure from the territory. Tests have shown a high incidence of dengue among the populations of Martinique and Guadeloupe. Serological conversions in the group B observed were due to yellow fever vaccination received on arrival in Guyane. Circulation of viruses of groups A and B is very weak, and the cases are always sporadic.

2.1.2. Martinique. 659 sera of children have been studied, of which 378 were from children 0-4 years of age. Dengue antibody was not found. There has been no circulation of the virus since the last epidemic in 1969.

2.1.3. Guadeloupe. 181 sera were studied. They gave results identical to those of Martinique.

2.2 Animal sera.

A study was carried out on 200 sera of bovines in the province of Guadeloupe. None showed antibodies.

Studies on the sera of rodents collected in Guyane showed that these animals play an important role in the cycle of the CA An 410 d virus of the VEE complex.

(J-P. Digoutte)

Continuing dengue-2 activity in Puerto Rico

Sporadic cases and localized outbreaks of dengue-2 (DN-2) have been documented annually since the 1969 epidemic which involved virtually the entire northern half of Puerto Rico (1-4). The most recent outbreak occurred in Villalba in the late summer and fall of 1973, affecting an estimated 4,000 persons (5).

The first half of 1974 was unusually dry, and only 4 laboratory confirmed cases (San German, 2; Santa Isabel, 1; Villalba, 1) were documented through September. Some weeks following the onset of rains in August, Aedes aegypti house indices increased, and during October 1974, surveillance conducted by the Puerto Rico Health Department (PRHD) and the San Juan Laboratories, Center for Disease Control, indicated an increase in dengue-like illness in the southwestern part of the Island. A total of 38 dengue cases were confirmed in seven communities during October 1974 through January 1975 (Table). Control activities directed against A. aegypti mosquitoes were initiated by the PRHD.

To assess an apparent outbreak in Tallaboa Alta near Peñuelas, on December 3, 1974, survey teams visited every tenth occupied house in the community to collect morbidity information and diagnostic specimens. Thirty-eight households were visited, clinical information obtained on 204 family members, and serum obtained from 45 persons reporting febrile illness during the previous 2 months. Eleven of the serum specimens were collected from individuals reporting onset of illness within 2 days, and from 3 of these DN-2 virus has been isolated and identified by complement fixation.

Convalescent serum specimens were requested 5 weeks after the first survey, and of 36 serum pairs obtained, 18 (50%) showed seroconversion to dengue virus by complement fixation and/or hemagglutination inhibition test. An additional 5 (14%) of the 36 pairs showed very high titers of dengue antibodies, indicating recent infection. Eighty-three of the 204 household members (41%) experienced febrile illness during the 2-month period prior to the survey.

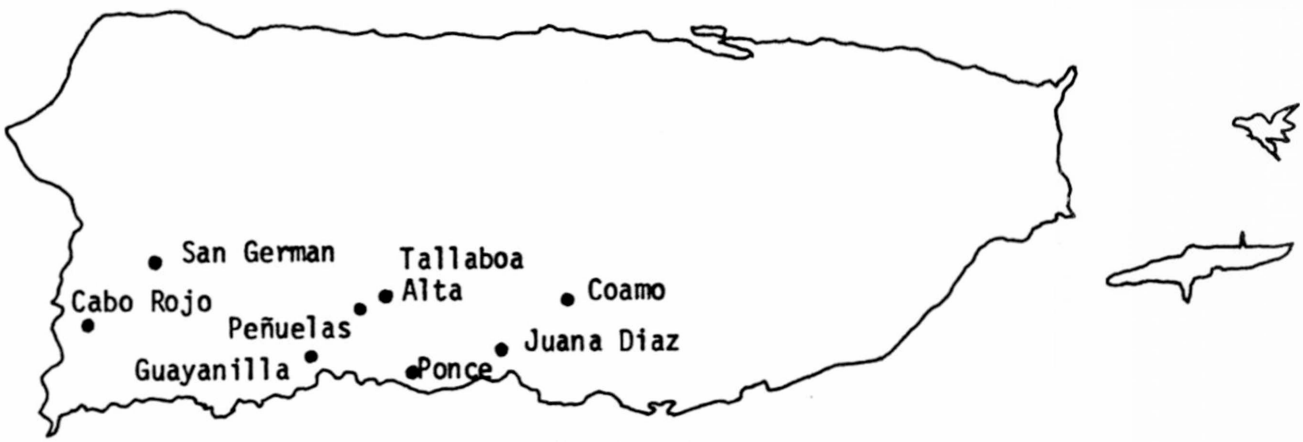
A. aegypti control was initiated by the PRHD in the community, and apparently no new cases have occurred since mid-December 1974. Sporadic cases of dengue-like illness continue to occur, however, in other areas of southwest Puerto Rico. The location of communities with confirmed dengue during October 1974 through January 1975 is shown in the figure. In view of the abundance of A. aegypti vectors and of susceptibles in the human population, it is not clear why the limited activity described above has not become more widespread.

Submitted by:
Staff, San Juan Laboratories
Bureau of Laboratories, CDC

TABLE
LABORATORY CONFIRMED CASES OF DENGUE
DETECTED BY SURVEILLANCE SYSTEM,
OCTOBER 1974 THROUGH JANUARY 1975,
PUERTO RICO

Town	Oct	Nov	Dec	Jan
Cabo Rojo	3	-	-	-
Coamo	-	1	5	3
Guayanilla	1	2	-	6
Juana Diaz	2	-	-	-
Peñuelas	8	1	4	-
Ponce	-	1	-	-
San German	-	1	-	-
Total	14	6	9	9

FIGURE
COMMUNITIES WITH CASES OF LABORATORY CONFIRMED DENGUE
OCTOBER 1974 THROUGH JANUARY 1975
PUERTO RICO



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Preliminary report on an outbreak of encephalitis occurring in Hermosillo, Sonora, Mexico, in 1974 probably caused by St. Louis encephalitis virus

Between the first of August and the 25th of September 1974, a total of 51 cases of encephalitis which required hospitalization were presented in the township of Hermosillo. The cases were scattered geographically, with most of them occurring within the city limits but also in a total of 13 localities in the surrounding township.

The case fatality rate was 20%; males and females were attacked equally, with 69% of the cases being children under 15 years of age.

Hermosillo is in a warm desert valley, a large part of which is irrigated by water from the Abelardo Rodriguez Dam at the east side of the city. The whole area has 230,000 inhabitants of whom about 205,000 are living in the city of Hermosillo. A prosperous chicken industry exists in the region. The veterinarians have reported no epizootics in either domestic or wild animals at this time.

The first cases occurred in residents of the city with more than half being in the northeast section. It is probable that many more cases with mild symptoms occurred but were not reported.

The clinical course of the disease was characterized by fever, vomiting, headache, nuchar rigidity and somnolence; in 5 patients tracheotomy was necessary.

In patients who recovered, the hospitalization period averaged 10 days. There was no intrafamily or intrainstitutional spread of the disease from person to person.

We received 276 sera from persons of different population groups, from affected areas and from nonaffected areas; 23 sera were from patients (from eight of whom there were two sera, the first one from the acute phase and the second one obtained 10 days later).

As shown in Table 1, 100% of the sera from patients were positive by HI with the SLE antigen. 65% of the sera were positive with VEE antigen, which is in accordance with an outbreak of VEE in August 1972 in which many human infections occurred and 1,167 equines became infected and died.

In the paired sera we showed fourfold increases in titers to SLE antigen. With VEE antigen no such increases were found.

Among the sera from the 23 patients, 74% showed positive titers for SLE of 1:40 or higher (Table 2); for VEE, only 17% of the sera attained this titer.

In the irrigated desert valleys of southern California which are similar to those of Hermosillo, arbovirus activity, SLE and WEE, was reported[1] which could be related to the present outbreak. It is probable that the large number of domestic fowl which are bred in the area could have played a role. There are records since 1950 of possible small outbreaks of encephalitis with 15 deaths in that year and successively each year. Neurological disease has occurred in humans coincident with the rainy season and increases in the mosquito population and environmental temperature. Even in the absence of rainfall, suitable conditions for arbovirus transmission prevail, as there is constant presence of irrigation water.

Studies of CF and NT antibodies in these sera are also being made, as well as a search for antibodies to other group B viruses. We intend to isolate in later work the etiologic agents from new clinical cases and from natural reservoirs.

TABLE 1. HI TEST ON 23 SERA FROM PATIENTS SUSPECTED TO HAVE VIRAL ENCEPHALITIS.

	POSITIVE SERA	
	No.	%
VEE	15	65
SLE	23	100

TABLE 2. HI TITERS OF 23 SERA FROM PATIENTS WITH ENCEPHALITIS.

	TITER[a]			
	20		40	
	No.	%	No.	%
VEE	12	52	3	17
SLE	6	26	17	74

a. Reciprocal of the dilution of the sera.

(Dra. Maria Luisa Zarate A., Dr. Abel Gonzalez Cortes, Dr. Jesus Guzman, and Margarita Guerrero Sarinana)

[1]Morbidity and Mortality Weekly Report, 1974, CDC, Atlanta, Ga. 23:34:293-294.

REPORT FROM THE FLORIDA MEDICAL ENTOMOLOGY LABORATORY
STATE DIVISION OF HEALTH, VERO BEACH, FLORIDA

A 6-year study of the seasonal feeding pattern of Culex nigripalpus, the primary vector of SLE virus in Florida, revealed that the summer shift from avian to mammalian hosts corresponded with the onset of the rainy season. During two of the six years studied the rains, which normally start around late May, did not begin until mid-August and the shift to mammalian hosts also was about 3 months delayed during the same two summers. Further study of flight behavior strongly suggests that C. nigripalpus mainly fly in damp wooded habitats (where avian hosts roost) during dry periods but on nights following afternoon showers, the usual pattern of summer rain, they readily invade open terrain where cattle and other mammals, but very few birds, are found.

The feeding patterns of engorged Culex (Melanoconion) and (Mochlostyrax) spp. collected in Mahogany Hammock, Everglades National Park, were studied by precipitin and hemoglobin crystallization tests on the blood meals of females identified to species. This South Florida site was shown to be an enzootic focus of VE virus (involving native rodents and C. (Melanoconion) mosquitoes not identified to species) through studies conducted by CDC scientists in the 1960's. Culex pilosus fed principally on lizards, Culex iolambdis on birds, and Culex opisthopus on rodents (40%) and deer (20%). Other hosts of C. opisthopus included raccoon, opossum, amphibian, and passerine bird. Test on mosquitoes from Vero Beach showed a similar host-feeding pattern for these 3 species and also indicated that Culex erraticus fed principally on birds and Culex peccator on poikilotherms. Based on these feeding patterns it appears that C. opisthopus is the only Melanoconion suited to serving as an enzootic vector among rodents in the Park area. The specific rodent hosts of C. opisthopus were then determined from a second group of engorged females by employing the hemoglobin crystallization test described by Washino. Among rodent feedings, the cotton mouse (= white-footed deer mouse) was the most common blood found (63%), followed by the cotton rat (29%), rice rat (5%), and wood rat (3%). These feeding percentages are in line with the population estimates for these rodents in the Park area as reported in the CDC studies. The

number of deer feedings found suggests that this host should at least be considered a potential dispersal mechanism for the virus between the widely scattered hammocks in the everglades.

Efforts to develop a histological technique for the recognition of multiple blood feeding by mosquitoes are continuing. The key to the method is the peritrophic membrane which is secreted almost immediately after blood-feeding and becomes a distinct layer around the blood bolus within 6 hours. Sections of double meals, representing many time combinations, were examined and the presence of two separate meals in the midgut could be accurately diagnosed 80-90% of the time. Even two meals separated by as little as 1 hour were often recognizable. Plans are to use this method to assess the multiple feeding behavior of a field population of C. nigripalpus this year.

(Maurice W. Provost)

REPORT FROM THE FLORIDA DIVISION OF HEALTH
JACKSONVILLE, FLORIDA

As part of general virus diagnostic services provided to the medical community in Florida, we tested sera from 767 patients against a battery of antigens associated with central nervous system diseases during the period January 1974 - December 1974. Sera from four patients showed low-level Group B Arbovirus HI antibodies when tested against both SLE and Dengue antigens. All four patients were prior residents of a Dengue endemic area. One case of EEE was detected in a two month old infant.

Sera from 616 wild mammals were tested as part of our ongoing statewide surveillance. Twenty-four VEE and five Group B reactors were found in a South Florida area where VEE (Fe 3-7C) is endemic.

Human and Animal Sera Screened by the HI Technique with Arbovirus Antigens* January 1974 - December 1974

Species	Number of Sera	Reactors
Human	767	1 EEE ** 8 Group B ***
Horses	34	3 WEE 8 EEE 3 Group B
Field Specimens (mammals)	616	5 Group B 24 VEE °
Sentinel Fowl Sera	318	0
Total	1,735	52

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

** The EEE represents a two month old infant.

*** Four patients' paired sera had constant low level HI antibodies with both SLE and Dengue antigens. Patients were prior residents of a Dengue endemic area.

° Field specimens, South Florida study areas, where VEE (Fe3-7c) is endemic.

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

REPORT FROM THE VIRAL AND RICKETTSIAL PRODUCTS BRANCH
BIOLOGICAL PRODUCTS DIVISION, BUREAU OF LABORATORIES,
CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

Production methods for mouse ascitic fluid reference reagents

In the production of large volumes of immune mouse ascitic fluids for distribution as reference reagents, the volume of final product obtained from a given number of mice is of considerable economic importance. After mice have been immunized and inoculated with sarcoma cells, ascitic fluid can be collected several times before the mice die from the ascitic condition. If the quality of antibody content of these serially collected fluids is satisfactory, the cost of production is greatly reduced.

When ascitic fluids are processed by the methods still employed in many laboratories, a significant loss in volume occurs. The methods referred to consist of removing fibrinogen clots by repeated freezing, thawing, and centrifuging before filtration. The removal of fibrinogen by acid precipitation as described by Chiewsilp and McCown (1) minimizes the reduction in volume of the final product.

We were interested in comparing antibody titers of ascitic fluids collected at weekly intervals after development of ascites. We also decided to determine the effect of clot-removing treatment on antibody titers.

Six-week-old female mice (Swiss-Webster, ICR) were immunized with Venezuelan equine encephalomyelitis, strain TC-83 (VEE) antigen prepared from infected suckling mouse brains. The immunizing antigen consisted of a 10%, W/V, brain suspension in phosphate buffered saline (pH 7.8). The suspension was blended for 2 min at the highest setting of the Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.) and left in an ice bath for 30 min. It was then clarified by centrifugation for 20 min at 1060 x g. The supernatant fluid was used as the immunizing antigen. It had an LD_{50} titer of $10^{7.5}$/0.02 ml in suckling mice by the intracranial route. Mice were immunized by the schedule shown in table 1.

Ascitic fluids were collected from all distended mice on days 35, 41, and 48. After clot formation at room temperature, the clots were broken up with a pipette and fluids were centrifuged for 1 hr at 16,318 x g (2). Clarified fluids were stored at 4 C overnight and then divided into equal volumes. One-half of the fluid was centrifuged again as described above and the other half was treated with 2 M glacial acetic acid until the pH was lowered to pH 4.8 by the method of Chiewsilp and McCown (1). The precipitated fibrinogen was sedimented by centrifugation for 1 hr at 16,318 x g. The clarified fluid was then neutralized to pH 7.2 with 2 N NaOH in an ice bath with constant stirring. Centrifuged ascitic fluids were stored at -20 C. At weekly intervals, all batches of ascitic fluids were thawed and clarified by centrifugation. Samples were collected at each freeze-thaw interval for antibody testing.

The ascitic fluids that were not acid precipitated continued to form clots during each freeze-thaw cycle; whereas, no clot formation occurred after acid precipitation.

All of the fluids were sterilized by filtration through 0.22 μ Millipore filters. Acid precipitated fluid was filtered successively through 8.0 μ, 3.0 μ, 0.45 μ, and 0.22 μ filters. Nonacidified fluid had to be prefiltered before passage through the above series of filters. The time required to filter acid precipitated fluid was about one-half that required for the unprecipitated fluid. Based on the ascitic fluid collected from 400 mice, there was about a 26% loss in volume of fluid processed by the centrifugation procedure. The volume loss in fluid subjected to acid precipitation was about 16% of that collected from mice.

Antibody titers are presented in table 2 as measured by complement fixation (CF), hemagglutination inhibition (HAI), and neutralization (LNI). There was no effect on any of the antibodies measured with respect to (a) time ascitic fluid was collected, (b) method of removing clot, and (c) number of freeze-thaw cycles.

It can be concluded from this investigation that mouse ascitic fluid for VEE can be produced economically by multiple paracentesis followed by acid precipitation and filtration. This procedure is currently being used in this laboratory for the production of ascitic fluids for other arboviruses and also viruses in other classification groups.

(W. Adrian Chappell, William C. Gamble, and Edwin H. George)

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Table 1. Immunization Schedule

Day of Injection	Volume of Inoculum	Route of Injection	Composition of Inoculum
0	1.0 ml	intraperitoneal	Equal volumes of antigen and FCA*
14	0.2 ml	"	Equal volumes of antigen and FCA*
21	0.2 ml	"	Sarcoma cells**
24	0.2 ml	"	Antigen only

* Freund's complete adjuvant (2)
** Sarcoma 180/TG (2)

Table 2. Effect of Treatment on VEE Antibody Titer in Mouse Ascitic Fluid

Day Ascitic Fluid Collected	Tap No.	Clot Removing Treatment	Number of Freeze-Thaw Cycles	Antibody Titer of Ascitic Fluid		
				CF*	HAI**	LNI***
35	1	Centrifugation	0	1:256	1:80	4.3
35	1	Acid precipitation	0	1:256	1:80	4.3
35	1	Centrifugation	1	1:128	1:80	4.3
35	1	Acid precipitation	1	1:128	1:80	4.4
35	1	Centrifugation	2	1:256	1:80	4.0
35	1	Acid precipitation	2	1:128	1:80	4.3
35	1	Centrifugation	3	1:256	1:160	4.3
35	1	Acid precipitation	3	1:256	1:80	4.1
41	2	Centrifugation	0	1:128	1:80	4.3
41	2	Acid precipitation	0	1:128	1:80	4.2
41	2	Centrifugation	1	1:128	1:40	4.3
41	2	Acid precipitation	1	1:64	1:80	4.1
41	2	Centrifugation	2	1:128	1:80	4.0
41	2	Acid precipitation	2	1:64	1:80	4.0
48	3	Centrifugation	0	1:256	1:40	4.2
48	3	Acid precipitation	0	1:128	1:80	3.9
48	3	Centrifugation	1	1:128	1:40	4.2
48	3	Acid precipitation	1	1:128	1:80	4.0

* CF titer
** Hemagglutination inhibition titer
*** Log neutralization index

REPORT FROM LIFE SCIENCES DIVISION
MELOY LABORATORIES, INC.
SPRINGFIELD, VIRGINIA 22151

We have previously described the isolation of temperature sensitive (ts) mutants of dengue virus type 2 (DEN-2, TH36 isolate). These mutants were obtained by propogation of cell culture-adapted virus (P3T11) in primary hamster kidney cell culture (HKCC) containing 5-azacytidine (25-100 µg/ml). Clones were isolated by the direct immunofluorescent technique in HKCC. Temperature sensitivity was assessed by titration of clones at the permissive (33.5°C) and non-permissive (40°C) temperatures, by titration of 33.5 and 40°C yields at 33.5°C, and by inoculation of suckling mice. P4 refers to virus in its fourth suckling mouse brain passage.

By these various tests, 7 ts mutants were isolated from 138 clones tested. Of these, 5 have proven sufficiently stable to permit partial characterization. Complementation analyses were performed as follows. Lab-Tek 8-well dishes were inoculated with 0.2 ml of a given ts clone, or 0.1 ml of each of 2 different mutant clones. Two replicates per experiment were performed. Each possible combination was tested from 4 to 7 times. The yield from each well was titrated by the direct immunofluorescent (FFU) method at 33.5°C. Table 1 shows that ts-1 and ts-7 appear to form a single complementation group, while ts-2 and ts-3 each appear to form a separate complementation groups of their own. Mutant ts-5 did not significantly complement any of the other 4 clones and may represent a non-complementable or multiple mutation.

In attempts to ascertain whether these mutations had any correlation with quantitatable biochemical or biophysical properties, several experiments were planned. Heat stability at 40, 47, and 56°C did not yield much useful information, due to the low titer of the mutants and the relatively rapid inactivation rates at the higher temperatures. Assessment of hemagglutinin formation at the permissive and non-permissive temperatures is currently in progress. After a number of failures, a system for measuring viral RNA synthesis under permissive and non-permissive was devised. This method is a modification of that developed by Stollar and Stollar, employing a quantitative complement fixation assay specific for double stranded (ds) RNA. Normal LLC-MK2 cells were found to have a sufficiently low background of dsRNA to permit easy detection of viral material in infected cells. LLC-MK2 cultures were infected with various mutants or wild type (wt) clones, and harvested at 24, 48, and 72 hr. Cytoplasmic extracts were assayed immunochemically for dsRNA. The results shown in Table 2 for 24 hr harvests are similar to data for 48 and 72 harvests, and demonstrate that ts-1, ts-3, and ts-7 are RNA^+, while ts-2 and ts-5 are RNA^-. This does not conflict with any of the complementation data.

In summary, mutants of DEN-2 TH36 isolate have been chemically induced, isolated, and partially characterized. Complementation among some of the mutants has been detected, and tentative complementation groupings have been established. An immunochemical assay has been employed to successfully classify the RNA phenotype of the mutants. Previously, we reported that of the 7 ts mutants, only ts-1 and ts-2 induced antibody synthesis in rhesus monkeys without producing detectable viremia. It is therefore concluded that the vaccine potential of a given mutant depends strictly on the properties of the expressed mutation, and not on which cistron bears the mutation.

A. S. Lubiniecki (Meloy Laboratories) and G. C. Tarr (Department of Microbiology, University of Pittsburgh, Pittsburgh, PA).

Table 1. Complementation Indices[a] for Combinations of ts Mutants in HKCC at 40°C

	ts-1	ts-2	ts-3	ts-5	ts-7
ts-1	(1.22)[b]	5.46[a] <0.0005[c]	4.49 <0.0005	0.80 >0.10	1.23 >0.10
ts-2		(0.78)	3.71 <0.0005	0.52 >0.025	2.04 <0.005
ts-3			(0.73)	0.48 <0.0005[d]	2.02 <0.005
ts-				(1.07)	1.68 >0.025
ts-7					(0.82)

[a] Complementation index (CI) = $\frac{\text{FFU (tsA x tsB)}}{\text{FFU (tsA) + FFU (tsB)}}$, geometric mean of 4-7 determinations.

[b] $\log_{10}$ FFU/0.1 ml yield of singly-infected cultures at 40°C, geometric means. Yields of 33.5° single infections are 4.32, 4.34, 3.98, 3.51, and 3.18 $\log_{10}$ FFU/0.1 ml, respectively.

[c] Probability that the 2 mutants do not complement each other. Calculations involve the use of Student's t-test on pooled $\log_{10}$ CI data for each combination.

[d] In this case, ts-5 appears to significantly inhibit the replication of ts-3.

Table 2. Levels of dsRNA in Cytoplasmic Extracts of LLC-MK2 Cells Infected with Various DEN-2 ts Mutant or wt Viruses

	ng RNA/10^6 cells at			
Virus(V)	33.5°C	40°C	$X'_{40}/X'_{33.5}$[a]	Phenotype
ts-1	234	231	0.51	RNA^+
ts-2	114	121	0	RNA^-
ts-3	125	187	0.72	RNA^+
ts-5	94	152	0	RNA^-
ts-7	145	266	1.57	RNA^+
5i2(wt)	135	234	1.32	RNA^+
P4	181	224	0.69	RNA^+
CON	69	147	-	-

[a] $X'_{40}/X'_{33.5} = (\text{ng } RNA_V - \text{ng } RNA_{CON})_{40}/(\text{ng } RNA_V - \text{ng } RNA_{CON})_{33.5}$. A given value was considered positive (i.e., $X' = \text{ng } RNA_V - \text{ng } RNA_{CON} > 0$) if the value of X' exceeded the upper 95% confidence interval about the control value.

REPORT FROM THE DEPARTMENT OF VIRUS DISEASES
DIVISION OF COMMUNICABLE DISEASE AND IMMUNOLOGY
WALTER REED ARMY INSTITUTE OF RESEARCH, WASHINGTON, D. C., U. S. A.

This laboratory has used the M 1/311 strain of Japanese encephalitis virus in plaque reduction neutralization tests (PRNT) for JEV antibody. This strain was isolated from C. tritaeniorhynchus in Japan in 1951 and was utilized in neutralization tests in studies defining the epidemiology of JEV in birds, swine, and humans in Japan (1-3). The M 1/311 strain was originally selected for these early studies because of its specificity in neutralization (N) tests, a characteristic necessary to separate JEV infections from infections by other flaviviruses indigenous to Asia.

Recent experience in this laboratory has re-emphasized that the price of increased specificity is often decreased sensitivity. Sera from 4 marines stationed in Thailand who developed Japanese encephalitis were tested for JEV antibody. All 4 patients had rises to JEV (Nakayama) antigen in HAI and CF tests, but had minimal or no rise in N antibody against the M 1/311 strain (Table 1). Using the Nakayama strain, however, all 4 patients had greater than 4-fold rises in JEV N antibody.

In order to explore this discrepancy, mouse hyperimmune ascitic fluid (MHAF) raised to the M 1/311 strain and rabbit antisera and MHAF (obtained from YARU) to the Nakayama strain, were used in PRNT against M 1/311, Nakayama, and the Peking JEV strains. Results are shown in Table 2. Antibody induced by M 1/311 recognizes Nakayama and Peking identically to M 1/311. Antibody to Nakayama recognizes Peking as identical to Nakayama, but neutralizes M 1/311 to a significantly lower titer.

Similar findings have been documented by Japanese workers. Yoshioka, et al have described identical antigenic relations in PRNT between Nakayama (NIH) and JaGAr-01 (4). From inspection of their data, it is likely that M 1/311 is antigenically similar to JaGAr-01; however, we have not tested this hypothesis. It would seem essential to use broadly reacting JEV strains like Nakayama, rather than M 1/311, for serologic diagnosis of human infections.

(F. H. Top, Jr., J. W. McCown, and W. E. Brandt)

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TABLE I

JEV Antibody Titer (reciprocal)

atient	Serum	HAI (Nakayama)	CF (Nakayama)	Neutralization M 1/311	Neutralization Nakayama
PM	acute	320	< 8	< 10	59
	conv.	5120	32	< 10	720
PK	acute	160	8	< 10	< 10
	conv.	640	32	< 10	190
JM	acute	< 20	< 8	< 10	10
	conv.	320	8	16	320
SP	acute	160	16	< 10	16
	conv.	5120	64	19	115

TABLE II

Cross Neutralization Tests (PRNT)

irus:	Serum: M 1/311 (MHAF)	Serum: Nakayama (MHAF)	Serum: Rabbit
M 1/311	900*	90	70
Nakayama	700	390	470
Peking	1100	420	1000

* reciprocal of PRNT titer

Report from the United States Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21701

A Preliminary Report on the Cross Protection by Arenaviruses of the Tacaribe Complex Against Machupo Virus

We carried out a series of experiments to measure the degree of cross protection provided by arenaviruses of the Tacaribe complex against Machupo virus infection of rhesus monkeys. Machupo virus produces a severe and usually fatal infection in rhesus monkeys that we have found to be similar to Bolivian hemorrhagic fever in humans.

Six different arenaviruses listed in Table 1 were inoculated into rhesus monkeys at the specified dosages. The monkeys were held for various periods of time as indicated and then challenged with Machupo virus. The responses to challenge as shown in the Table were measured. Only two of the viruses given alone protected well against challenge. Tacaribe virus given at a dose of 6.3 $\log_{10}$ 60 days before Machupo virus challenge protected the two recipients against severe disease and detectable viremia (Group I). However, a tenfold lower dose, 5.3 $\log_{10}$, of the same virus gave less protection when the monkeys were challenged 58 days later, as evidenced by severe illness in the monkeys and the subsequent death of one (Group II). Nevertheless this lower dose of Tacaribe virus protected monkeys in Groups III and IV challenged with Machupo virus 5 or 21 days after Tacaribe virus inoculation. A dose of 8 $\log_{10}$ of Tacaribe virus given at the time of Machupo virus inoculation did not prevent severe disease although one of the two monkeys survived (Group V).

Tamiami virus also protected against severe disease following Machupo virus infection. Each of two monkeys in Group VI which received 6.0 $\log_{10}$ of Tamiami virus were protected against severe disease when challenged with Machupo virus 2 months later. These two monkeys exhibited little or no viremia and their clinical signs were mild to moderate.

One of the two monkeys in Group VII inoculated with Amapari virus showed no clinical response to virus challenge 58 days later, whereas the other became severely ill and died relatively early, day 14. We do not as yet know whether the survivor developed Machupo virus neutralizing antibody following challenge. All other surviving monkeys in the study developed neutralizing antibody to Machupo virus by day 28.

Pichinde, Latino and Parana viruses given alone did not protect the monkeys from severe disease following Machupo virus challenge 2 months later (Groups VIII, IX and X). Two other monkeys, Group XI, given Parana and Latino viruses simultaneously were partially protected against Machupo virus challenge. They exhibited only moderate clinical signs and little or no viremia.

None of the monkeys showed evidence of clinical disease during the 2 months following inoculation of Pichinde, Parana, Amapari, Tacaribe or Tamiami virus. One of two monkeys inoculated with Latino virus developed edema of the face. We do not know whether this was related to Latino virus inoculation.

Although these studies were carried out with small groups of monkeys, they indicate a significant cross protection of monkeys by some of the viruses of the Tacaribe complex against Machupo virus. The protection may be relatively brief and may be closely associated with the dose of cross protecting virus. Our data are not sufficiently complete as yet to allow us to determine with certainty whether all of the Tacaribe complex viruses infected the monkeys, but to date they indicate that most of the monkeys responded serologically to the original virus prior to challenge. Our preliminary evidence at this time suggests that the protection afforded does not correlate well with either neutralizing or complement fixing antibody against Machupo virus.

(Gerald A. Eddy)

TABLE 1. CROSS PROTECTION AGAINST MACHUPO VIRUS IN RHESUS MONKEYS BY OTHER VIRUSES OF THE TACARIBE COMPLEX

Group	Cross Reacting Virus			Response to Machupo Challenge[a]		
	Virus	Dose Log_{10}	Day Inoculated	Severity of Illness (No.)	Viremia[b]	Dead/ Total
I	Tacaribe	6.3	-60	Mild (2)	None	0/2
II	Tacaribe	5.3	-58	Severe (2)	Intermittent to typical	1/2
III	Tacaribe	5.3	-5	None to mild (2)	None	0/2
IV	Tacaribe	5.3	-21	Mild (2)	None to intermittent	0/2
V	Tacaribe	8.0	0	Severe (2)	Typical	1/2
VI	Tamiami	6.0	-58	Mild to moderate (2)	None to intermittent	0/2
VII	Amapari	6.0	-58	None (1) Severe (1)	None Typical	1/2
VIII	Pichinde	7.3	-60	Severe (2)	Typical	1/2
IX	Latino	5.4	-60	Severe (2)	Typical	2/2
X	Parana	6.8	-60	Severe (2)	Typical	2/2
XI	Parana + Latino	6.8 5.4	-60	Mild to moderate (2)	None to intermittent	0/2
XII	Controls	None	-	Severe (6)	Typical	6/6

a. All monkeys were challenged with approximately 1000 pfu of Machupo virus on day 0.

b. Viremia was measured on days 7, 10, 14 and 17. Typical viremia consisted of detectable circulating virus on at least 3 of the 4 days sampled. Intermittent viremia was defined as detectable circulating virus on at least one but less than 3 of the 4 days tested.

REPORT FROM THE STATE OF NEW JERSEY DEPARTMENT OF HEALTH
TRENTON, NEW JERSEY

There were no isolations of arboviruses made in the New Jersey Department of Health laboratories during the first and second quarters of 1974 (January 1 through March 31, 1974, and April 1 through June 30, 1974, respectively).

The following table summarizes and tabulates findings concerning arboviruses and arboviral disease in the Woodbine, New Jersey, area for the third quarter of 1974.

No evidence of arboviral disease was detected in humans or horses during this period.

No isolations of arboviruses were made during the fourth quarter of 1974, October 1 through December 31, 1974.

(Martin Goldfield)

Isolations from Arthropods in New Jersey — 3rd Quarter

Group	Virus and No. of Strains		Isolated from	Collected in	Month	Year
	EE	WE				
A		1	C. melanura	Woodbine	June	1974
A		3	C. melanura	Woodbine	July	1974
A		14	C. melanura	Woodbine	August	1974
A		1	A. crucians	Woodbine	August	1974
A		1	C. salinarius	Woodbine	August	1974
A		2	C. melanura	Woodbine	September	1974
Totals		22				

Artificial feeding technique for mosquitoes:

Certain possibly insect-transmitted viruses pose experimental difficulties as a satisfactory recipient host is not readily available for virus transmission studies. Accordingly various techniques were explored to overcome this difficulty. One method will be described. Aedes aegypti was the experimental host.

Attempts to feed "free" or unconfined mosquitoes on defibrinated blood in finely-drawn capillaries (1 mm outer diameter) were accomplished by inserting the entire proboscis into the capillary and permitting the mosquito to dangle free.

Using sharply-pointed jewelers forceps and an entomological microscope, a lightly-anesthetized mosquito is firmly grasped at the base of its two wings. Shifting the closed forceps to the left hand and taking a "charged" capillary needle in the right hand, the capillary is quickly inserted over the proboscis. The capillary and proboscis are held together for a few seconds and then very gently, the forceps are relaxed from the wings and the capillary with dangling mosquito is transferred to a styrotex rack containing a series of holes into one of which the capillary is inserted and the mosquito allowed to feed. This system works as the serum quickly hardens around the capillary orifice, thus holding fast the proboscis in position. Care must be taken to remove the first and second pairs of legs to prevent the female from using its legs as levers to free itself.

Results. It may take 15-20 minutes to properly position a mosquito in the beginning but with practice the time can be reduced to 4-5 minutes.

In one experiment 80% of 49 exposed mosquitoes engorged on the capillary blood meal. No enhancement of feeding was noted when mosquitoes were exposed to blood meals over a 37°C water bath. A drawback to the technique is that some ejected virus may be taken up again by the feeding mosquito as there is no provision for blood circulation. Nevertheless, with this technique, low-passage dengue 2 virus has been recovered from the capillary blood meal after being exposed to a feeding infected mosquito. (See Figures 1 and 2.)

(Thomas H.G. Aitken)

Antigenic similarity between Ganjam and Nairobi sheep disease (NSD) viruses:

Dr. F.G. Davies, Veterinary Research Laboratory, Kabete, Kenya, informed the Director of YARU in December, 1974, that he had detected a strong reaction between NSD virus and a polyvalent grouping AF supplied to him by YARU at an earlier date. The AF, designated "Polyvalent Congo", had been prepared by immunizing mice with Congo, Bhanja, Dugbe, Ganjam, and Hazara viruses; the reaction between this AF and NSD virus was found by "an indirect fluorescent antibody method and by an indirect hemagglutination test".

Specific immune sera and AFs for the above viruses have been sent to Dr. Davies in order that he may test them against NSD virus; in the meantime and for urgent administrative and veterinary health reasons, an effort was made at YARU to determine which of the above viruses was related to NSD virus. A sample of mouse antiserum against NSD virus had been sent to us, on request, by Dr. R. West, East Africa Virus Research

Institute, in May, 1967, with a stated titer of 1:128 - 1:256 by CF test.

A CF test was done with the following reagents: polyvalent Congo AF (an ampoule of the same material shipped to Dr. Davies), NSD mouse immune serum (Dr. West), and as control for specificity a mouse immune serum for JE virus; and antigens for Congo, Bhanja, Dugbe, Ganjam, Hazara and controls, JE and Whataroa (group A). The sera, AF and antigens were used in two-fold dilutions, beginning at 1:4. The result of the test is shown in Table 1.

There is no question about the close relationship between NSD and Ganjam viruses in the test; even the slight reaction between NSD serum and Dugbe antigen is significant, as the two viruses, Ganjam and Dugbe, are part of an antigenic group.

All subsequent identification work involving NSD virus in its relation to Ganjam will be done by Dr. Davies.

(Jordi Casals)

Table 1

Similarity between NSD and Ganjam (I619) viruses by CF test

Antigen	Serum or AF		
	Poly-Congo	NSD	JE
Congo	128/32+	0	0
Bhanja	64/32+	0	0
Dugbe	128/32+	4/4	0
Ganjam	128/32+	128+/32+	0
Hazara	64/32+	0	0
JE	0	0	8+/32+
Whataroa	0	0	0

Reciprocal of serum titer/reciprocal of antigen titer;

0, negative reaction at dilution 1:4.

Figure 1. General view of blood-feeding mosquitoes on a capillary holding rack.

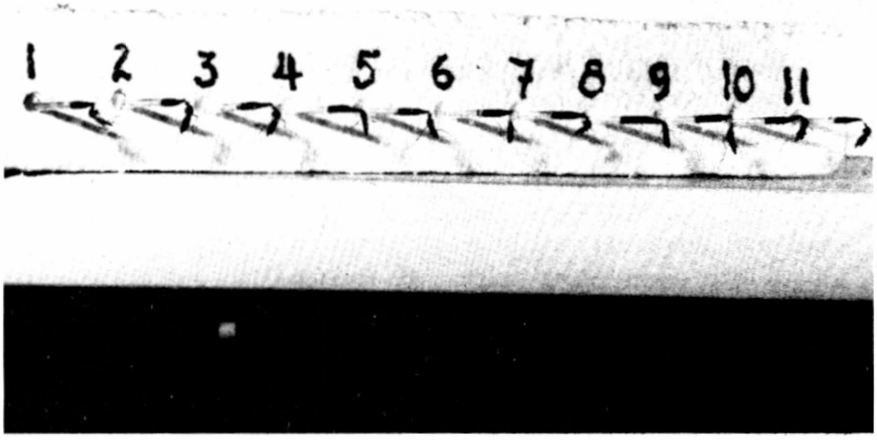
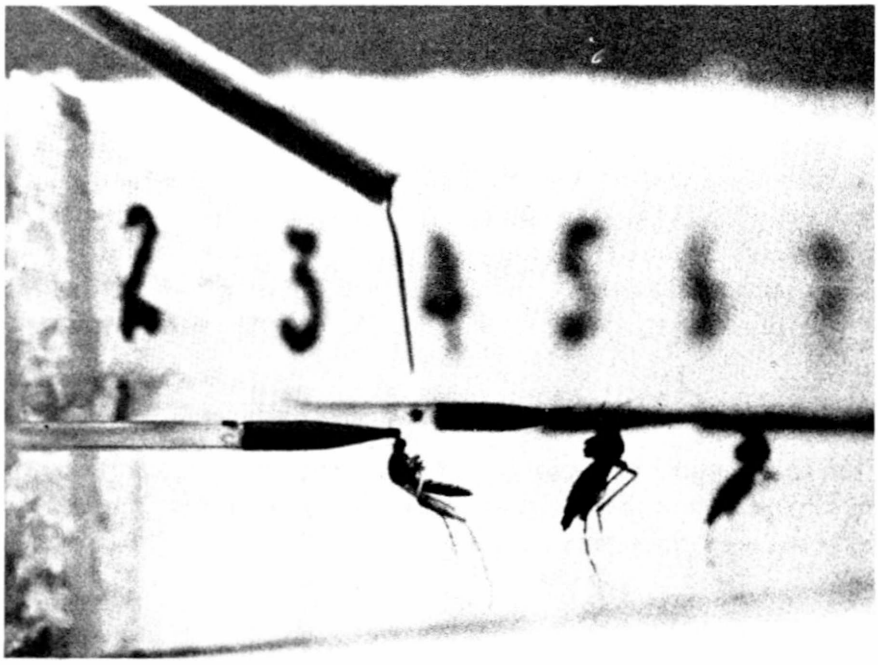


Figure 2. Enlarged view of feeding mosquitoes. The black mark (indicated by the pointer) shows the original blood level.



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

Arbovirus Summary - 1974

There were 15 isolations of California encephalitis group viruses from mosquitoes this year, more than the total for all previous years, although the number of mosquitoes tested (150, 000) was only 50% greater than in 1973 (Table 1). Isolations came from Albany, Cattaraugus, Essex, Hamilton, Livingston, Schenectady and Suffolk Counties and included 2 isolations from mosquitoes collected in Quebec Province at the request of the Canadian authorities. Six species of mosquitoes were involved, including Aedes aurifer. This is the first time a California group virus has been isolated from this species. Infection rates ranged from 1:3642 for Aedes cantator in Suffolk County in July to 1:16 for Aedes triseriatus in Albany County in August.

In spite of this evidence of widespread mosquito involvement, only 3 human cases of presumptive California encephalitis infection were detected this year from serum specimens submitted from 413 cases of central nervous system (CNS) disease (Table 2). Two boys aged 2 1/2 and 4 years from different places in Albany County had onset dates in August and June respectively, and an 18-year-old girl from Wyoming County had onset in early October. For various reasons, the specimens submitted were not adequate to establish more than a presumptive diagnosis.

Specimens from a total of 332 CNS patients were tested for California virus neutralizing antibody, and 36 of the patients were positive. Past experience has shown that clinically apparent cases of California virus infection are restricted to the age group 18 and under. The antibody-positive rate in this age group of CNS patients was 8%, while for patients 19 and over it was 13%. In a current sample of 144 healthy people from the eastern half of the state, the rates were 6% and 16% respectively. These rates are compatible with an endemic situation in which the infection rate increases with age. There is an indication that the rate is higher in the 18-and-under age group of patients than in the comparable group of controls. However, adequate paired sera were received from only 5 of these patients, and in only the 3 patients described above could even a presumptive diagnosis of California virus infection be made.

One human case of meningitis had high antibody titers to Powassan virus. This was an 8-year-old boy from Otsego County, with onset in July. In addition to the signs of meningitis he had a rash, the first time this has been reported in a presumptive case of Powassan meningoencephalitis. Only one blood specimen was received.

Eastern equine encephalitis infection again appeared in Oswego and adjoining counties in August, and by the end of September there had been 14 horse deaths but no human cases. Virus was isolated from the brain of 3 of the horses, and serologic confirmation was obtained from 6 others. Virus was also isolated from 5 pools of Culiseta melanura mosquitoes (infection rate 1:95), 2 pools of Aedes canadensis (1:61), a pool of the Culex restuans (1:89), and the viscera of a fox sparrow (1/102 birds tested from Oswego County in September). The virus is transmitted between birds by Cul. melanura and from birds to horses and man by Aedes canadensis. The outbreak was controlled by aerial spraying of 48,600 acres.

Other arboviruses isolated this year from mosquitoes were 5 strains of Flanders virus and a second strain of the new virus NY 73-51694/5, first isolated in 1973 from Aedes cantator from Suffolk County and now found in Aedes communis from Schenectady County. There were no isolations from 1672 Simulidae or 2049 ticks tested. California virus has been isolated by other laboratories from the overwintering larvae of Aedes triseriatus. We did not succeed in isolating any virus from 200 larvae or 4595 adult mosquitoes reared from wild-caught larvae of Aedes triseriatus from Monroe County nor from 1561 reared adults of the associated species Culex restuans.

(John P. Woodall and Margaret A. Grayson)

Table 1
Arboviruses Isolated in New York State, 1974

Virus	No. of Strains	County	Month	Species	Infection* Rate
CE complex	1	Livingston	June	Ae. aurifer	1:162
	1	Suffolk	July	Ae. cantator	1:3642
	1	Suffolk	Aug.	Ae. cantator	1:108
	3	Essex	June	Ae. communis	1:262
	1	Essex	Aug.	Ae. communis	1:128
	1	Hamilton	June	Ae. communis	1:540
	2	Quebec**	July	Ae. communis	1:403
	1	Cattaraugus	July	Ae. stimulans	1:468
	2	Albany	Aug.	Ae. triseriatus	1:16
	1	Schenectady	Aug.	Ae. vexans	1:4204
	1	Livingston	Aug.	Ae. vexans	1:273
EEE	1	Oswego	Aug.	equine	-
	2	Oswego	Sep.	equine	-
	1	Oswego	Sep.	fox sparrow	1:19
	2	Oswego	Sep.	Ae. canadensis	1:61
	1	Oswego	Sep.	C. restuans	1:89
	4	Oswego	Sep.	Cu. melanura	1:98
	1	Onondaga	Sep.	Cu. melanura	1:84
Flanders	1	Chemung	July	C. pipiens	1:400
	1	Chemung	Aug.	C. pipiens	1:2419
	1	Suffolk	July	Cu. melanura	1:876
	1	Oswego	Sep.	Cu. melanura	1:391
	1	Suffolk	July	Coq. perturbans	1:28768
New virus	1	Schenectady	July	Ae. communis	1:89

* Ratio, infected to uninfected mosquitoes
** Canada

Table 2
Human Arbovirus Infections, New York State, 1974

Case #	Age (Years)	Sex	1974 Date of Onset	Diagnosis	Agent Indicated	Days after Onset	Antibody Titer		
							CF	HI	NI
1	4	M	6.23	Meningitis	California encephalitis group	9	<4	10	positive
						24	<4	10	4.3
2	8	M	7.15	Meningitis	Powassan	19	8	2560	≥3.9
3	2 1/2	M	8.18	Encephalitis	California encephalitis group	6	<4	5-10	4.0
4	18	F	10.8	Meningitis	California encephalitis group	2	<4	<10	2.3
						16	<4	10	3.6

CF = complement-fixation
HI = hemagglutination-inhibition
NI = $\log_{10}$ neutralization index

REPORT FROM THE ZOONOSES RESEARCH UNIT, UNIVERSITY OF WISCONSIN,
DEPARTMENT OF PREVENTIVE MEDICINE, MADISON, WISCONSIN

Studies of the natural distribution of LaCrosse virus:

Fourteen cases of California encephalitis with fourfold or greater rises in antibody titer to LaCrosse (LAC) were diagnosed in Wisconsin during the 1974 season. The age and sex distribution was: 7 boys and 7 girls, 1 to 12 years of age. Ten were residents of Wisconsin, 1 from southeastern Minnesota, and 3 from northeastern Iowa. Four cases had dates of onset during July, 5 during August and 5 during September. The distribution of these cases is similar to that of 189 cases diagnosed during previous years in Wisconsin and associated with histories of exposure of these children to Aedes triseriatus mosquitoes in the hardwood deciduous forest such as are found in the southwestern third of Wisconsin where LAC virus has been continuously re-appearing season after season.

The frequency and distribution of LAC virus overwintering in Aedes triseriatus diapause eggs was studied during the 1974 season by following 64 tree-hole oviposition sites in four enzootic hardwood forest areas. A direct fluorescent antibody (FA) technique proved to be a rapid, reliable and economical tool for ascertaining true field infection rates.

Virus was found in larvae from each of the four study areas before the seasonal emergence of adults and detected in 10 (0.6%) of 1698 individually processed adults reared from collected larvae. In one of these study areas, all 12 located tree-holes were enclosed with screen before the seasonal emergence of adults, to ensure that all collected from these sites originated from overwintered eggs. Of 1280 Aedes triseriatus processed from this area throughout the season, 16 (1.2%) contained virus. Isolates were found in overwintered Aedes triseriatus throughout the summer months, demonstrating the role of these tree-holes as foci for both overwintering and continuing summer season source of LAC virus in these forested areas.

In transovarial transmission experiments with LAC virus in Aedes triseriatus, using dissections and sections of larvae, pupae and adults, virus is commonly observed in alimentary tract and malpighian tubules with the FA technique. Virus has also been detected in other tissues, including eggs developing inside gravid females, and in the accessory sexual organs of a transovarially infected adult male.

The distribution of LAC virus in transovarially infected adults is being compared to that in adults infected while engorging blood-virus mixtures.

(Wayne Thompson and Barry Beaty)

Field Studies with LaCrosse virus vectors:

Since earlier studies indicated that relatively few tree holes contained LAC-infected larvae, five areas in southwestern Wisconsin were studied to more precisely determine the incidence and characteristics of LAC virus overwintering sites. Larval Aedes triseriatus were collected in the spring of 1974 prior to adult emergence in mid-June and assayed for presence of virus. Transovarially infected larvae were found in 3 of the 5 areas. Virus identification is incomplete, but presumably is LAC. The highest incidence of infected treeholes in any one area was 7 of 18, but the incidence of infection in individual larvae appeared to be low with only 11 of 138 (8%) of the pools positive. In the other two positive areas 2 of 10 and 2 of 11 treeholes produced infected larvae, with 8 of 150 pools (5.3%) and 7 of 72 pools (9.7%), respectively, positive. Treeholes that supported successful virus overwintering were of no one type, for both large and small cavities produced infected larvae.

In studies to elucidate the competitive relationship of A. triseriatus and the LAC virus non-vector, A. hendersoni, vertical oviposition preferences were tested. Data showed that the ratio of eggs deposited by A. hendersoni in ovitraps increased with increasing height above ground. The percent A. hendersoni at various heights was as follows: 0' - 69%, 10' - 80%, 20' - 89% and 30' - 94%. Arboreal and basal treeholes monitored concurrently in no case showed A. hendersoni to be present in percentages as high as from ovitrap collections.

LAC Virus Variation:

Studies have continued with prototype LAC virus and the association of different plaque sizes, observed in both Vero and BHK_{21} cells, with endemic maintenance cycles in vertebrates. As previously reported, prototype LAC virus, containing a mixture of plaque sizes, when passed in chipmunks results in selection for predominantly large plaque type virus. When this material is passed in gray squirrels there is a selection for small plaque type LAC virus which remains small on subsequent passage in chipmunks.

Plaque purification of prototype LAC virus into predominantly large and small plaque type populations was accomplished by terminal dilution selection. Subcutaneous inoculation of gray squirrels with 2.7 or 4.8 $\log_{10}$ $SMICLD_{50}$ of purified large and small plaque type virus resulted in no detectible viremia and a low titered neutralizing antibody response. Chipmunks developed viremias after subcutaneous inoculation with 2.6 or 4.5 logs of large plaque type 3.6 or 6.2 log $SMICLD_{50}$ of small plaque type virus. Examination of the plaque size distribution produced by viremic chipmunk blood indicated no change in the plaque type inoculated. Subcutaneous inoculation of gray squirrels with 4.0 logs $SMICLD_{50}$ of chipmunk passaged large and small plaque type virus has so far resulted in no detectible viremia.

(G.R. DeFoliart, M. Lisitza, B. Miller, C. Gary, T. Yuill, W. Hansen, R. Gebhart and D. Nassif)

REPORT FROM DIAGNOSTIC VIROLOGY SECTION
VETERINARY SERVICES LABORATORY
VETERINARY SERVICES, ANIMAL AND PLANT HEALTH INSPECTION SERVICES
UNITED STATES DEPARTMENT OF AGRICULTURE
AMES, IOWA

Venezuelan Equine Encephalitis Surveillance 1972-1974

A Venezuelan equine encephalitis (VEE) surveillance program was established by the United States Department of Agriculture following the 1971 outbreak in Texas. This laboratory provided diagnostic support for this program. A summary of the 3 phases of the program and the results follow. There has been no evidence that VEE has become established in the United States.

1. Field Investigations

All suspected cases of equine encephalitis were investigated and specimens were submitted for laboratory confirmation. Virus isolation, by intracerebral inoculation of suckling mice, was attempted on all serum samples and tissues submitted. Hemagglutination-inhibition and neutralization tests were performed on all serum samples submitted. The horse appears to be the best known sentinel animal, therefore, this part of the program and these specimens received the most emphasis. The results of these investigations are shown in Table 1. There were no VEE positive cases in 1972, 1973 and 1974. In 1972 and 1974 a large number of WEE cases were diagnosed in Colorado, Kansas, Minnesota and South Dakota. In 1973 there were fewer WEE positive cases but there was an EEE outbreak in Massachusetts, New Hampshire and Michigan.

2. Serological Sampling

In 1972 a program was established to collect serum samples from wild mammals and domestic dogs primarily in a belt from coast to coast across southern U.S. The samples were screened using the hemagglutination-inhibition test and all positive titers were confirmed using a neutralization test in primary duck embryo fibroblast cell culture. A sample was positive if the 1:10 dilution produced 90% reduction in the number of virus plaques. The number of samples collected and results are shown in Tables 2 and 3. The reasons for the 6 VEE positive samples from outside Texas could not be determined with any certainty. The samples were all from dogs which could not be traced and there was no history available. They may have originated in Texas or they may have been exposed to

vaccine virus. The small number of VEE positive samples indicated that the virus had not become established in the United States. This program was therefore curtailed in 1973 and 1974. Dr. Vincent Kelly, Bureau of Veterinary Public Health, Texas State Department of Health, Harlingen, TX, submitted serum samples collected from wild mammals in Texas along the lower Rio Grande Valley. In 1973, 171 samples were submitted with 3 positive for antibody against VEE, 6 for WEE, and none for EEE. In 1974, 242 samples were submitted, 15 were positive for VEE, 29 positive WEE, and 1 for EEE. All the VEE positive samples were from wildlife that would have been in the area where the 1971 outbreak occurred.

An outbreak of EEE occurred in the New England area in 1973. Bird tissues and serum samples were collected from this area and submitted for equine encephalitis virus isolation and serology. The number of specimens and results are shown on table 4.

3. Mosquito Collection

In 1973 and 1974 Veterinary Services and Public Health personnel collected mosquitoes at points along the Mexican border and submitted them for virus isolation. The mosquito pools were inoculated intracerebrally into suckling mice. In 1973 a total of 190 pools were processed 88 from Texas, 63 from Arizona, 20 from California, 9 from New Mexico. WEE virus was isolated from 5 pools from Arizona.

In 1974 virus isolation was attempted from 244 mosquito pools, 52 from Arizona, 40 from California, and 152 from Texas. WEE virus was isolated from 1 pool from Texas.

(J. E. Pearson, D. A. Senne, E. A. Carbrey and B. M. Mathis)

State	Calendar Year 1972 Cases Submitted	Positive Cases* VEE	EEE	WEE
Alabama	4	0	0	0
Arizona	4	0	0	1
Arkansas	4	0	0	0
California	0	0	0	0
Colorado	101	0	0	77
Connecticut	11	0	10	0
Delaware	0	0	0	0
Florida	12	0	3	0
Georgia	6	0	1	0
Hawaii	1	0	0	0
Idaho	32	0	0	15
Illinois	25	0	0	5
Indiana	15	0	0	1
Iowa	39	0	0	16
Kansas	91	0	0	71
Kentucky	2	0	0	0
Louisiana	14	0	0	0
Maine	4	0	0	0
Maryland	12	0	1	0
Massachusetts	2	0	0	0
Michigan	4	0	0	1
Minnesota	124	0	1	86
Mississippi	16	0	0	0
Missouri	1	0	0	1
Montana	46	0	0	29

* Positive virus isolation and/or serology

TABLE I.

of Equine Encephalitis Cases

Calendar Year 1973				Calendar Year 1974			
Cases	Positive Cases*			Cases	Positive Cases*		
Submitted	VEE	EEE	WEE	Submitted	VEE	EEE	WEE
10	0	5	0	13	0	1	1
11	0	0	3	3	0	0	0
0	0	0	0	3	0	0	0
0	0	0	0	0	0	0	0
60	0	0	38	62	0	0	51
0	0	0	0	0	0	0	0
3	0	1	0	0	0	0	0
17	0	12	0	7	0	0	0
15	0	7	0	3	0	0	0
0	0	0	0	0	0	0	0
31	0	0	13	21	0	0	13
8	0	0	2	31	0	0	4
32	0	4	2	59	0	1	4
10	0	0	3	38	0	0	19
20	0	0	8	43	0	0	32
0	0	0	0	9	0	0	0
16	0	3	0	25	0	15	0
6	0	3	0	0	0	0	0
2	0	0	0	0	0	0	0
70	0	47	0	4	0	0	0
61	0	36	1	5	0	4	0
36	0	1	7	75	0	0	62
19	0	10	0	21	0	0	0
5	0	0	0	1	0	0	1
10	0	0	3	36	0	0	17

Investigations of Equine Encephalitis Cases (Continued)

State	Calendar Year 1972				Calendar Year 1973				Calendar Year 1974			
	Cases Submitted	Positive Cases* VEE	EEE	WEE	Cases Submitted	Positive Cases* VEE	EEE	WEE	Cases Submitted	Positive Cases* VEE	EEE	WEE
Nebraska	32	0	0	19	5	0	0	3	21	0	0	16
Nevada	3	0	0	1	9	0	0	1	2	0	0	0
New Hampshire	0	0	3	0	74	0	38	0	3	0	0	0
New Jersey	0	0	0	0	0	0	0	0	0	0	0	0
New Mexico	15	0	0	3	5	0	0	3	1	0	0	0
New York	0	0	0	0	0	0	0	0	1	0	0	0
North Carolina	33	0	10	1	34	0	19	0	14	0	6	0
North Dakota	29	0	0	21	4	0	0	2	30	0	0	20
Ohio	15	0	0	1	1	0	0	0	7	0	0	0
Oklahoma	32	0	0	7	17	0	1	1	49	0	0	16
Oregon	15	0	0	5	22	0	0	12	11	0	0	5
Pennsylvania	3	0	0	0	2	0	0	0	3	0	0	0
Rhode Island	2	0	2	0	4	0	0	0	0	0	0	0
South Carolina	11	0	0	0	26	0	0	0	20	0	0	0
South Dakota	54	0	0	38	5	0	0	1	20	0	0	18
Tennessee	5	0	0	0	13	0	0	0	7	0	0	0
Texas	61	0	0	5	29	0	2	8	39	0	0	22
Utah	2	0	0	0	0	0	0	0	2	0	0	0
Vermont	3	0	0	0	2	0	1	0	2	0	0	0
Virginia	8	0	0	0	9	0	6	0	5	0	1	0
Washington	27	0	0	21	11	0	0	6	14	0	0	4
West Virginia	1	0	0	0	1	0	0	0	2	0	0	0
Wisconsin	0	0	0	0	0	0	0	0	4	0	0	0
Wyoming	41	0	0	21	8	0	0	2	13	0	0	7
Virgin Islands	0	0	0	0	0	0	0	0	1	0	0	0
TOTAL	962	0	31	446	723	0	209	119	749	0	34	312

* Positive virus isolation and/or serology

TABLE II.

Calendar Year 1972
Equine Encephalitis Serological Surveillance by States

State	Positive Samples VEE	EEE	WEE	Total Samples Submitted
Alabama	1			25
Arizona			5	536
Arkansas				2
California				104
Florida				639
Georgia		4		276
Louisiana	3			846
Maryland				10
Minnesota	2			59
Mississippi				35
New Mexico			4	226
New York				69
North Carolina				5
Ohio				50
Oregon				2
South Carolina				207
Texas	10	1	92	6296
Utah				57
TOTAL	16	5	101	9444

TABLE III.

Calendar Year 1972
Equine Encephalitis Serological Surveillance by Species

	Canine	Rabbit	Raccoons	Opossum	Other	Total
VEE Positive	16	0	0	0	0	16
EEE Positive	4	0	0	1	0	5
WEE Positive	31	63	2	1	4*	101
Total Samples Submitted	5049	1378	260	114	2643	9444

* 2 badgers, 1 porcupine, 1 bobcat

TABLE IV.

Calendar Year 1973
Equine Encephalitis Surveillance in New England

State	Pheasant No. EEE Pos*/No. Submitted	Pigeon No. EEE Pos*/No. Submitted	Sparrow No. EEE Pos*/No. Submitted
Maine	11/14	. . .	. . .
Massachusetts	4/7	0/1	0/2
New Hampshire	34/40	1/5	1/1
TOTAL	49/61	1/6	1/3

* EEE positive by virus isolation or serology (neutralizing antibody)

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

Arbovirus activity in Texas remained low and undetectable during 1974.

Serology	No. of Sera	Positive
Wildlife (assorted)	81	0
Isolation		
Mosquitoes	3573(225 pools)	0
Horse brains (all were rabies specimens)	14	0

(Charles E. Sweet)

Characterization of avirulent WEE strains

Since 1972, numerous isolations of an aberrant strain of WEE virus have been made from birds (Passer domesticus, Petrochelidon pyrrhonota) and bugs (Oeciacus vicarius) collected at Ft. Morgan in eastern Colorado. Characteristically, these isolates produce plaques in duck embryo (DE) and Vero cells but do not kill suckling mice upon primary isolation attempts; passage in cell cultures increases mouse virulence. Whereas typical WEE strains from other areas produce more plaques in DE than Vero cells upon primary isolation, these aberrant strains from Ft. Morgan produce more plaques in Vero cells than in DE cells. Further, these strains produce plaques which are roughly half the size of those produced by more typical Western strains in DE cells. All strains including those from Ft. Morgan appear to be virulent for suckling hamsters even without previous passage from nature.

An attempt was made to detect markers other than virulence, hemagglutinin presence and patterns, and characteristics in cell cultures, in order to more fully characterize these strains and compare them with more typical strains of WEE virus. First, a thermal inactivation curve was determined. Strains CMA4-368 (from a bird), CM4-146 (from a bug) and 67V5009 (from a mosquito), the former two viruses without prior passage in the laboratory and the third at the DE 3 level, were tested at 37°, 42° and 60°C for from 5 to 120 minutes and titered in both DE and Vero cells. Results are presented in Table 1. The only difference between titrations in DE and in Vero appears to be the greater sensitivity of Vero cells for the field strains from the bird and the bug and the greater sensitivity of DE for the mosquito strain. At 37° and 42°, 67V5009 is stable for up to 2 hrs., while at 60°, all detectable virus was lost within 10 mins. after incubation. This contrasts with the results obtained with the bird and bug isolates in that both 37° and 42° virus was increasingly lost with time and neither were stable for even 5 mins. at 60°. Both CMA4-368 and CM4-146 lost very little if any virus through the first 30 mins. of incubation at 37°, but, thereafter, the titer decreased through the first 90 mins. and was undetectable at 120 mins.

An attempt was then made to compare CMA4-368 and CM4-146 viruses by their kinetics of replication. The results are not complete, but an attempt is made here to summarize the results thus far. Both isolates were passed into DE and Vero fluid cultures, into a litter of suckling mice, and into a litter of suckling hamsters, both of the latter by the intracerebral route. At 40 hrs. the cell cultures and suckling animals were harvested and stored frozen. Aliquots of the

DE harvest were then titrated in both DE and Vero cells and incubated at either 37° or 42°C. The inoculum remaining 30 and 60 mins. after infection and harvests at intervals to 192 hrs. after infection were titrated in DE cells. There appears to be little or no significant difference between virus passed once in DE and once in Vero and virus passed twice in DE cells. However, there is a significant difference in the quantity of virus detectable by time after inoculation, in that cells incubated at 42° generally yielded less virus than those incubated at 37°. Within 48 hrs. after infection, little or no virus was detectable, whereas, harvests from cells infected and held at 37° yielded virus up to 120 hrs. after infection. Since the initial stages of virus replication appear to be identical in both rate and maximum titer, we feel that this is further evidence that both field strains are thermolabile but not temperature sensitive (TS). Additional results of these and other studies will be reported in a later edition of the Arbovirus Information Exchange.

Charles H. Calisher, John S. Lazuick,
and Thomas P. Monath

TABLE 1. Thermal Inactivation of WEE Strains CMA4-368, CM4-146 and 67V-5009 at 37°, 42° and 60°C Titrated in Duck Embryo and Vero cells.

CMA4-368

	DE			Vero		
Mins.	37°	42°	60°	37°	42°	60°
0	5.0	5.0	5.0	5.4	5.4	5.4
5	4.6	4.5	<2.7	5.5	5.3	<3.0
10	4.5	4.3	<2.7	5.3	5.0	<3.0
15	4.6	4.0	<2.7	5.3	4.8	<3.0
20	4.4	3.4	<2.7	5.2	4.6	<3.0
30	4.4	3.0	<2.7	5.1	4.1	<3.0
50	4.0	<2.7	<2.7	5.0	<3.0	<3.0
60	3.7	<2.7	<2.7	5.0	<3.0	<3.0
90	2.7	<2.7	<2.7	4.3	<3.0	<3.0
120	<2.7	<2.7	<2.7	<3.0	<3.0	<3.0

CM4-146

	DE			Vero		
Mins.	37°	42°	60°	37°	42°	60°
0	2.5	2.5	2.5	4.4	4.4	4.4
5	2.3	1.9	<1.3	4.2	4.3	<2.4
10	2.4	1.6	<1.3	4.3	4.1	<2.4
15	2.4	2.2	<1.3	4.0	4.0	<2.4
20	2.3	2.0	<1.3	4.1	4.1	<2.4
30	2.3	2.2	<1.3	4.3	4.1	<2.4
50	1.3	1.3	<1.3	3.8	3.9	<2.4
60	2.1	<1.3	<1.3	3.5	3.6	<2.4
90	1.9	<1.3	<1.3	3.9	3.1	<2.4
120	<1.3	<1.3	<1.3	3.1	<2.4	<2.4

67V-5009

	DE			Vero		
Mins.	37°	42°	60°	37°	42°	60°
0	>4.0	>4.0	>4.0	4.9	4.9	4.9
5	>4.0	>4.0	5.4	4.9	4.9	3.7
120	>4.0	>4.0	<5.4	4.7	4.7	<2.6

Pathogenesis Studies on Avirulent WEE Strains.

A. Experimental Infection of Adult House Sparrows (*Passer domesticus*).

Preliminary studies have been conducted on the responses of adult sparrows to primary infection with a WEE strain originally recovered from a nestling sparrow in Morgan Co., Colorado. The virus strain used, designated CMA4-368, is representative of numerous other isolates recovered from nestling sparrows, cliff swallows, and from *Oeciacus vicarius* bugs inhabiting their nests in 1973 and 1974 (see Arbo Info Exchange No. 27 p. 76-77, Sept., 1974). By HI, CF, and NT these virus strains are closely related to WEE viruses isolated from humans, equines, and *C. tarsalis* mosquitoes in the western U.S., but differ dramatically in their biological characteristics. In particular, they are non-pathogenic or have a very low pathogenicity for baby mice and newly-hatched chicks.

In order to better define the virus-host relationships in the natural transmission cycle, experimental infection of nestling and adult sparrows was considered. Initial studies utilized original (unpassaged) CMA4-368 virus (a diluted sparrow blood field specimen) as well as first Vero cell culture passage material. Parallel studies were conducted using virulent WEE strains (67V5009 and CM4-885) recovered from *C. tarsalis* in Morgan Co. Adult *P. domesticus* were first tested for N antibody to CMA4-368 and to *C. tarsalis*-WEE virus. Sparrows were inoculated subcutaneously with 0.1 ml of virus suspension and were bled from the jugular

vein daily for 10 days and periodically thereafter. Preliminary results are shown below:

Virus (passage)	Dose (PFU/0.1 ml)	No. Viremic/ No. Tested	VIREMIA		
			Maximum Titer (PFU/0.1 ml)	Titer Range on Day 1 P.I.	Duration (days)
67V5009* (DECC 3)	$>10^{7.0}$	11/11	>6.7	3.8->6.7	Pending
CM4-885* (Vero 1)	$10.^{4.0}$	7/7	>7.7	5.5->7.7	3-5
CMA4-368 (Orig.)	$10^{2.4}$	0/10	--	--	--
CMA4-368 (Vero 1)	$10^{5.0}$	4/9	2.5	1.0-2.5	1

**Culex tarsalis* isolates from Morgan Co., Colo.

Sparrows inoculated with CMA4-368 were challenged 1 month later with CM4-885 virus. Results are presently available only for the group initially given $10^{2.4}$ PFU of original CMA4-368 virus. Eight birds were challenged with $10^{4.0}$ PFU; two failed to produce detectable viremia, while the remaining 6 birds had high titered and prolonged viremia resembling birds with primary CM4-885 infection. Further studies are in progress.

These results again indicate a marked difference in the pathogenic determinants between 'classical' WEE virus and strains isolated from nestling birds and *O. vicarius* bugs in Colorado. They suggest that the adult sparrow, unlike the nestling, is not an important host in the transmission cycle. Although they are incomplete, the challenge experiments suggest that prior infection

with the avirulent strains may partially protect birds against 'classical' mosquito-borne WEE virus. The importance of this in terms of WEE virus transmission and amplification in nature remains to be elucidated; however a 1974 serosurvey of over 100 adult sparrows from Morgan Co. failed to show antibody to CMA4-368, indicating that few infected nestling birds survive or that long-lasting N antibody is not produced.

Inoculation of Immunosuppressed Mice.

In order to determine what role host defense mechanisms may play in determining the relative avirulence of WEE strains from Morgan Co., Colorado, CMA4-368 virus was inoculated I.P. into immunosuppressed 3-4 week (150-200 gram) male white mice. Mice were given either 0.9% saline or cyclophosphamide (Cy) (0.1 mg/g) on the day before, the day of, and 3 days after virus inoculation. The effect of Cy was determined by demonstrating a profound leukopenia compared to saline-inoculated controls 24 hours after the second Cy dose. Mice received $10^{5.9}$ PFU of CMA_4-368 virus (Vero 1 passage). None of 72 mice treated with Cy and none of 12 controls developed a detectable viremia during the duration of the experiment (24 days). Organ titrations on daily-sacrificed mice, interferon, and antibody studies are underway. Despite the absence of viremia, CMA4-368 virus probably replicated in inoculated mice, since cross-protection was provided against challenge at 20 days with CM4-885 (*C. tarsalis*-WEE), as shown below:

Inoculum	Treatment	Intracerebral Challenge (Day 30 P.I.)	Mortality
CMA4-368	Cytoxan	CM4-885	1/12
CMA4-368	Saline	CM4-885	0/3
None	Cytoxan	CM4-885	12/12

The results indicate that immunosuppression did not markedly increase replication of CMA4-368 virus in the mouse, and suggest that host defense mechanisms (cellular and humoral immunity, and possibly interferon) are less important in determining the low pathogenicity of the Morgan Co. WEE strains than are virulence or tissue tropism characteristics of the virus itself.

Infection of Organ Cultures.

As a preliminary step in developing technics for investigation of the virulence determinants of WEE virus strains, their tissue tropism, and the role of host defense mechanisms, hamster organ cultures have been studied. Suckling hamsters are susceptible to lethal infection with CMA4-368 virus administered by the peripheral or intracerebral routes. Organ cultures were thus prepared from tissues of infected suckling hamsters harvested at the time of onset of illness. Two virus strains were used: CMA4-368 Vero 1 passage (see above) and CM4-146 (original, unpassaged) isolated from a pool of *O. vicarius* bugs from Morgan Co. Tissue explants placed in plastic cell culture trays were incubated at either 30 or 33° C in Medium 199 containing 20% fetal calf serum, growth

estimated on a semiquantitative scale (1 to 4+), and culture fluid exchanged and preserved (-70° C) for titration at 72 to 96 hour intervals. Results for both virus strains were similar. Cultures of skeletal muscle and kidney demonstrated chronic productive infections for the duration of the preliminary study (24 days), whereas spleen and liver did not yield viral replication and brain supported yielded virus for only 6 days. Culture fluids, exchanged at 72-96 hour intervals and assayed in Vero cells, contained virus at a titer of 3.3-4.6 log PFU/0.1 ml for muscle and 1.6-3.3 log PFU/0.1 ml for kidney. Kidney and spleen explants grew out into nearly confluent monolayers by day 16, whereas muscle explants showed minimal (1+) or no growth.

Both the virus stock used to infect hamsters and the infected culture fluid harvests contained a mixed population of small and large plaques. The proportion of large to small plaques did not vary over the 24-day period of observation of the organ cultures.

In order to determine whether chronically-infected organ cultures were productive of a temperature-sensitive virus population, virus assays were performed in Vero cells at 30, 37, and 41° C. No difference in titer or proportion of large to small plaques was observed. This confirms other observations from our laboratories that the naturally-occurring avirulent WEE strains are not TS.

(T. P. Monath, G. S. Bowen, M. Gresikova)

Laboratory Studies on the ability of Oeciacus vicarius to serve as a host and vector of western encephalitis (WE) virus.

As discussed in previous reports (Arthropod-borne Virus Information Exchange No. 26:118; No. 27:76-77), atypical strains of WE virus have been recovered from nestling house sparrows (Passer domesticus), nestling cliff swallows (Petrochelidon pyrrhonota), and cliff swallow bugs (Oeciacus vicarius) from a bridge collecting site over the So. Platte River in Morgan County, northeastern Colorado.

In 1974, the first WE virus isolations were made from cliff swallow bugs in May. Although virus was not recovered from these hemipterans collected in July and August, WE virus has been recovered from pools tested in each succeeding month through January.

Preliminary experimental studies with the cliff swallow bug and a strain of WE virus (CMA4-368) recovered from a nestling house sparrow have been initiated. In an attempt to obtain a viremic host for feeding the cliff swallow bugs, original nestling blood from isolate CMA4-368 was inoculated subcutaneously into wet chicks ($\leq$0.5 day). Chicks were bled at closely spaced intervals for three days following inoculation and blood samples from 5 of the chicks tested. Four of the 5 chicks were negative, however, a single chick had a titer $\geq 10^{4.7}$/0.1 ml at 18 hrs. and $10^{1.9}$ at 36 hrs. All chicks survived without apparent signs of distress or illness. The 18 hr. blood sample from the single viremic chick was then inoculated subcutaneously into 6 additional wet chicks, which all died between 24 and 30 hrs. Blood samples collected at 18 and 24 hrs. from these 6 chicks had titers ranging from $10^{8.1}$-$10^{9.5}$/0.1 ml blood. Three additional wet chicks were then inoculated with the 18 hr. blood sample from the original viremic

chick and O. vicarius bugs fed on these chicks at 24 hrs. After 7 days incubation at 75°F, 2 pools each of 5 adult and 5 nymphal bugs were tested in primary duck embryo cell culture (DECC) and found positive. The suspension from the nymphal bugs also killed 5/5 wet chicks, 8/8 suckling mice, and 8/8 suckling hamsters. After 14 days incubation, individual bugs were tested in DECC and 3/5 adults and 2/5 nymphs were positive. Studies on replication and transmission of WE virus in the cliff swallow bugs are in progress.

Two points of interest emerge from this very preliminary work with the CMA4-368 strain of WE virus recovered from a nestling sparrow. The first is the very rapid and marked change in pathogenicity and virulence for laboratory hosts resulting from a single passage of the original nestling blood through wet chicks, and the second is the suggestion that the virus replicates in the cliff swallow bugs following ingestion of viremic blood.

D.B. Francy and W.A. Rush

Human Infection with Bhanja Virus: During studies with strain #7 of Bhanja virus from ticks collected in Yugoslavia (see this issue, report from the Andrija Stampar School of Public Health) a laboratory worker (CHC) became ill. On April 25, 1974, 34 days after the first suckling mice were inoculated in Ft. Collins, CHC awoke with mild myalgia and arthralgia, slight elevation of temperature (37.4°C), a mild frontal headache and mild photophobia. A blood specimen was drawn and the serum separated and inoculated into SM by the ic route on the same day. The mice were housed, handled and maintained in separate, but adjoining, quarters from those used for mice inoculated with specimens from Yugoslavia. Symptoms in the patient lasted approximately 48 hours, but were not so severe as to cause absenteeism. No sequalae were observed. On the 6th day after inoculation, 14 of 14 SM showed signs of infection characterized by weakness, ataxia, hyperpnea, anorexia, and tremors. By the following day, two were dead and the remaining 12 prostrate. All mice were collected, their brains harvested and pooled and the isolate designated R-1819. The virus later was reisolated from the serum drawn April 25, this serum had titers of 10^6 $SMICLD_{50}$/ml and $10^{6.2}$ plaque forming units per ml in Vero cells. The patient was bled 31 and 185 days after onset of symptoms. A serum fortuitously drawn 3 days prior to onset, the sera drawn 31 and 185 days after onset, a mouse immune serum prepared against Bhanja strain #7, and two reference mouse hyperimmune ascitic fluids prepared against Bhanja virus strains from India (IG 690) and Nigeria (IbAr 2709) were tested by complement fixation against crude antigens prepared from strain #7, an antigen prepared from isolate R-1819 and sucrose acetone extracted antigens of the reference strains. All reference materials were kindly provided by Drs. Robert E. Shope and Jordi Casals, Yale Arbovirus Reference Unit, New Haven, Connecticut.

The results, presented in Table 1 show identity of strain #7, isolate R-1819 and the two reference strains of Bhanja virus and demonstrate a diagnostic rise in titer to Bhanja virus in the sequential sera from the patient.

Both strains #7 and R-1819 were tested against the three sera from the patient and the mouse serum prepared against strain #7 in a serum dilution plaque reduction neutralization test performed in Vero cells, using 100 plaque forming units of each virus. As may be seen from Table 2, both Bhanja strains #7 and R-1819 reacted with the mouse serum and the patient's sera drawn 1 and 6 months after onset, demonstrating diagnostic rises in antibody in the patient.

The patient handled both viruses 34, 22, 20, 13, 9, 5, 4 and 2 days prior to onset of his symptoms. At best, one may estimate the incubation period for from 2 to 13 days prior to onset; however, the most probable frame of reference is 2 to 9, 4, 5 or 9 days as these were the times when virus was worked with most intensely. It must be pointed out that all mice on test were examined daily and infection of the patient could have occurred through contaminated water, feed, bedding, feces and urine or other fomites within the area housing the animals.

Since this virus had never been in this laboratory prior to the isolation of the two strains from Yugoslavia, it appears reasonable to relate the patient's illness, the isolation of Bhanja virus from the patient's serum, the fact that all mice showed signs of illness after inoculation with patient's serum R-1819 drawn the day of onset of symptoms, reisolation of the virus from the patient's serum, the similar CF and N reactivities of strains #7 and R-1819 with the patient's sera, the mouse serum and the reference Bhanja-immune ascitic fluids, and the diagnostic rise to Bhanja virus one and six months after onset of symptoms. It can be concluded that the patient had been infected with Bhanja virus and that his illness was a consequence of the infection.

Bhanja was first isolated from Hemaphysalis intermedia ticks from a paralyzed goat in India, but it was not possible to associate the paralysis to the virus. Since that time, Bhanja virus has been isolated from only two mammals, an African ground squirrel (Xerus erythropus) and an African hedgehog (Atelerix albiventris), although goats, sheep and other mammals have been shown to have antibody in serosurveys in India and Italy. This then is the first report of a human infection with Bhanja virus and serves to demonstrate the importance of taking precautions when working with poorly characterized viruses, even for highly experienced personnel working with excellent facilities and immunized against a battery of arboviruses known to cause disease in man.

C. H. Calisher

TABLE 1. CROSS-REACTIVITY OF STRAIN R-1819 BY COMPLEMENT FIXATION

Antigen \ Serum	C.H. Calisher sera			MIS	From YARU		
	4-22-74	5-23-74	10-24-74	Bhanja #7	Bhanja (IbAr2709) HIAF	Bhanja (IG690) HIAF	Normal Mouse Serum
Bhanja #7	<8	16	32	128	64	32	<8
R-1819	<8	32	32	128	128	64	<8
Bhanja (IbAr2709)	<8	16	32	64	64	32	<8
Bhanja (IG690)	<8	16	64	128	64	64	<8
Normal	<8	<8	<8	<8	<8	<8	<8

TABLE 2. CROSS-REACTIVITY OF STRAIN R-1819 BY NEUTRALIZATION

Virus \ Serum	C.H. Calisher sera			MIS	
	4-22-74	5-23-74	10-24-74	Bhanja #7	Normal Mouse Serum
Bhanja #7	<40	≥640	320	320	<10
R-1819	<40	160	40	80	<10

Field studies of Colorado tick fever in Rocky Mountain National Park

During 1973 and 1974, the Vector-borne Diseases Division documented 143 cases of human Colorado tick fever (CTF). A large number of the patients were exposed to CTF virus in or around Estes Park and the Rocky Mountain National Park. In 1974 extensive field studies were initiated in Rocky Mountain National Park in attempts to elucidate tick and mammalian involvement in the CTF maintenance cycle in this area of apparent high human exposure. Ecological parameters examined included

1. Focality and intensity of CTF transmission
2. Altitudinal variation in CTF transmission, vectors and reservoirs
3. Differential involvement of mammalian species
4. Tick ectoparisitism on mammal species
5. Infection rates in several tick species and stages and their relationship to mammalian hosts
6. Seasonal variation in the above factors

Animals and ticks were collected in seven locations ranging in elevation from 7,500 to 10,500 feet. Two areas (Upper Moraine Park, Beaver Meadow) were undisturbed natural settings of south-facing slopes, meadow and north-facing slopes. One area (Rock Ridge) was undisturbed tundra. Four areas (Moraine Park Campground, Aspenglen Campground, Many Parks and Rainbow) were ecologically disturbed by human campgrounds or animal feeding areas. Marked differences were found in levels of CTF transmission between the different areas studied (Table 1). The highest virus isolation rates from mammal blood samples and immature ticks were found at Upper Moraine Park, a relatively undisturbed area contiguous to Moraine Park Campground where the next highest virus isolation rates were found. Twenty-one per cent of larval and nymphal tick pools and 6.5% of animal blood samples were virus positive at Upper Moraine Park vs. 11.4% and 4.5%, respectively, at Moraine Park Campground. Adult tick virus isolation rates, however, were highest at Moraine Park Campground (50% of pools positive).

More moderate levels of virus activity were found at Aspenglen Campground, Many Parks, and Beaver Meadow. No virus isolations were made at Rainbow or Rock Ridge, both of which were above 10,000 ft. elevation. Significant virus activity was seen at Many Parks (Elev. 9,300 ft.) as well as the four areas at 7,500 ft.

Table 2 shows virus isolation rates from the blood of the four most abundant mammalian species (Eutamias minimus, the least chipmunk; Spermophilus lateralis, the golden-mantled squirrel; Spermophilus richardsoni, Richardson's ground squirrel; and Peromyscus maniculatus, deer mouse) and the 90% plaque reduction neutralization serology from the same animals. Virus isolation and seropositivity rates were highest overall in E. minimus, followed by S. lateralis. In the areas of highest virus activity (Upper Moraine Park, Moraine Park Campground), virus isolation rates were much higher in E. minimus (19.2% vs. 5.6%) but seropositivity rates were comparable in both species (35.2% vs. 37.7%). The results imply that viremia at titer high enough to be detected was more persistent in E. minimus. At Many Parks, where S. lateralis and E. umbrinus in place of E. minimus were collected, virus activity occurred only in S. lateralis.

Virus isolations from ticks were made almost entirely from Dermacentor andersoni. Twenty-eight CTF isolations were made from adult D. andersoni collected by dragging. Over one hundred CTF isolations were made from nymphal D. andersoni collected as ectoparasites from mammals. Only three isolates came from D. andersoni larval pools. Two isolations were made from pools of larval Ixodes spp. ticks, all stages of which were taken as ectoparasites from trapped mammals. D. andersoni collected as ectoparasites were almost entirely nymphs between April and July. In August and September the larval stage predominated.

The highest percentage of animals infested with ticks (49.5%) and the highest tick load per infested animal (6.2 ticks/infested animal) were seen in S. lateralis. Additionally, 22.5% of tick pools from S. lateralis were virus positive vs. 17.2% of pools taken from E. minimus.

Monthly variation was evident for some of the parameters studied. Adult ticks were abundant only in April-June and were very difficult to obtain from July-September. Peak infection rates of ectoparasite D. andersoni nymphs were in May and June. In the Moraine Park area, virus isolation rates from blood samples of E. minimus were highest in April, May, July and August (18-30%). Lower numbers collected in June may have biased results negatively for this month. A definite fall in proportion of viremic chipmunks took place in September, during which month only 2 of 40 (5%) of these animals bled were viremic. Rates of virus isolation in S. lateralis remained between 5% and 7.5% during May to August when these animals were above ground.

Further studies to relate vertebrate and vector population parameters to virus maintenance are in progress for 1975. Experimental studies of CTF infections in the above vertebrate hosts in the laboratory are also being carried out since seropositive viremic animals have been found, and since only about 50% of a small number of viremic animals were seropositive on recapture one or more months after initial capture.

(G. Stephen Bowen, Robert G. McLean, D. Bruce Francy, Allen M. Barnes, Werner L. Jakob, and Charles H. Calisher)

TABLE 1

CTF VIRUS ISOLATIONS IN 7 RMNP STUDY AREAS

	Type of Specimen Tested								
	Adult Tick			Immature Tick			Mammal Blood		
Area Studied	No. Pools Tested	No. Pos.	% Pos.	No. Pools Tested	No. Pos.	% Pos.	No. Pools Tested	No. Pos.	% Pos.
Upper Moraine Park	42	6	14	406	87	21	518	34	6.5
Moraine Park Campground	30	15	50	183	21	11.4	463	21	4.5
Aspenglen Campground	2	0	0	235	5	4.8	242	8	3.3
Many Parks	13	4	31	43	1	2.3	70	4	5.7
Beaver Meadow	10	3	30	113	5	4.4	240	4	1.6
Rainbow	4	0	0	5	0	0	155	0	0
Rock Ridge	-	-	-	0	-	-	28	0	0

TABLE 2

CTF VIRUS ISOLATIONS AND SEROLOGY FROM FOUR ABUNDANT MAMMALS OF RMNP

	Type of Specimen Tested					
	Virus Isolation			Plaque Reduction Neut. Serology		
Species	No. Bloods Tested	No. Positive	% Positive	No. Bloods Tested	No. Positive	% Positive
E. minimus	218	34	15.5	208	70	33.6
S. lateralis	398	22	5.5	367	80	21.7
S. richardsoni	351	9	2.5	333	5	1.5
P. maniculatus	604	5	0.8	580	56	9.6

TABLE 3

TICK ECTOPARISITISM AND VIRUS ISOLATIONS FROM FOUR MAMMAL SPECIES IN RMNP

Species	# Animals Collected	# Ticks Infested	% Tick Infested	# Ticks Collected	# Ticks/Infested Animal	# Ticks Tested	# Pools Tested	# Pos. Pool	% Pos. Pool
E. minimus	293	110	37.5	434	3.9	392	128	22	17.2
S. lateralis	519	257	49.5	1605	6.2	1529	363	82	22.5
S. richardsoni	459	136	29.6	265	1.9	231	145	8	5.5
P. maniculatus	953	177	18.6	429	2.4	315	169	5	2.9
Other	241	19	7.8	99	5.2	86	30	1	3.3

REPORT FROM ENVIRONMENTAL AND ECOLOGY BRANCH, U.S. ARMY
DUGWAY PROVING GROUND, DUGWAY, UTAH

During July, August, and September 1974 insects were collected by CDC miniature light traps at three sites along the western border and four sites along the southern border of Utah. Insects were sealed in vials, frozen on dry ice, and transported to Dugway where they were pooled on a CDC chill table prior to intracerebral inoculation into suckling mice. From western Utah 25,470 insects were obtained with 123 trap nights for an average of 207 insects per trap. There were 45 California Group (CAL) viruses, of which 41 were from Aedes dorsalis from Blue Lake near Wendover, Nevada, and three unidentified isolates. Viruses not yet identified were one each from A. dorsalis, Culex tarsalis, and Culicoides variipennis from Callao, Utah. From southern Utah 5863 insects were collected with 252 trap nights for an average of 23 insects per trap. The only three viruses isolated were from Anopheles freeborni from Beaver Wash, Arizona, near the Utah-Nevada border and these have not yet been identified.

(G. T. Crane, R. E. Elbel, and K. L. Smart)

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

Surveillance for mosquito-borne encephalitis during 1974 again confirmed the persistance of western encephalitis (WEE) and St. Louis encephalitis (SLE) viruses in mosquitoes, but they were detected much less frequently than in previous survey years. Only 4 strains of WEE virus were isolated from sites in Riverside County, and only 2 strains of SLE virus from Imperial County. There were 29 strains of Turlock virus isolated from Colusa, Imperial, Kern, Placer, Riverside, San Bernardino, Shasta, Solano, and Sutter Counties, and from Mojave and Yuma Counties in Arizona. Nine strains of Hart Park virus were isolated from Kern, Placer, Solano, Stanislaus, Tehama and Yuba Counties. All viral isolates were from Culex tarsalis except for 2 isolates of Turlock virus from Culex erythrothorax. A total of 67,299 mosquitoes in 1,690 pools were collected and tested from April 10 through November 22 from study sites in 26 California counties and from Mojave and Yuma Counties in Arizona. A limited budget required this smaller effort as compared with 1973, when 4,838 pools were tested (75 strains of SLE virus and 97 strains of WEE virus isolated), or 1972 when 6,336 pools were tested (64 strains of SLE virus and 42 strains of WEE virus isolated).

The apparently low level of WEE and SLE virus activity during 1974 was reflected in an unusually low incidence of human and equine arbovirus disease. Although the usual extensive screening program of suspect encephalitis cases was carried out statewide (643 persons tested serologically for WEE and SLE by the Viral and Rickettsial Disease Laboratory and by 5 County Public Health Laboratories), for the first time since specific laboratory tests were developed in the 1940's and accurate records have been kept, there were no proven human cases of WEE or SLE acquired in California. One case of SLE

was confirmed in an 87 year old woman in Bakersfield, Kern County. She had arrived for a visit on August 23, having left her home in Alvin, Brazori County, Texas, on August 20. Mosquito exposure in Texas was her presumptive source of infection. Onset of illness was August 28, with fever, earache, and "cold" symptoms. She was hospitalized when she developed fever to 104° F, inability to walk, then coma. The cerebrospinal fluid had 68 cells (predominantly lymphocytes) and 70 mgm% protein. The complement-fixation test for SLE showed a rising antibody titer from 1:4 to 1:32. The indirect fluorescent antibody test showed SLE antibody titers of 1:256, and the hemagglutination-inhibition test showed titers of 1:640 in both the acute- and convalescent-phase sera. The mouse neutralization index was 2.8 in the acute-phase serum and 3.3 in the convalescent-phase serum. The patient recovered completely and returned home to Texas.

Only 2 cases of WEE in equines had laboratory confirmation during the year: a 6 year old horse from Bakersfield, Kern County, with onset July 13 (presumptive positive); and a 1 year old horse from Temecula, Riverside County, with onset August 31. There were 59 other suspect equine cases reported to the Department, but laboratory proof of the etiology could not be obtained.

The other arbovirus disease of significance during 1974 was Colorado tick fever: 14 cases were confirmed in persons exposed to tick bite in Alpine, Lassen, Modoc, Mono, Placer, and Plumas Counties and in Colorado. This brings the total proven cases since record-keeping began in 1954 to 179. It is undoubtedly under-reported, and may be the commonest vector-borne infectious disease in California.

(R.W. Emmons)

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

California encephalitis (CE) virus isolates from wild-caught adult Aedes communis mosquitoes (74-Y-234) and Aedes sp. larvae (74-L-82) in the Yukon Territory during summer 1974 have propagated in laboratory-bred A. aegypti mosquitoes after intrathoracic injection of 100 mouse LD_{50} of CE isolate and incubation both at 55°F and 70°F. Infectivity increments were detected in thoraces, and CE-specific immunoperoxidase staining of cytoplasm of acinar cells of salivary glands was demonstrated in mosquitoes after 6 to 27 days incubation at 55°F for the 74-Y-234 strain, after 6 to 21 days incubation at 70°F and after 30 days' incubation at 55°F for the 74-L-82 strain. The minimum infectivity dose of the 74-Y-234 strain for A. aegypti by intrathoracic injection was 0.1 mouse LD_{50} after incubation for 12 days at 70°F and 55°F. CE specific immunoperoxidase staining and infectivity increments were detected after 3 days' incubation at 70°F, 95°F and 102°F, following intrathoracic injection of A. aegypti with 100 mouse LD_{50} of the 1971 Yukon prototype strain (Marsh Lake 23) of CE virus (snowshoe hare subtype).

A recent Alaskan Bunyavirus isolate, Northway (NOR) virus, has propagated after intrathoracic injection of A. aegypti with 100 mouse LD_{50}, following 6 and 13 days incubation at 55°F, 2 to 20 days at 70°F, 2 and 3 days at 95°F and 2 and 3 days at 102°F, and NOR-specific immunoperoxidase reactions were observed by light microscopy of salivary glands from infected mosquitoes.

Murray Valley encephalitis (MVE) replication was demonstrated in A. aegypti mosquitoes 3 to 6 days after intrathoracic injection of 300 mouse LD_{50} following incubation at 70°F, after 2 to 6 days at 95°F and after 2 to 6 days at 102°F, and MVE-specific immunoperoxidase reactions were detected in salivary glands of infected mosquitoes. After injection of 10 and 1 mouse LD_{50} viral replication and immunoperoxidase staining were observed following 19 and 26 days' incubation at 55°F. The minimum infectivity dose of MVE virus for A. aegypti incubated for 19 days at 70°F was 0.1 mouse LD_{50}.

(D.M. McLean)

REPORT FROM THE PACIFIC RESEARCH SECTION
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH, HONOLULU, HAWAII

A. Transovarial transmission of La Crosse virus by Aedes albopictus.

Recent reports of isolations of La Crosse, Keystone, and snowshoe hare viruses from wild caught Aedes sp. mosquito larvae as well as laboratory demonstration of La Crosse virus transovarial transmission by A. triseriatus suggest that vertical transmission in mosquitoes may be a mechanism common to many viruses of the California serogroup. These reports prompted us to test whether LAC virus transovarial transmission might occur in other mosquito species. We also considered whether vertical transmission might occur with several related, non-California group viruses.

Groups of female A. albopictus and C. fatigans were inoculated with each of the following viruses: La Crosse, Cache Valley, Batai, Arumowat, Itaporanga and VSV-Indiana. After incubation at 32°C for 8 or 10 days, the mosquitoes were allowed to feed on clean laboratory mice. The subsequent eggs were collected, hatched and the resultant adult offspring were tested individually for the presence of virus. Results are summarized in Table 1.

Transovarial transmission of La Crosse virus by experimentally infected A. albopictus females to 2.7% of their F_1 generation offspring was demonstrated. Progency of both sexes were infected. Mean virus titers in parent mosquitoes and infected F_1 generation adults were $10^{4.6}$ and $10^{3.4}$ PFU/insect, respectively. The La Crosse infected offspring were randomly distributed among the female parents. After 2 serial passages in A. albopictus, a marked change occurred in the plaque morphology of the virus but this had no apparent effect on the subsequent vertical transmission rate. In contrast, transovarial transmission did not occur in La Crosse infected Culex fatigans or with A. albopictus and C. fatigans infected with VSV-Indiana, Cache Valley, Batai, Arumowot and Itaporanga viruses. Results of this experiment indicate that the La Crosse model might be useful in studying the mechanism of transovarial transmission in additional mosquito species. The data also suggest that while vertical transmission may be a mechanism common to the California serogroup, it does not occur with all Bunyaviridae.

(R.B. Tesh and D.G. Gubler)

Table 1

Summary of viruses tested in

A. albopictus and *C. fatigans* for transovarial transmission

Virus tested	Mosquito species	Mean virus titer of parents post-oviposition	Number of F_1 offspring tested	Transovarial transmission rate
La Crosse	*A. albopictus*	4.6*	1,022	2.7%
La Crosse	*C. fatigans*	4.8	316	0
Cache Valley	*A. albopictus*	4.2	166	0
Cache Valley	*C. fatigans*	3.8	21	0
Batai	*C. fatigans*	4.2	237	0
Arumowot	*A. albopictus*	4.3	92	0
Itaporanga	*A. albopictus*	2.5	87	0
Itaporanga	*C. fatigans*	3.7	122	0
VSV-Indiana	*A. albopictus*	2.5	173	0

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* Log 10 plaque forming units per insect as assayed in Vero cell cultures.

B. Failure of rubella virus to replicate in mosquitoes.

Rubella virus recently has been included in the togavirus family because of physicochemical and morphologic relationships to the alpha-and flaviviruses. Although rubella virus is antigenically distinct from arboviruses of the A and B serogroups, several workers have suggested that it might be an arbovirus. In order to determine whether rubella virus replicates in arthropods, survival of the virus was studied following parenteral inoculation into mosquitoes.

Rubella virus used was the M-33 strain, obtained from the American Type Culture Collection, passed 3 times in BSC-1 and twice in Vero cell cultures. Mosquitoes utilized were 1 to 3 day old female A. albopictus and C. fatigans from our laboratory colony. Each insect was inoculated intrathoracically with approximately 0.0003 ml of undiluted virus suspension. Immediately after inoculation, 6 mosquitoes of each species were frozen at -70°C for subsequent virus titration. The remaining insects were maintained on 10% sucrose solution at 32°C and were sampled 7, 14 and 21 days post-inoculation.

Assay of rubella virus was done in tube cultures of the RK_{13} continuous line of rabbit kidney cells, provided by Dr. Paul D. Parkman, Bureau of Biologics, Food and Drug Administration. Mosquitoes were triturated individually in sterile Ten Broeck tissue grinders with 2.0 ml of phosphate buffered saline, pH 7.4, containing 0.5% gelatin, 25% heated calf serum (56°C for 30 minutes), 800 units penicillin and 800 µg streptomycin. After centrifugation of the mosquito suspension at 8,800 g for 30 minutes at 5°C, serial 10-fold dilutions of the supernatant were prepared. Then 0.1 ml of each dilution was inoculated into 4 RK_{13} tube cultures. After absorption for 1 hour at 37°C, 1.0 ml of maintenance medium was added to each tube, and the cultures were incubated at 32°C for 21 days. The medium was changed every 3 to 4 days. Cultures were observed regularly for 21 days for rubella cytopathic effect. Virus titers were calculated as the tissue culture cytopathic dose 50 ($TCID_{50}$) per mosquito by the method of Reed and Muench.

Table 2 summarizes results of this experiment. Rubella virus titers in mosquitoes tested immediately after inoculation (day 0) varied from $10^{1.8}$ to $10^{3.4}$ $TCID_{50}$ per insect. In contrast, no virus was detected in mosquitoes sampled on days 7, 14 and 21 post-inoculation. These data indicate an inability of rubella virus to replicate or to survive in mosquito tissues (A. albopictus and C. fatigans). This we interpret as further evidence that rubella is not an arbovirus.

(R. B. Tesh and L. Rosen)

Table 2

Culture results of mosquitoes inoculated with rubella virus

Day Post-inoculation	A. albopictus			C. fatigans		
	No. pos. / No. tested	Range of titers	Mean titer	No. pos. / No. tested	Range of titers	Mean titer
0	6/6	$10^{2.5}$ - $10^{3.5}$*	$10^{2.9}$	6/6	$10^{1.8}$ - $10^{2.8}$	$10^{2.3}$
7	0/6	0**	-	0/6	0	-
14	0/6	0	-	0/6	0	-
21	0/9	0	-	0/3	0	-

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* $TCID_{50}$ per insect as titrated in RK_{13} tube cultures.

** 0 = $<10^{1.3}$ $TCID_{50}$

A method to study transmission of dengue virus by mosquitoes

Transmission studies with dengue viruses have been limited in the past by the lack of a suitable vertebrate laboratory host. Both man and lower primates have been used for this purpose, but both have obvious disadvantages. Moreover, in transmission studies using these hosts, it is not possible to determine the amount of virus expelled by the mosquito while feeding. We would like to describe a simple method of measuring the amount of virus a mosquito may expel.

After an incubation period sufficient to allow virus to replicate in the salivary glands, infected mosquitoes are transferred to individual 9 dram vials covered at the top with fine mesh nylon organdy and starved for 24 hours. A drop (0.025 ml) of feeding suspension consisting of equal parts of washed human erythrocytes, heat inactivated calf serum, and 10% sucrose is placed on the nylon organdy of each vial using a "Biopette" automatic pipetter. The vials containing individual mosquitoes are placed in a row so that an observer can watch each mosquito as it either feeds or probes the drop. After feeding, the amount of blood ingested by each insect is recorded. A drop of phosphate buffered saline (PBS) (pH 7.4) containing 30% heat inactivated calf serum is then added to the drop of feeding suspension with the automatic pipetter using a clean tip for each mosquito. The PBS and feeding suspension is then mixed thoroughly with a Pasteur pipette and transferred to a labeled 1 dram vial. This mixture contains approximately 0.05 ml and can be stored at -80°C until assayed for virus content. If desired, the virus content of the entire mosquito or its salivary glands can then be studied, either by virus assay or a direct fluorescent antibody technique.

The feeding drop for each infected mosquito is assayed quantitatively for virus content using the mosquito inoculation technique described by Rosen and Gubler. Briefly, serial dilutions of the feeding drop are inoculated intrathoracically into Aedes albopictus. The inoculated mosquitoes are held for 10 days at 32°C and then tested for the presence or absence of virus. At least 5 mosquitoes are tested for each dilution and the mosquito ID_{50} calculated by the method of Reed and Muench.

The data presented in Table 1 show that the majority (83.3%) of the mosquitoes with all salivary gland lobes fully infected will transfer virus to the feeding drop and presumably would have transmitted the infection to a susceptible animal host. The amount of virus transferred ranged from $<10^{2.5}$ to over $10^{4.0}$ MID_{50}. Even mosquitoes which probed the suspension without feeding were capable of virus transfer (mosquito no. 32689N). The reason that 2 mosquitoes failed to transfer virus is not known. This occurred in an early experiment and could have been due to faulty technique.

Obviously, this method could also be used to estimate the vector competence of mosquitoes for viruses other than dengue without employing laboratory animals.

(D.J. Gubler and L. Rosen)

Table 1

Transfer of dengue 2 virus by Aedes albopictus to an artificial feeding suspension

Mosquito number*	Amount of blood ingested**	Transmission	Amount of virus expelled (MID_{50})
32525Y	3/4	+	$3x10^2$
32525BB	1/2	+	10^3
32525JJ	<1/2	+	$3x10^2$
32525KK	<1/2	+	$\geq 10^3$
32907G	F	-	-
32907H	3/4	-	-
32689K	F	+	$5x10^3$
32689L	F	+	$<3x10^2$
32689M	1/2	+	$5x10^2$
32689N	trace	+	$5x10^2$
32842P	F	+	$\geq 10^4$
Total	-	83.3%	$8x10^2$

* Dengue antigen was observed by the direct fluorescent antibody technique in all tissue of the six salivary gland lobes of each mosquito.

** F = fully engorged

fractions = estimated fraction of potential complete blood meal ingested by mosquito

trace = mosquito probed but took little blood.

REPORT FROM THE VETERINARY RESEARCH LABORATORY, NAIROBI, KENYA

By CF and indirect FA tests carried out using Nairobi Sheep Disease (NSD) antigen and mouse ascitic fluids prepared against NSD and Ganjam viruses, the viruses seem to be identical or closely related. CF tests with Ganjam antigen and NSD antiserum confirmed this relationship.

(F.G. Davies)