ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 30 March 1976

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

COMMENTS FROM THE EDITOR

The 1975 Annual Report on the working catalogue is included in this issue of the Information Exchange, pages 2-59. It represents a careful updating of Dr. T.O. Berge's 1974 Annual Report (Info Exchange No. 28) by Nick Karabatsos, the current Catalogue Editor.

Also of special note is Fred Murphy's announcement of the opening of the WHO Centre for Collection and Evaluation of Data on Comparative Virology, page 60. It is quite likely that the American Committee on Arthropod-borne Viruses will have considerable contact in the future with this new Centre.

Some new contributors are recognized in this issue of the Information Exchange; they are most welcome and we hope they will continue to submit reports of current work.

Be reminded that the deadline for receipt of reports for issue No. 31 is September 1, 1976. Please keep the reports coming in. We would especially like to receive reports from those of you who have rarely contributed. The continued success and vitality of the Info Exchange depends on a sharing of information by all.

Send your reports to:

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The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1975 ANNUAL REPORT ON THE CATALOGUE OF ARTHROPOD-BORNE AND SELECTED VERTEBRATE VIRUSES OF THE WORLD*

by

THE SUBCOMMITTEE ON ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

I. Objectives:

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

II. Materials and Methods:

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

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

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

Distribution of Catalogue Material: At the start of 1975, 164 mailings of Catalogue material were being made. During the year, 11 participants were dropped and 4 new participants were added to the mailing list. At the end of the year, 157 mailings of Catalogue material were being made, including 61 within the U.S.A. and 96 to foreign addresses. Distribution by continent was: Africa 14, Asia 17, Australasia 6, Europe 33, North America 70, and South America 17.

Abstracts and Current Information: A total of 722 abstracts or references were coded by subject matter and distributed to participants during 1975. Of this total, 543 were obtained from Biological Abstracts, 170 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 9 from current journals, personal communications, or other sources. A total of 9,594 references or units of information have been issued since the start of the program.

Change in the Status of Registered Viruses: At the request of Dr. R. Doherty, the Samford virus registration was dropped. Recent information indicated that it was antigenically identical to Aino virus which had been isolated earlier.

Registration of New Viruses: Eighteen viruses were submitted for consideration for registration during 1975. Of this number, 11 viruses have been registered during the year and the remaining 7 are in various stages of review or processing prior to official registration. As of December 1974, there were 359 registered viruses in the Catalogue. With the deletion of Samford and the addition of the 11 new registrations, the total number of registered viruses increased to 369. The viruses submitted and registered during 1975 are listed below:

Virus Name	Recommended Abbreviation	Country	Source	Antigenic Group
Bagaza	BAG	Cent. Afr. Rep.	Mosquitoes	B
Bobaya	BOB	Cent. Afr. Rep.	Bird	
Eyach	EYA	W. Germany	Ixodid ticks	CTF
Ippy	IPPY	Cent. Afr. Rep.	Rodent	
Nugget	NUG	Macquarie Island, So. Ocean	Ixodid ticks	KEM
Orungo	ORU	Uganda	Mosquitoes	
Oubangui	OUB	Cent. Afr. Rep.	Mosquitoes	
Salanga	SGA	Cent. Afr. Rep.	Rodent	
Sebokele	SEB	Cent. Afr. Rep.	Rodent	
Snowshoe Hare	SSH	U.S.A.	Hare	CAL
Taggert	TAG	Macquarie Island, So. Ocean	Ixodid ticks	SAK

All of the above viruses were isolated between 1959 and 1972. Two were isolated in 1959 (ORU, SSH), one each in 1966 (BAG) and 1970 (IPPY), two in 1971 (BOB, SGA) and five in 1972 (EYA, NUG, OUB, SEB, TAG).

Antigenic Grouping: The name of the Ganjam antigenic group has been changed, and it has acquired an additional member with the observed antigenic relationship of Nairobi Sheep Disease to Ganjam virus. The NSD antigenic group, composed of NSD, Ganjam, and Dugbe, supersedes the Ganjam group, which was composed of Ganjam and Dugbe viruses.

The Colorado tick fever antigenic group has been constituted with the registration of Eyach virus, which was isolated in West Germany. The group presently consists of Eyach and CTF viruses, and it is added to the list of antigenic groups now represented in the Catalogue.

Publication of the Catalogue: During 1975, a second edition of the Arbovirus Catalogue was published. This edition was entitled the "International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates," and represented the working Catalogue as of December 1974. The second edition of the published Catalogue contains information on 359 registered viruses. The published Catalogue was available for distribution in early June 1975. For about a year before publication, individual registrations were reviewed and/or revised in preparation for printing.

Revised Virus Registration Forms: The revised virus registration forms were printed and made available to the Catalogue Editor in early fall of 1975. Notice of their availability has been publicized, and they are currently being distributed to new registrants.

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.

As the revised registration forms become more widely used and with the eventual reactivation of the computer program for storage and retrieval of information on registered viruses, it is anticipated that additional other types of analyses will be presented.

Table 1. Alphabetical listing of registered viruses. Table 1 lists in alphabetical order the 369 viruses registered in the Catalogue as of December 1975. 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, 48 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 (58 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.

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

Viruses discovered initially in the first three decades of this century, and many of those first recognized in the 1940's 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; almost 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 (103), followed by North America (73), South America (71), Asia (64), Australasia (36), and Europe (22).

The countries which have yielded the largest number of registered viruses are the United States of America (46), Brazil (40), Australia (30), India (22), the Central African Republic (27), 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 other arthropods. 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.

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 physicochemical characteristics, group B viruses can be subdivided according to their principal vectors.

The largest subgroup, 28 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, 7 others only from bats, 2 from man, and one from 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 12 registered members. The serologic evidence for the inclusion of Bocas virus in the California group remains minimal. As with group C viruses, all members of the California group are associated with mosquito vectors; most of them (10 of 12) 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). Three 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 12 registered California group viruses have been examined by SIRACA; the Bocas, snowshoe hare, 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 Cross, 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 15 members of the Simbu group have been isolated either from culicine mosquitoes or from culicoides; of these recoveries from arthropods, 4 viruses have been isolated from mosquitoes alone; 3 only from culicoides; and 5 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 Para, 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 9 as possible arboviruses.

Table 14. Tete group and unassigned (SBU) viruses. Little is known of the 4 Tete group viruses which originally were isolated only from wild-caught birds in Europe (Cyprus, Italy), Africa (Egypt, Nigeria, South Africa), and Asia (Japan). Since then, both Bahig and Matruh viruses have been isolated from ixodid ticks in Egypt. 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. Fourteen of the agents have been isolated from phlebotomine flies; two (Icoaraci, Chagres) have been recovered from both sandflies and mosquitoes in nature. Two others have been isolated from wild-caught mosquitoes but never from phlebotomine flies; these two, 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, 7 are probably arboviruses, while in regard to the remaining 10, 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 morphological characteristics 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 Kaisodi group consists of 3 members, 2 of which have been isolated in Asia from ticks collected in forest undergrowth or from forst rodents, and one in North America from snowshoe hares and their ticks.

The Nairobi Sheep Disease group which previously was designated the Ganjam group, now consists of 3 members. Demonstration of a close antigenic relationship between NSD and Ganjam viruses has led to the inclusion of NSD virus in the group and the constitution of the Nairobi Sheep Disease antigenic group. Two of the members (Dugbe, Ganjam) have been recovered repeatedly from ixodid ticks and, rarely, from culicine mosquitoes. All three viruses have been associated with sporadic cases of febrile illness in man, while both NSD and Ganjam have been implicated in laboratory infections. In addition, NSD causes serious disease with high mortality in sheep.

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. The lower portion of table 17 lists the 16 presently registered members of the growing Kemerovo group of tick-borne non-group B viruses. The Kemerovo group

members differ 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. Eleven of the viruses in the KEM group were recovered initially from ticks taken from nesting areas of marine birds (9 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, South America, and Australasia, and 2 in more than one continent. Of the 16 viruses registered, 2 have been rated as probable arboviruses with 14 classified as possible arboviruses.

The Colorado Tick Fever group, shown at the top of Table 17, is a newly constituted antigenic group which consists of two members. Eyach virus, which was isolated in W. Germany, was shown to be closely related to CTF virus, and it was registered during 1975. Colorado tick fever virus causes considerable human disease yearly in the Western United States, and it has been classified taxonomically as an orbivirus.

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. Both members of the Quaranfil group now are classified as arbovirus.

The newly created Sakhalin group consists of 4 viruses which again have been isolated from ticks taken from nesting areas of sea birds, with one also recovered from a young herring gull. Three are considered possible arboviruses, and one (Avalon) has been upgraded to a probable arbovirus.

Tables 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 cannot 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 wild-caught lower vertebrates. Taciuma virus has been recovered from man on one occasion. 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 physicochemical 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 rhabodoviruses, 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 5 others as probably arboviruses. The remaining 4, 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 21 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 (15 of 18) are probably tick-borne in nature, but 2 have been isolated only from biting midges and one from phlebotomine flies. One tick-borne virus is considered to be a proved arbovirus; the African swine fever virus, an iridovirus, is an important cause of veterinary disease.

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 30 viruses in this category, 10 were isolated from birds, 5 from bats, 7 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 3.8 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 84 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 and 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. 191 (52%) of all registered viruses have been recovered from mosquitoes, 81 (about 22%) from ticks, and 54 (15%) from all other classes. 74 viruses have never been isolated from any arthropod host, including 16 members of the large group B, and 30 of the ungrouped category. By far the largest number of viruses which have been isolated from any arthropod, 264 of 295 (89.5%) 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 236 or 63.6%). 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, 25.7 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 151 registrations (41%) 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 193 registrations, or 52%, data are lacking which would permit classification other than as possible arboviruses.

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TABLE 1

ALPHABETICAL LISTING OF 369 VIRUSES REGISTERED AS OF 31 DEC. 1975
WITH RECOMMENDED ABBREVIATIONS AND ANTIGENIC GROUPING

NAME	ABBR.	ANTI-GENIC GROUP
ABSETTAROV	ABS	B
ABU HAMMAD	AH	DGK
ACADO	ACD	COR
ACARA	ACA	CAP
AFRICAN HORSESICKNESS	AHS	AHS
AFRICAN SWINE FEVER	ASF	
AGUACATE	AGU	PHL
AINO	AINO	SIM
AKABANE	AKA	SIM
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
BAGAZA	BAG	B
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
BOBAYA	BOB	
BOCAS	BOC	CAL

NAME	ABBR.	ANTI-GENIC GROUP
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 ENC.	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	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	NSD
EAST. EQUINE ENC.	EEE	A
EDGE HILL	EH	B
ENTEBBE BAT	ENT	B
EP. HEM. DIS.	EHD	EHD
EUBENANGEE	EUB	EUB

NAME	ABBR.	ANTI-GENIC GROUP
EVERGLADES	EVE	A
EYACH	EYA	CTF
FLANDERS	FLA	
FRIJOLES	FRI	PHL
GAMBOA	GAM	SBU
GANJAM	GAN	NSD
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
IPPY	IPPY	
IRITUIA	IRI	CGL
ISRAEL TURKEY MEN.	IT	B
ISSYK-KUL	IK	
ITAPORANGA	ITP	PHL
ITAQUI	ITQ	C
JAMESTOWN CANYON	JC	CAL
JAPANAUT	JAP	
JAPANESE ENC.	JE	B
JERRY SLOUGH	JS	CAL
JOHNSTON ATOLL	JA	QRF
JOINJAKAKA	JOI	
JUAN DIAZ	JD	CAP
JUGRA	JUG	B
JUNIN	JUN	TCR
JURONA	JUR	SBU
JUTIAPA	JUT	B
KADAM	KAD	B
KAENG KHOI	KK	SBU
KAIRI	KRI	BUN
KAISODI	KSO	KSO

NAME	ABBR.	ANTI-GENIC GROUP
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	
KETEPANG	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 FOR. 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
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

*Rabies related

NAME	ABBR.	ANTI-GENIC GROUP
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
MONT. MYOTIS LEUK.	MML	B
MORICHE	MOR	CAP
MOSSURIL	MOS	MOS
MOUNT ELGON BAT	MEB	
M'POKO	MPO	TUR
MUCAMBO	MUC	A
MURRAY VALLEY ENC.	MVE	B
MURUTUCU	MUR	C
NAIROBI SHEEP DIS.	NSD	NSD
NARIVA	NAR	
NAVARRO	NAV	
NDUMU	NDU	A
NEGISHI	NEG	B
NEPUYO	NEP	C

NAME	ABBR.	ANTI-GENIC GROUP
NGAINGAN	NGA	
NIQUE	NIQ	PHL
NKOLBISSON	NKO	
NODAMURA	NOD	
NOLA	NOLA	SIM
NORTHWAY	NOR	BUN
NTAYA	NTA	B
NUGGET	NUG	KEM
NYAMANINI	NYM	
NYANDO	NDO	NDO
OKHOTSKIY	OKH	KEM
OKOLA	OKO	
OMSK HEM. FEVER	OMSK	B
O'NYONG NYONG	ONN	A
ORIBOCA	ORI	C
OROPOUCHE	ORO	SIM
ORUNGO	ORU	
OSSA	OSSA	C
OUANGO	OUA	
OUBANGUI	OUB	
PACORA	PCA	
PACUI	PAC	PHL
PAHAYOKEE	PAH	PAT
PALYAM	PAL	PAL
PARANA	PAR	TCR
PATA	PATA	EUB

NAME	ABBR.	ANTI-GENIC GROUP
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
RUSS.SPR.SUM.ENC.	RSSE	B
SABO	SABO	SIM
SABOYA	SAB	B
SAGIYAMA	SAG	A
SAINT-FLORIS	SAF	
SAKHALIN	SAK	SAK
SALANGA	SGA	
SALEHABAD	SAL	PHL
SAN ANGELO	SA	CAL
SANDFLY F.(NAPLES)	SFN	PHL
SANDFLY F.(SICILIAN)	SFS	PHL
SANDJIMBA	SJA	SIM
SANGO	SAN	SIM
SATHUPERI	SAT	SIM
SAWGRASS	SAW	
SEROKELE	SEB	
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 HEM. FEV.	SHF	
SINDBIS	SIN	A
SIXGUN CITY	SC	KEM
SNOWSHOE HARE	SSH	CAL
SOKOLUK	SOK	B
SOLDADO	SOL	HUG
SORONOCA	SOR	BUN

NAME	ABBR.	ANTI-GENIC GROUP
SPONDWENI	SPO	B
ST. LOUIS ENC.	SLE	B
STRATFORD	STR	B
TACAIUMA	TCM	ANA
TACARIBE	TCR	TCR
TAGGERT	TAG	SAK
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
VEN.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. EQUINE ENC.	WEE	A
WEST NILE	WN	B
WHATAROA	WHA	A
WITWATERSRAND	WIT	
WONGAL	WON	KOO
WONGORR	WGR	

NAME	ABBR.	ANTI-GENIC GROUP
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

NAME	ABBR.	ANTI-GENIC GROUP

Table 2. Antigenic Groups of 369 Viruses Registered in Catalogue

Antigenic Group	Abbreviation	No. Registered Viruses in Group		%
A	A		20	5.4
African horsesickness	AHS		1	0.3
Anopheles A	ANA		3	0.8
Anopheles B	ANB		2	0.5
B	B		58	15.7
Bakau	BAK		2	0.5
Bluetongue	BLU		1	0.3
Boteke	BTK		2	0.5
Bunyamwera Supergroup			87	23.6
Bunyamwera	BUN	18		
Bwamba	BWA	2		
C	C	11		
California	CAL	12		
Capim	CAP	6		
Guama	GMA	6		
Koongol	KOO	2		
Patois	PAT	4		
Simbu	SIM	15		
Tete	TETE	4		
Unassigned	SBU	7		
Changuinola	CGL		2	0.5
Colorado tick fever	CTF		2	0.5
Congo	CON		2	0.5
Corriparta	COR		2	0.5
Dera Ghazi Khan	DGK		5	1.4
Epizootic hemorrhagic disease	EHD		1	0.3
Eubenangee	EUB		2	0.5
Hughes	HUG		4	1.1
Kaisodi	KSO		3	0.8
Kemerovo	KEM		16	4.3
Kwatta	KWA		1	0.3
Malakal	MAL		2	0.5
Mapputta	MAP		3	0.8
Matariya	MTY		3	0.8
Mossuril	MOS		2	0.5
Nairobi sheep disease	NSD		3	0.8
Nyando	NDO		1	0.3
Palyam	PAL		4	1.1
Phlebotomus fever	PHL		20	5.4
Qalyub	QYB		2	0.5
Quaranfil	QRF		2	0.5
Sakhalin	SAK		4	1.1
Tacaribe	TCR		9	2.4
Thogoto	THO		1	0.3
Timbo	TIM		2	0.5
Turlock	TUR		3	0.8
Uukuniemi	UUK		5	1.4
Vesicular stomatitis	VSV		5	1.4
Warrego	WAR		2	0.5
Ungrouped viruses			80	21.7
		Total	369	

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,ORU
	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,SSH,TUR,VSNJ
	S. America	Argentina	JUN
		Brazil	APEU, AURA, BSQ, CAP,CAR,CATU, GJA,GMA,ITQ,MAG,MIR,MOJU,MTB,MUC, MUR,ORI,TCM,UNA.
		Colombia	GRO,NAV
		Trinidad	ARU,BIM,BSB,IERI,KRI,LUK,MAN,MAY, MEL,NEP,ORO,TCR,TNT.

* Isolated in U.S.A. Laboratory

Table 3. (Continued)

Decade	Continent	Country	Virus
1960-69	Africa	Cameroon	NKO,OKO
		Cent.Afr.Rep.	BAG,BGN,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, SEP,STR,TRU,UPO,WAR,WON
		New Zealand	WHA
		Pacific Island	JA*
	Europe	Czechoslovakia	CVO,KEM,LIP,TRB
		Finland	INK,UUK
		France	GA,PTV
		West Germany	MBG
	N. America	Canada	SIL
		Guatemala	JUT*
		Mexico	MNT,TLA*
		Panama	AGU,CHG,CHI,CGL,FRI,GAM,JD,LAT,MAD, MAT,OSSA,PAR,PAT,PT*,ZEG
		U.S.A.	BUT,CR,EVE,FLA,GL,HUG,JC,JS,KC,KEY 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
1970-75	Africa	Cent.Afr.Rep.	BBO,BGI,BOB,GAR,GOM,GOR,IPPY,KOL,LJA, NOLA,OUA,OUB,SAE,SAL,SEB,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,NUG,TAG,WAL,WGR
	Europe	Germany	EYA,TET
		Scotland	CM,CW
		U.S.S.R.	BAKU
	N. America	Canada	AVA,BAU*,GI*
		Panama	CAC,CAI,NIQ
		U.S.A.	BAU,GI,NOR,YH

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

Table 4. Initial Isolation of 369 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.					10	17	27
	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	6	4		17
	Totals	4	4	5	28	41	21	103
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-ASIA and PACIFIC ISLANDS	Australia				1	25	5	30
	Hawaii			1				1
	Johnston Island					1		1
	New Guinea			1				1
	New Zealand					1		1
	Philippines				2			2
	Totals			2	3	27	5	36
EUROPE	Czechoslovakia			1	2	4		7
	Finland				1	2		3
	France					2		2
	West Germany					1	2	3
	Italy			2				2
	Scotland	1					2	3
	U.S.S.R.(West)				1		1	2
	Totals	1		3	4	9	5	22
NORTH AMERICA	Canada				1	1	3	3
	Guatemala					1		1
	Mexico					2		2
	Panama				3	15	3	21
	U.S.A.	1	3	3	10	25	2	46
	Totals	1	3	3	14	44	8	73
SOUTH AMERICA	Argentina				1			1
	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	108	181	46	369

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	SIAS 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: 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	SFAS RATING *	TAXONOMIC STATUS
Alfuy	+										+							+						20	Flavivirus
Bagaza	+														+	+								22	"
Banzi	+							+							+	+							+	20	"
Bouboui	+	+							+							+								22	"
Bussuquara	+							+		+					+					+	+			20	"
Dengue-1	+							+	+							+	+	+			+	+	+	20	"
Dengue-2	+							+	+							+	+	+			+	+	+	20	"
Dengue-3	+							+									+	+				+		20	"
Dengue-4	+							+									+	+				+		20	"
Edge Hill	+	+																+						20	"
Ilheus	+							+			+				+					+	+	+	+	20	"
Japanese encephalitis	+	+						+			+	+		+			+					+	+	20	"
Jugra	+											+					+							22	"
Kokobera	+																	+						21	"
Kunjin	+							+			+							+					+	20	"
Murray Valley 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				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																			
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														ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																				
Apoi										+		+					+						+	22	Flavivirus
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: 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
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															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		
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	"
Snowshoe Hare	+									+				+	+					+				20	"
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															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																			
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		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																				
Aino	+					+											+	+					22	Bunyavirus
Akabane	+					+											+	+					21	"
Buttonwillow						+								+						+			20	"
Ingwavuma	+										+			+		+	+						20	"
Manzanilla									+												+		22	"
Mermet											+									+			22	"
Nola	+																						20	"
Oropouche	+							+						+		+					+	+	21	"
Sabo						+								+		+							22	"
Sango	+					+								+		+							22	"
Sathuperi	+					+								+		+	+						22	"
Shamonda						+								+		+							22	"
Shuni	+					+		+						+		+					+		22	"
Simbu	+															+							21	"
Thimiri											+					+	+						22	"

* See footnote Table 5

TABLE 14. 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																					
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
Aguacate					+															+	+			21	Bunyavirus-like
Anhanga														+ +							+			22	"
Arumowot	+									+ +						+					+			22	"
Bujaru					+															+	+			22	"
Cacao					+															+				21	"
Caimito					+															+				22	"
Candiru								+													+	+		22	"
Chagres	+				+			+												+		+		21	"
Chilibre					+															+				21	"
Frijoles					+															+				22	"
Gordil										+						+								22	"
Icoaraci	+	+			+					+	+				+						+			21	"
Itaporanga	+										+		+		+		+				+			20	"
Karimabad					+																			22	"
Nique					+															+				22	"
Pacui					+					+											+			21	"
Punta Toro					+			+												+	+	+		21	"
Salehabad					+												+							22	"
SF-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				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																			
CHF-CONGO GROUP																								
Congo			+			+		+						+		+	+		+			+	+	20	Bunyavirus-like
Hazara			+														+							22	"
KAISODI GROUP																								
Kaisodi			+								+						+							22	Bunyavirus-like
Lanjan			+														+							22	"
Silverwater			+											+						+				21	"
NAIROBI SHEEP DISEASE																								
Dugbe	+		+			+		+		+				+		+						+		22	Bunyavirus-like
Ganjam	+		+					+									+					+	+	22	"
Nairobi Sheep Disease			+					+						+		+						+	+	20	"
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: 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
COLORADO TICK FEVER																									
Colorado Tick Fever			+	+				+		+				+						+		+	+	20	Orbivirus
Eyach			+																+					22	"
KEMEROVO																									
Baku				+															+					22	Orbivirus
Bauline			+																+					22	"
Cape Wrath			+																+					22	"
Chenuda				+												+								22	"
Great Island			+																	+				22	"
Huacho				+																	+			22	"
Kemerovo			+					+			+					+	+					+	+	21	"
Lipovnik			+																+					22	"
Mono Lake				+																+				22	"
Nugget			+															+						22	"
Okhotskiy			+														+							22	"
Seletar			+														+							22	"
Sixgun City				+																+				22	"
Tribec			+							+					+				+					21	"
Wad Medani			+													+	+			+				22	"
Yaquina Head			+																	+				22	"

* See footnote Table 5.

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

	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																			
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				+														+						20	Unclassified
Quaranfil				+				+			+					+						+		20	"
SAKHALIN GROUP																									
Avalon			+								+									+				21	Unclassified
Clo More			+																+					22	"
Sakhalin			+														+			+				22	"
Taggert			+															+						22	"

* See footnote Table 5

TABLE 19. MINOR ANTIGENIC GROUPS OF VIRUSES

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

* See footnote Table 5

TABLE 20. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anopheline	Ticks Ixodid	Ticks Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: Africa	Asia	Australasia	Europe	North America	South America	HUMAN DISEASE: Natural Infection	Lab Infection	SEAS RATING *	TAXONOMIC STATUS
AFRICAN HORSESICKNESS																									
African horsesickness						+								+		+	+		+					20	Orbivirus
BLUETONGUE GROUP																									
Bluetongue						+								+		+	+		+	+				20	Orbivirus
CHANGUINOLA GROUP																									
Changuinola					+			+												+		+		21	Orbivirus
Irituia										+											+			22	"
CORRIPARTA GROUP																									
Acado	+															+								22	Orbivirus
Corriparta	+										+							+						22	"
EHD GROUP																									
Epizootic hem. dis.														+						+				21	Orbivirus
EUBENANGEE GROUP																									
Eubenangee	+2	+2																+						22	Orbivirus
Pata	+															+								22	"
PALYAM GROUP																									
D'Aguilar						+												+						22	Orbivirus
Kasba	+																+							22	"
Palyam	+																+							22	"
Vellore	+																+							22	"
WARRIEDO 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		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																					
KWATTA GROUP																									
Kwatta	+																				+			22	Rhabdovirus
MOSSURIL GROUP																									
Kamese	+															+								22	Rhabdovirus
Mossuril	+										+					+								22	"
VESICULAR STOMATITIS GR.																									
Chandipura					+			+						+		+	+					+		20	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
Zirgilamo											+					+								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				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																			
Amapari							+			+											+			24	Arenavirus
Junin							+	+		+				+		+					+	+	+	24	"
Lassa								+		+											+	+	+	24	"
Latino								+		+											+			24	"
Machupo								+		+											+	+	+	24	"
Parana										+											+			24	"
Pichinde			+				+			+											+			24	"
Tacaribe	?	?										+									+			24	"
Tamiami										+										+				24	"

* See footnote Table 5

TABLE 24. UNGROUPED MOSQUITO-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		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																					
Rift Valley fever	+							+						+		+						+	+	20	Bunyavirus-like
Tataguine	+	+						+								+						+		21	"
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: 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
Arkonam	+	+															+							22	Unclassified
Aruac	+																				+			21	"
Bangoran	+										+					+								22	"
Belmont	+																	+						22	"
Gomoka	+	+									+					+								22	"
Ieri	+																				+			22	"
Kowanyama		+																+						22	"
La Joya	+																			+				22	"
Minnal	+																+							22	"
Nkolbisson	+															+								22	"
Okola	+															+								22	"
Orungo	+	+														+						+		22	"
Oubangui	+															+								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		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																					
Bhanja			+					+		+				+		+	+		+				+	22	Bunyavirus-like
Lone Star			+																	+				22	"
African swine fever				+										+		+			+		+**			20	Iridovirus
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	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	"
Bobaya											+					+								22	"
Gossas												+				+								23	"
Ippy										+						+								22	"
Kammavanpettai											+						+							22	"
Kannamangalam											+						+							22	"
Keuraliba										+						+								22	"
Kolongo											+					+								22	"
Landjia											+					+								22	"
Le Dantec								+								+								22	"
Marburg								+											+			+	+	23	"
Marco														+							+			22	"
Ouango											+					+								22	"
Saint-Floris										+						+								22	"
Salanga										+						+								22	"
Sandjimba											+					+								22	"
Sebokele										+						+								22	"
Sembalam											+						+							22	"
Simian Hemorrh. fever									+								+				+			24	"
Tanjong Rabok															+		?							22	"
Thottapalayam														+			+							22	"
Toure										+						+								22	"
Yogue												+				+								22	"

*See footnote Table 5.

TABLE 28. CONTINENTAL DISTRIBUTION OF GROUPED AND UNGROUPED VIRUSES

Antigenic Group		Total in Group	Africa	Asia	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		58	18	24	12	7	10	6	44	10	3	1	0
BAK		2	0	2	0	0	0	0	2	0	0	0	0
BLU		1	1	1	0	1	1	0	0	0	0	1	0
BTK		2	2	0	0	0	0	0	2	0	0	0	0
Bunyamwera Supergroup	BUN	18	4	1	0	2	8	6	15	3	0	0	0
	BWA	2	2	0	0	0	0	0	2	0	0	0	0
	C	11	0	0	0	0	5	8	9	2	0	0	0
	CAL	12	1	0	0	2	9	2	10	2	0	0	0
	CAP	6	0	0	0	0	3	5	4	2	0	0	0
	GMA	6	0	0	0	0	2	5	5	1	0	0	0
	KOO	2	0	0	2	0	0	0	2	0	0	0	0
	PAT	4	0	0	0	0	4	0	4	0	0	0	0
	SIM	15	9	5	2	0	2	2	9	6	0	0	0
	TETE	4	3	1	0	2	0	0	2	2	0	0	0
	SBU	7	1	1	0	0	2	3	7	0	0	0	0
CGL		2	0	0	0	0	1	1	2	0	0	0	0
CTF		2	0	0	0	1	1	0	2	0	0	0	0
CON		2	1	2	0	1	0	0	1	0	1	0	0
COR		2	1	0	1	0	0	0	2	0	0	0	0
DGK		5	2	3	0	0	0	0	5	0	0	0	0
EHD		1	0	0	0	0	1	0	1	0	0	0	0
EUB		2	1	0	1	0	0	0	2	0	0	0	0
HUG		4	0	1	0	0	1	3	3	1	0	0	0
KSO		3	0	2	0	0	1	0	3	0	0	0	0
KEM		16	3	4	1	4	6	1	14	1	1	0	0
KWA		1	0	0	0	0	0	1	1	0	0	0	0
MAL		2	1	1	0	0	0	0	2	0	0	0	0
MAP		3	0	0	3	0	0	0	3	0	0	0	0
MTY		3	3	0	0	0	0	0	3	0	0	0	0
MOS		2	2	0	0	0	0	0	2	0	0	0	0
NSD		3	2	1	0	0	0	0	2	0	0	0	0
NDO		1	1	0	0	0	0	0	1	0	0	0	0
PAL		4	0	3	1	0	0	0	4	0	0	0	0
PHL		20	4	4	0	2	8	6	18	0	2	0	0
QYB		2	2	0	0	0	0	0	2	0	0	0	0
QRF		2	1	0	1	0	0	0	2	0	0	0	0
SAK		4	0	1	1	1	2	0	3	1	0	0	0
TCR		9	1	0	0	0	1	7	9	0	0	0	0
THO		1	1	0	0	1	0	0	0	1	0	0	0
TIM		2	0	0	0	0	0	2	2	0	0	0	0
TUR		3	1	1	0	0	1	1	2	1	0	0	0
UUK		5	0	2	0	3	0	0	5	0	0	0	0
VSV		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		80	36	17	10	4	10	10	72	2	2	0	0
Totals		369	112	85	42	33	85	86	311	43	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		58	28	15	0	0	1	1	39	3	0
BAK		2	2	1	0	0	0	0	1	1	0
BLU		1	0	0	0	1	0	0	1	0	0
BTK		2	1	0	0	0	0	0	1	0	0
Bunyamwera Supergroup	GUN	18	17	0	0	2	0	0	17	1	0
	BWA	2	2	0	0	0	0	0	2	0	0
	C	11	11	0	0	0	0	0	11	0	0
	CAL	12	12	0	0	0	0	1	11	1	0
	CAP	6	5	0	0	0	0	0	5	0	0
	GMA	6	5	0	1	0	0	0	4	1	0
	KOO	2	2	0	0	0	0	0	2	0	0
	PAT	4	3	0	0	0	0	0	3	0	0
	SIM	15	9	0	0	8	0	0	7	5	0
	TETE	4	2	0	0	0	0	0	1	0	0
	SBU	7	5	0	0	0	0	0	5	0	0
CGL		2	0	0	1	0	0	0	1	0	0
CTF		2	0	2	0	0	0	0	2	0	0
CHF-CON		2	0	2	0	1	0	0	1	1	0
COR		2	2	0	0	0	0	0	2	0	0
DGK		5	0	5	0	0	0	0	5	0	0
EHD		1	0	0	0	0	0	0	0	0	0
EUB		2	2	0	0	0	0	0	2	0	0
HUG		4	0	4	0	0	0	0	4	0	0
KSO		3	0	3	0	0	0	0	3	0	0
KEM		16	0	16	0	0	0	0	16	0	0
KWA		1	1	0	0	0	0	0	1	0	0
MAL		2	2	0	0	0	0	0	2	0	0
MAP		3	3	0	0	0	0	0	3	0	0
MTY		3	0	0	0	0	0	0	0	0	0
MOS		2	2	0	0	0	0	0	2	0	0
NSD		3	2	3	0	1	0	0	1	1	1
NDO		1	1	0	0	0	0	0	1	0	0
PAL		4	3	0	0	1	0	0	4	0	0
PHL		20	4	0	13	0	0	0	15	1	0
QYB		2	0	2	0	0	0	0	2	0	0
QRF		2	0	2	0	0	0	0	2	0	0
SAK		4	0	4	0	0	0	0	4	0	0
TCR		9	1	1	0	0	3	0	3	1	0
THO		1	0	1	0	0	0	0	1	0	0
TIM		2	0	0	0	0	0	0	0	0	0
TUR		3	3	0	0	0	0	0	3	0	0
UUK		5	0	5	0	0	0	0	5	0	0
VSV		5	2	0	2	0	1	1	2	2	0
WAR		2	0	0	0	2	0	0	2	0	0
Ungrouped		80	34	15	2	2	0	1	44	4	0
Totals		369	191	81	19	20	9	6	265	25	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
ANA		3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANR		2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B		58	26	3	17	14	14	1	5	6	27	6	5	4	2	1
BAK		2	0	1	0	0	0	0	0	0	1	0	0	0	0	0
BLU		1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
BTK		2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Bunyamwera Supergroup	BUN	18	5	1	3	0	0	0	1	3	9	2	0	0	0	0
	BWA	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0
	C	11	9	0	8	0	1	5	0	1	2	6	2	1	0	0
	CAL	12	2	0	3	0	1	0	0	1	5	1	0	0	0	0
	CAP	6	0	0	3	0	0	1	0	0	2	1	0	0	0	0
	GMA	6	2	0	5	0	2	3	0	0	2	1	0	2	0	0
	KOO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	PAT	4	0	0	3	0	0	0	0	0	3	0	0	0	0	0
	SIM	15	2	1	0	3	0	0	6	2	8	3	0	0	0	0
	TETE	4	0	0	0	4	0	0	0	0	4	0	0	0	0	0
	SBU	7	0	0	0	1	1	0	0	0	2	0	0	0	0	0
CGL		2	1	0	1	0	0	0	0	0	2	0	0	0	0	0
CTF		2	1	0	1	0	0	0	0	0	0	1	0	0	0	0
CON		2	1	0	0	0	0	0	1	1	0	0	1	0	0	0
COR		2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
DGK		5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EHD		1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
EUB		2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HUG		4	0	0	0	1	0	0	0	0	1	0	0	0	0	0
KSO		3	0	0	0	1	0	0	0	1	2	0	0	0	0	0
KEM		16	1	0	1	1	0	0	0	0	1	1	0	0	0	0
KWA		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAL		2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAP		3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MTY		3	0	0	0	3	0	0	0	0	3	0	0	0	0	0
MOS		2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
NSD		3	3	0	1	0	0	0	1	1	1	1	1	0	0	0
NDO		1	1	0	0	0	0	0	0	0	1	0	0	0	0	0
PAL		4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHL		20	5	0	5	2	0	1	0	2	9	3	0	0	0	0
QYB		2	0	0	1	0	0	0	0	0	1	0	0	0	0	0
QRF		2	1	0	0	1	0	0	0	0	0	1	0	0	0	0
SAK		4	0	0	0	1	0	0	0	0	0	0	0	0	0	0
TCR		9	3	0	8	0	1	0	0	1	6	2	1	0	0	0
THO		1	1	0	0	0	0	0	1	0	0	1	0	0	0	0
TIM		2	0	0	0	0	0	0	0	2	2	0	0	0	0	0
TUR		3	0	0	0	2	0	0	0	1	1	1	0	0	0	0
UUK		5	0	0	1	1	0	0	0	0	0	1	0	0	0	0
VSV		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		80	9	1	12	14	8	0	4	3	39	3	0	0	0	0
Totals		369	86	9	80	59	30	17	29	30	150	41	11	10	3	2

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

Antigenic Group	Total in Group	In Nature	Lab Infection	Either or Both	
				Number	Percent
Group A	20	10	7	11	55.0
Afr.horsesickness	1	0	0	0	
Anopheles A	3	0	0	0	
Anopheles B	2	0	0	0	
Group B	58	27	22	29	50.0
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	12	4	0	4	33.0
Capim	6	0	0	0	
Guama	6	2	0	2	33.3
Koongol	2	0	0	0	
Patois	4	0	0	0	
Simbu	15	2	1	2	13.3
Tete	4	0	0	0	
SBU	7	0	0	0	
Changuinola	2	1	0	1	50.0
Colorado tick fever	2	1	1	0	50.0
CHF-Congo	2	1	1	1	50.0
Corriparta	2	0	0	0	
Dera Ghazi Khan	5	0	0	0	
Epizoot.hem.dis.	1	0	0	0	
Eubenangee	2	0	0	0	
Hughes	4	0	0	0	
Kaisodi	3	0	0	0	
Kemerovo	16	1	1	1	6.3
Kwatta	1	0	0	0	
Malakal	2	0	0	0	
Mapputta	3	0	0	0	
Matariya	3	0	0	0	
Mossuril	2	0	0	0	
Nairobi sheep dis.	3	3	2	2	100.0
Nyando	1	1	0	1	100.0
Palyam	4	0	0	0	
Phlebot. fev.	20	5	0	5	25.0
Qalyub	2	0	0	0	
Quaranfil	2	1	0	1	50.0
Sakhalin	4	0	0	0	
Tacaribe	9	3	3	3	33.3
Thogoto	1	1	0	1	100.0
Timbo	2	0	0	0	
Turlock	3	0	0	0	
Uukuniemi	5	0	0	0	
Vesic. stom.	5	3	3	4	80.0
Warrego	2	0	0	0	
Ungrouped	80	9	5	9	12.5
Totals	369	89	50	92	25.7

TABLE 32. EVALUATION OF ARTHROPOD-BORNE STATUS OF 369 REGISTERED VIRUSES (SEAS)

Antigenic Group		Total in Group	Arbovirus	Probably an Arbovirus	Possible Arbovirus	Probably not Arbovirus	Not an Arbovirus	Arbo or Probably Arbo No.	Arbo or Probably Arbo %	Not or Probably Not Arbo No.	Not or Probably Not Arbo %
A		20	14	3	3	0	0	17	85.0	0	
AHS		1	1	0	0	0	0	1	100.0	0	
ANA		3	0	2	1	0	0	2	66.7	0	
ANB		2	0	0	2	0	0	0		0	
B		58	29	7	15	2	5	36	62.0	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	
	BWA	2	1	1	0	0	0	2	100.0	0	
	C	11	10	1	0	0	0	11	100.0	0	
	CAL	12	7	2	3	0	0	9	75.0	0	
	CAP	6	3	1	2	0	0	4	66.7	0	
	GMA	6	4	0	2	0	0	4	66.7	0	
	KOO	2	0	2	0	0	0	2	100.0	0	
	PAT	4	1	1	2	0	0	2	50.0	0	
	SIM	15	3	3	9	0	0	6	40.0	0	
	TETE	4	0	0	4	0	0	0		0	
	SBU	7	1	1	5	0	0	2	28.6	0	
CGL		2	0	1	1	0	0	1	50.0	0	
CTF		2	1	0	1	0	0	1	50.0	0	
CON		2	1	0	1	0	0	1	50.0	0	
COR		2	0	0	2	0	0	0		0	
DGK		5	0	0	5	0	0	0		0	
EHD		1	0	1	0	0	0	1	100.0	0	
EUB		2	0	0	2	0	0	0		0	
HUG		4	0	1	3	0	0	1	25.0	0	
KSO		3	0	1	2	0	0	1	33.3	0	
KEM		16	0	2	14	0	0	2	12.5	0	
KWA		1	0	0	1	0	0	0		0	
MAL		2	0	0	2	0	0	0		0	
MAP		3	0	1	2	0	0	1	33.3	0	
MTY		3	0	0	3	0	0	0		0	
MOS		2	0	0	2	0	0	0		0	
NSD		3	1	0	2	0	0	1	33.3	0	
NDO		1	0	1	0	0	0	1	100.0	0	
PAL		4	0	0	4	0	0	0		0	
PHL		20	3	5	12	0	0	8	40.0	0	
QYB		2	0	0	2	0	0	0		0	
QRF		2	1	0	1	0	0	1	50.0	0	
SAK		4	0	0	4	0	0	0		0	
TCR		9	0	0	0	0	9	0		9	100.0
THO		1	0	0	1	0	0	0		0	
TIM		2	0	0	2	0	0	0		0	
TUR		3	1	1	1	0	0	2	66.7	0	
UUK		5	1	1	3	0	0	2	40.0	0	
VSV		5	2	1	2	0	0	3	60.0	0	
WAR		2	0	0	2	0	0	0		0	
Ungrouped		80	3	9	59	6	3	12	15.0	9	11.3
Totals		369	97	53	193	8	17	151	40.9	25	6.8

OPENING OF THE WHO CENTRE FOR COLLECTION AND EVALUATION OF DATA ON COMPARATIVE VIROLOGY

The opening of the WHO Centre for Collection and Evaluation of Data on Comparative Virology at the Ludwig-Maximilians University in Munich, West Germany took place on October 15-17, 1975. Approximately 30 consultants from seven countries participated in the opening ceremony and a workshop. It is intended that the Centre establish means of rapid collection, interpetation and dissemination of data concerning animal viruses. Dr. Peter Thein is the Director of the new Centre, which will work closely with the WHO/FAO Comparative Virology Programme (Dr. James Gillespie, Ithaca, New York, Chairman). The objectives of the Centre and the Programme are as follows:

1. To establish and maintain detailed information on viruses affecting man and animals. This will involve completion of elaborate questionnaire and a computerized data system.

2. To determine (in collaboration with the Working Teams of the Comparative Virology Programme) which viruses represent particular health problems and those which require international reference reagents. Reference reagent production is undertaken by Team members.

3. To identify (in collaboration with the Working Teams) gaps in knowledge which need to be filled and to identify needed lines of research.

4. To furnish information to all individuals and groups interested in viruses affecting man and animals and in particular, to the Working Teams of the WHO/FAO Comparative Virology Programme.

The community of arbovirologists was represented at this opening and workshop by Dr. Paul Brés, WHO - Geneva, and by myself. The role that the programs of the ACAV have had in the construction of this Centre and the Comparative Virology Programme should be a source of pride for all who have been involved in the ACAV. In many instances, the Munich Centre will be using schemes tried and proven by the ACAV. In some cases, additional programs (such as an Information Exchange/Newsletter) were recommended for the first time. The undertaking of the Centre is massive, and progress will take time, but in the long run, ACAV programs will have to have a stronger link to the WHO Centre. For the foreseeable future, arboviruses (and arenaviruses) will not be included in the WHO Comparative Virology Programme--the reliance upon the ACAV for arbovirus informational services was unanimous. As means of continuing contact between the Centre and ACAV, 1) a large amount of ACAV material has been sent to Munich (Catalogues, Info Exchanges, etc.), and the Centre will be added to all ACAV mailing lists, 2) as a member of the Comparative Virology Board (through the International Committee on Taxonomy of Viruses), I will be able to serve as liason at future Board

meetings, and 3) Dr. Peter Thein will visit Dr. Roy Chamberlain (Subcommittee on Information Exchange) and Dr. Nick Karabatsos (Catalogue Editor) next year.

I believe we can look forward to a very productive cooperation between the WHO Centre and Comparative Virology Program and the ACAV.

(Frederick A. Murphy)
(Viral Pathology Branch, Center for Disease Control, Atlanta, Georgia, February 24, 1976)

REPORT FROM THE ARBOVIRUS LABORATORY, INSTITUT PASTEUR AND ORSTOM,

DAKAR, SENEGAL

During the second half of 1975, the activity of the arbovirus laboratory included virological studies on material from Senegal only and serological examination on specimens from Senegal and Gabon.

1. VIROLOGICAL STUDIES

1.1. Human blood samples

179 blood specimens collected from febrile patients, mostly children, in Dakar, Bandia andKedougou were processed for virus isolation.

One strain of Zinga virus was isolated from the blood of a laboratory technician girl who has worked at this virus 4 days before. The illness was mild : light fever but deep asthenia lasting three days, and she uneventfully recovered. Zinga virus has been isolated from Mansonia africana in Central African Republic in 1969 (Digoutte et al. Ann. Microbiol. (Ins. Pasteur) 1974, 125B, 107-118) and it is known as agent of febrile illness with a benign course (Digoutte et al., Bull. Soc. Path. Exot., 1974, 67, 451-457).

1.2. Wild vertebrate samples

140 blood specimens from monkeys caught in Kedougou were inoculated in suckling mice without success.

1.3 Arthropods

11.124 mosquitoes caught in Kedougou were processed in 375 pools for virus isolation. Numerous strains were isolated and 3 have been identified : Pongola virus from Aedes dalzieli mosquitoes, Sindbis virus (1) and Usutu virus (1) from Culex gr perfuscus mosquito pools. One isolate ArD 14701 from Aedes minutus, seems to be a new virus of the Flavivirus genus. A striking feature is the difficulty in obtaining a workable hemagglutinin with this virus.

2. SEROLOGICAL STUDIES

2.1. Human Sera

2.1.1. Senegal

A multipurpose serological survey was initiated in 1972 by a WHO-VDT team (WHO/IR 051 project) : aliquotes of sera were examinated for arbovirus antibodies. Results concerning Fleuve Senegal and Lower Casamance were reported previously.

Results concerning Diourbel, Sine-Saloum, Upper-Casamance and Eastern Senegal regions for the 1974-1975 period are summarized below.

- Yellow fever virus shows activity in restricted areas around Kedougou (Eastern Senegal) et Kolda (Upper Casamance).

- In each surveyed region, even in Diourbel where a yellow fever epidemic occured in 1965, less than 70% of the people have yellow fever antibodies.

- Chikungunya, Zika, Koutango and Wesselsbron viruses show some recent activity.

- Sindbis and West-Nile viruses do not seem active in the area.

2.1.2 Bandia

100 sera were collected from febrile children in Bandia village and tested for HI and CF antibodies. Chikungunya virus antibodies were not found in the sera of children less than seven years old. This fact confirms the lack of chikungunya activity in this area following the 1966-1967 chikungunya epidemic in Senegal. HI and CF group B antibodies appear early : Zika and Wesselsbron viruses show some activity around Bandia.

2.1.3 Diaganiao

Serological investigations have been conducted in Diaganiao village where numerous cases of hepatitis had occured with two deaths. No arbovirus can be involved. Search for HB_s antigen was negative too and we concluded that it was an Hepatitis A outbreak.

2.1.4. Gabon

1300 human sera collected in Gabon were examinated for HI CF and Neutralizing antibody. It was a "blind" survey and interpretation has to be done.

The only thing we can say is that more than 85% of these sera show yellow fever neutralizing antibodies, mostly due to effective vaccination.

2.2 Wild vertebrate sera

200 sera collected from monkeys in Kedougou have been examinated for HI, CF and neutralizing antibodies. Results show that yellow fever virus is moving around Kedougou focus.

Ch. JAN and Y. ROBIN, Institut Pasteur
J. COZ, M. CORNET, J. L. CAMICAS, ORSTOM

REPORT FROM ARBOVIRUS RESEARCH UNIT

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG

Lassa Virus

Between July and October 1972, 5 strains of virus were isolated in infant mice from the tissues of 5 Mastomys natalensis (Rodentia) collected at Mopéa Velha, near the mouth of the Zambesi River in central Mocambique. All these strains have a similar survival time in infant mice of 8-14 days and at the time of their isolation it was assumed that they were probably the same virus. One strain failed to cause illness in adult mice following IC inoculation. The 2 strains that were tested were sensitive to chloroform and both titred 4.0 logs when titrated IC in infant mice.

Recently one of these strains, AN 20410, was sent to the Centre for Disease Control, Atlanta, U.S.A. for identification. We have now been advised by Dr. Karl Johnson, Dr. Herta Wulff and Dr. Fred Murphy of the Virology Division, C.D.C., that AN 20410, in so far as they can presently identify it, is Lassa virus or a close relative. Lyophilized brain material from the 5th mouse passage produced typical Lassa-like cytopathic effect in Vero cell cultures, and fluorescent staining of infected cells was observed with anti-Lassa but not anti-lymphocytic choriomeningitis serum.

This finding extends considerably the potential area of Lassa-like virus distribution in Africa and underscores the need for the exercise of great care in working with wild Mastomys rodents for whatever purpose. If all 5 isolates are identical, infection rates were high as only 112 M. natalensis were sampled at Mopéa Velha.

The low pathogenicity of the Mocabique isolates for mice should be emphasised as it would seem that many of these isolates could be inadvertently discarded if mice are used as laboratory host.

(B.M. Mc Intosh)

REPORT FROM THE VETERINARY RESEARCH LABORATORY, SALISBURY, RHODESIA

A virus laboratory was started in the Department of Medical Microbiology at the University of Rhodesia, Salisbury, at the end of 1968. Among other projects, a survey was conducted on antibodies in human sera to 5 arthropod-borne viruses: RVF, WSL, WN, CHIK and SIN (Swanepoel and Cruickshank, 1974). A major outbreak of RVF in 1969 caused widespread abortion in sheep and cattle and mortality in young stock. This prompted long-term research on arbovirus diseases of livestock and the program was transferred to a newly established virology unit at the Veterinary Research Laboratory, Salisbury, at the end of 1972. The unit has been concerned chiefly with infertility and abortion of cattle, and investigation of arbovirus disease constitutes an integral part of the work.

Over 12 000 cattle sera have been screened for RVF and WSL HAI antibodies since 1969. It has emerged that in addition to well marked epidemics at intervals of years, small outbreaks of RVF probably occur in most years in defined areas along the north of the watershed in Rhodesia. In a study of potential reservoir hosts, 2 212 murids (rats and mice) of 14 species were trapped at 10 sites. Twenty seven insectivores (shrews) and 7 graphiurids (dormice) were also caught. Sera were screened for HAI antibodies to RVF and WSL as well as two viruses not known to occur in Southern Africa: LI and KAD. Results were as follows:-

Antigen	No. of Sera tested	No. of sera positive at:- 20	40	≥80
RVF	867	11	4	2
WSL	867	6	6	8
LI	549	0	0	0
KAD	549	1	2	1

Neutralisation (NT) tests on HAI-positive sera are not complete. The brains, spleens and livers of the rodents and shrews are being screened for viruses in pools of up to 10 individuals per species per site. Twenty five isolates of an unidentified virus have been obtained so far. Twenty two isolates came from pools of Praomys natalensis (i.e. Mastomys) and one isolate each from pools of Tatera leucogaster, Rhabdomys pumilio and Rattus rattus. Results to date are summarised as follows:-

Site	Isolates/pools tested
Mazoe	10/43
Sinoia	13/37
Vet. Lab.	2/15
Kariba	0/21
Mlezu	0/10
Matopos	0/4

The virus failed to cross-react in CF tests with nineteen of the YARU/NIH arbovirus grouping fluids as well as individual sera and antigens of the known Southern African arboviruses. The virus kills infant mice 10-14 days following ic inoculation, but is not lethal by the ip route. It fails to kill weaned mice. It is non-cytopathic in a variety of cell cultures and fails to produce plaques on BHK and Vero monolayers. It does not cross-react with Lassa CF antigen and serum from CDC Atlanta. The virus is tentatively designated Mazoe (prototype strain P25) and is of interest principally because it has also been isolated from two aborted cattle foetuses, two aborted sheep foetuses and the blood of sick cattle.

Rodents from litters reared in captivity are being used in RVF viraemia studies. Viraemias attaining an intensity of 10^4 pfu/ml or greater have been demonstrated in Aethomys chrysophilus, Rhabdomys pumilio, Saccostomys campestris and Lemniscomys griselda. Eighteen Praomys natalensis have been exsanguinated on each of the first 14 days following infection, without demonstrating viraemia. This is in contrast to the moderate viraemia demonstrated by McIntosh (1961) in two P. natalensis bled on alternate days following infection. Consequently, tests were conducted on individuals from a laboratory colony derived from South African P. natalensis and moderate viraemias were detected. A cytogenetics unit at the University of Rhodesia demonstrated that the South African-derived P. natalensis have 36 chromosomes whereas Rhodesian populations only have 32. The temptation to dismiss the laboratory culture as artificial must be tempered by the fact that 36-chromosome individuals have been found on the Rhodesia-Botswana border.

In the three years from October, 1972, through September, 1975, 470 aborted cattle and 42 aborted sheep foetuses have been tested in mice for arboviruses, in addition to routine culture for other organisms. Known and potential arboviruses obtained

from cattle foetuses include 10 isolates of RVF, 2 isolates of the Mazoe (rodent) virus mentioned above, 2 isolates of a virus tentatively designated Nyabira (prototype strain 792/73) and 1 isolate of a virus tentatively designated Gwebi (prototype strain 1220/74). The prototype strain of Nyabira virus was placed in the Palyam serogroup of viruses by Dr Casals of YARU on the basis of CF tests. NT tests are in progress here to determine whether Nyabira is a new member of the group. Nyabira is cytopathic for Vero cells and electron-micrographs have confirmed that it has Orbivirus morphology. Gwebi virus has failed to cross-react with YARU/NIH grouping fluids and produces plaques in Vero cells.

Another potential arbovirus was isolated from sick cattle. Tentatively designated Marandellas (prototype strain 1063/74), it has failed to cross-react with YARU/NIH grouping fluids and it is cytopathic for Vero cells.

Surveillance of cattle sera over the years has indicated that WSL infection is widespread in the country each year. WSL virus has been isolated from mosquitoes in Rhodesia (McIntosh, 1972) but never from vertebrates. The pathogenicity of WSL for sheep is well documented but information is lacking on its importance in cattle. Hence, experiments with cattle are in progress. The virus produced fever and viraemia in two pregnant heifers and six newborn calves, but no abortion or serious illness.

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(R. Swanepoel, N.K. Blackburn and J.B. Condy)

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE, ENTEBBE, UGANDA, AND THE INSTITUTE OF POLIOMYELITIS AND VIRUS ENCEPHALITIDES, AMS, MOSCOW, USSR

Preparation of complement-fixing, haemagglutinin and precipitin antigens of some tick-borne viruses (October 1974 - April 1975)

In this work, polyethyleneglycol - $HO(C_2H_4O)_nH$ (PLEG) - was used for concentrating the antigens. This substance is known to have been employed successfully by some authors for the concentration of poliomyelitis, tick-borne (Russian spring-summer) encephalitis, Omsk haemorrhagic fever, Crimean haemorrhagic fever (CHF), Congo and other viruses (1, 2, 3).

The procedure of concentration is as follows: to 100 ml of the original fluid containing antigen, 8 gms of PLEG is added. Then the suspension is shaken vigorously to dissolve the PLEG completely. For absorption of the antigen on the polymers of PLEG, the fluid is put in 4°C refrigerator for 18 hours. The sediment obtained after centrifugation is dissolved in a small volume of borate saline (pH 9.0) to obtain the required concentration (1).

Experience shows that by this method crude and sucrose-acetone mouse brain antigens can be concentrated 30-40 times (by volume) and tissue culture antigens by as much as approximately 1000 times.

Congo virus (v 3010 strain, 84th passage)

Crude suspension of Congo virus antigen in borate saline (original titre 1:32 in CF) was concentrated 40 fold by PLEG. The antigen obtained reacted in the CF test against its homologous antisera to titres higher than 1:1024. The same antigen was titrated by the agar gel diffusion precipitation (AGDP) test and gave a titre of 1:32, but did not react in the haemagglutination (HA) test with goose red cells.

Nairobi sheep disease (NSD) virus (SE 2175 strain, 16th passage)

10% crude borate saline suspension of NSD virus, prepared from the brains of infected suckling mice, had a titre of only 1:8 in the CF test against homologous antiserum (8/640). After 40-fold concentration with PLEG the titres increased significantly (up to 1:512-1024) and this concentrated antigen has been shown to give specific activity 1:2 in the AGDP test.

Kadam virus (AMP 6640 strain, 8th passage)

The antigen was prepared from the brains of infected infant mice by the method of acetone treatment of the crude borate saline antigen. Maximum titres of this antigen in CF and HA tests were 1:64 and 1:2560, respectively.

Supernatant fluid obtained after concentration by PLEG lost all specific activity. The antigen concentrated 40 times gave very high titres in CF and HA reactions, viz., up to 1:1024 and 1:91920, respectively, and 1:4 with homologous antiserum in AGDP test. Kadam virus brain antigen, prepared by the method of sucrose - acetone extraction followed by sonication has been shown to give titres of 1:2560 in HA test and 1:2 in AGDP test.

Bhanja virus (IbAR 2709 strain, 12-15 passages)

Bhanja sucrose - acetone brain antigen was treated with protamine sulphate and concentrated 20 fold by PLEG. Results obtained after concentration were as follows: HA test, 1:160; AGDP test, 1:4. Bhanja mouse brain antigens prepared by the method of sucrose - acetone extraction followed by sonication gave higher activity: up to 1:1028 in HA, 1:8 in AGDP and 1:512-1028 in CF test. Concentration of the sonified antigen 40 fold with PLEG gave no increase of HA and CF activity (which is surprising, because the supernatant fluid obtained after concentration had lost most of

its specific activity, but raised the titres of precipitin antigen up to 1:32).

Dugbe virus (AMP 5689 strain, 6-8 passages)

Results of testing of Dugbe brain virus antigens were as follows: sucrose - acetone antigen, 1:8 in HA test; sucrose - acetone antigen concentrated 20 fold by PLEG, 1:32 in HA test and negative in AGDP test; sucrose - acetone sonified antigen, 1:320 in HA test, 1:512 in CF test and 1:4 in AGDP test. After 10-fold concentration of this antigen by PLEG (by volume) the titre of its precipitin activity increased up to 1:8 only.

Dhori virus (IG 11313 strain, 5-9 passages)

Brain antigen

Sucrose - acetone sonified brain antigen gave a specific titre of 1:320 activity in the HA test (optimal pH 5.8) and negative results against homologous antiserum in the AGDP test.

Cell culture antigen

Primary syrian hamster kidney cell culture was infected with brain-adapted Dhori virus. After 4 days incubation at 37°C when CPE was estimated as 2+, the culture fluid was harvested and cleared by centrifugation for 15 mins at 4000 rpm. The supernatant fluid used as antigen gave a titre of 1:20 in the HA test at optimal pH 5.8 (titre of homologous Dhori antiserum tested against this antigen with the HI method was 1:320). After 100 fold concentration of this fluid with PLEG its HA activity increased up to 1:512. The presence of precipitin antigen was not determined.

The same procedure was employed for preparation Dhori virus antigen from tissue culture fluid of continuous BHK-21 (clone C-13) cell culture

infected with Dhori virus. On the 4th day after inoculation, when CPE was estimated as 3+, culture fluid was harvested, cleared by centrifugation and tested by the HA method. The highest titre (1:32) was found at optimal pH 5.8. Concentration 650 fold by PLEG led to an increase of the titre up to 1:2560 and removed all haemagglutinin activity from the supernate. This concentrated antigen had the capability to react against homologous immune serum in the AGDP test in a dilution of 1:2.

Jos virus (IbAn 17854 strain, 4-11 passages)

Jos virus CF antigen was prepared from liver of infected newborne mice using method of sucrose - acetone extraction and sonication. Titres of original and concentrated (20 times) Jos antigens in CF were 1:64 and 1:256, respectively. Supernatant fluid obtained after concentration lost antigenic activity. Precipitin liver antigen of Jos virus was not determined in the AGDP test.

Native culture fluid of BHK-21 (C-13) cell culture, harvested on day 7 after inoculation of Jos virus (CPE was absent) gave a titre of 1:4, and after concentration (by volume 620 times), 1:320 in the HA test. Because the BHK-21 cell monolayer was well preserved, the cells were collected from four 1 litre bottles by trypsin treatment, dissolved in maintenance media and exposed to sonication. This sonified antigen had a specific haemagglutinin titre of 1:128.

Thogoto virus (2A strain, 9-10 passages)

Thogoto sucrose - acetone sonified antigen, prepared from the liver of infected new-borne mice, gave a titre of 1:64 by CF test. While supernatant fluid obtained after 20-fold concentration by PLEG lost all specific CF activity, concentrated liver antigen gave no significant

increase in titre.

Thogoto HA antigen was prepared from BHK-21 (C-13 clone) culture fluid, harvested on the 4th day post inoculation and exposed to sonication treatment. Titres of unconcentrated and 500-fold concentrated antigens were 1:20 and 1:1280, respectively. Neither liver or culture Thogoto antigens had specific activity in the AGDP test.

In conclusion it may be said that precipitin antigens of CHF-Congo, Bhanja and Dugbe viruses were used by us successfully for detection of specific antibodies in the sera of livestock and wild animals in East Africa.

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(A. Butenko, E. Sekyalo, T. Minja, M. Mukuye)

REPORT FROM THE VIROLOGY DEPARTMENT, VETERINARY RESEARCH LABORATORY,

P. O. KABETE, KENYA

The serological relationships of Nairobi sheep disease have been studied by CFT, FAT, IHA and S/N and the results are summarised below:

a) Complement Fixation Tests

MAF used	Antigen NSD I 34 used at 1/16
NSD I 34	128*
Ganjam (G 619)	32
Dugbe Ib Ar 1792	4
Hazara J 1087	trace
Congo Ib Ar 10200	0
Bhanja IG 690	0
T 176/75	4
B 1341 Palyam	0
K 86 Ephemeral fever	0

reciprocal of serum dilution

b) Indirect Fluorescent Antibody

MAF used	BHK cells infected with NSD I 34 virus
NSD I 34	640
Ganjam	320
Dugbe	20
Hazara	20
Congo	10
Bhanja	0
T 176	20
B 1341 Palyam	0
K 86 EF	0

* reciprocal of highest serum dilution showing clear granular fluorescent particles indistinguishable from those seen with NSD MAF.

) Indirect Haemagglutination

MAF used	Antigen-NSD
NSD	10240*
Ganjam	2560
Dugbe	less than 20
Hazara	320
Congo	320
Bhanja	less than 20
T 176/75	320
B 1341	less than 20
K 86 EF	less than 20

* reciprocal of serum dilution at end point

) Serum Neutralisation

a) Infant mice S/N using constant serum, 37°C 1 hour.

	NSD using I 34 titre	Index
NSD I 34	4.7	1.2
Ganjam	4.9	1.0
Dugbe	5.9	0
Hazara	6.2	0
Congo	5.9	0
Bhanja	6.3	0
B 1341	5.9	0

b) S/N using constant virus, assayed in tissue culture, 37°C 1 hour.

NSD	32*
Ganjam	16
Dugbe	0
Hazara	0
Congo	0
Bhanja	0
T 176	0
B 1341	0

* titre of serum suppressing virus challenge of 80 $TCID_{50}$.

The relationship between NSD and Ganjam (G619) would seem to be very close in all the test systems, and the end point differences are due to the high titre of the immune I 34 NSD ascitic fluid. To clarify this relationship further it would be necessary to work with both viruses and we have not been able to do this. The indirect fluorescent antibody test and the indirect haemagglutination test have shown relationships between NSD and the other viruses examined which were not detected on neutralisation and were only evident at low titres on CFT. There is clearly an interesting field of study with these viruses and T 176, which appears more closely related to NSD than any of the others, and O-70/75 and O-249/75.

Summary of virus isolations from Culicoides midges

Species	Virus isolated	Times isolated	Total parous specimens	Total pools	Total
Mixed[o]	Ephemeral fever	1)			
	Palyam group A*	4)	153,000	28	
	group B*	1)			
C pallidipennis					
	Blue tongue type 1	2)			
	Blue tongue type 4	1)	12,100	36	
	Palyam group A	1)			
C tororensis					
	Blue tongue type 1	1)			
	Nairobi sheep disease	1)	250	12	
C milnei	Bluetongue type 1	1	3,340	21	
C cornutus	none		6,675	17	
C dekeyseri	none		280	2	
C grahami	"		50	2	
C magnus	"		1,800	16	
C schultzei	"		1,580	4	

* Palyam group A indicates the five Palyam group strains which appear identical, group B strain reacts with the Palyam grouping serum (YARU) but is serologically different from the other five. They have been submitted to YARU for identification.

[o] Details of the % composition of the mixed pools will be published later but they consisted largely of C pallidipennis, schultzei and zuluensis.

Bluetongue Virus

The strains of bluetongue virus isolated from clinically affected wool sheep in Kenya, fall into 9 serological groups. Six of these correspond with bluetongue serotypes known in other countries, three are not neutralised by any of the type sera available at Kabete (we do not have types 5, 9 and 16), although one is related to type 14. Serological evidence for the presence of seven further strains has been obtained from a sentinel herd study of cattle initiated in 1970. Interesting results of the challenge by bluetongue virus have been obtained from this.

African Horse Sickness

A suspicion that elephants may be involved in the natural history of this virus has been examined by titrating sera from a number of elephants and zebra from similar ecological zones. Complement fixing antibody was assayed using antigen prepared from strain 1 virus, the test is group specific and was standardised using a mouse hyperimmune ascitic fluid. The accompanying table summarises the results:

CFT carried out with AHS antigen and zebra, elephant and horse sera from areas considered to be endemic for AHS.

	Reciprocal of serum dilution										
	4	8	16	32	64	128	256	512	-	+	Total
Zebra	8	34	31	17	2	-	-	-	39	97	136
Elephant	4	12	42	41	22	8	1		24	130	154
Horse	-	-	2	5	1	-	-	-	-	8	8

The slightly lower titres obtained with the zebra sera may be due to inactivation at 60°C. The horse sera were included to indicate the titres obtained in horses in endemic areas. Many elephant sera contain complement fixing antibody to this antigen, if the reaction is not specific to AHS it must be to a very closely related viral antigen.

Neutralisation tests are being carried out with the positive sera in an attempt to demonstrate further new serotypes (Kabete G 75, a recent isolate is not neutralised by any of the known 9 type sera).

Virus isolates from Sheep

Two virus strains, O 70/75 and O 249/75 were isolated from sheep submitted to the diagnostic laboratory. One of these is related to Bhanja virus on indirect fluorescent antibody tests (FAT) but CFT shows that it is not identical. The results of the CFT are shown below:

Antigen	Mouse ascitic fluid							
0 70/75 °	0 70	0249	Bhanja	Congo	Ganjam	Dugbe	Hazara I 34	NSD
	128*	0	4	0	0	0	0	0
0 249/75	4	128	0	0	0	0	0	0

* reciprocal of serum titre
° antigens used at 1/8.

Viral Isolates from Ixodid Ticks

Approximately 10,000 ticks, most of which were removed from wild ungulates have been screened for viruses by the intracerebral inoculation of infant mice. Thirteen virus strains were recovered and the results of the initial identification procedures are shown in the accompanying table:

Virus	Tick pool	Animal host	Serological relations
T 39/74	Rhipicephalus pulchellus	Topi	Identical with 0-70/75 vide supra
T 45/74	" "	Wildebeest	Identical with T 83, 178 and 186
T 83/74	" "	Eland	
T 178/75	" "	Giraffe	
T 186/75	" "	from vegetation	Intra nuclear inclusions
T 176/75	Amblyomma cohaerens	Buffalo	Related to NSD vide supra
T 97/74	Rhipicephalus pulchellus	Rhinoceros	Identical with 100 101 103 129 and 163
T 100	" "	"	
T 101	" "	"	All have CF titres of 128-256 with Kadam IAF
T 103	" "	"	
T 129/75	Amblyomma variegatum	Buffalo	
T 163/75	" "	"	
Ngong/74	Rhipicephalus appendiculatus	Bovine	Thogoto virus on CFT

The strains have been submitted to YARU for identification.

(F.G. Davies, I. Solberg and A.R. Walker)

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

I. Search for antibodies against Tete and Matariya group arboviruses, first isolated from migratory birds in Egypt.

It was decided to explore several animals and human sera for antibodies against four "possible arboviruses" isolated in Egypt, in an attempt to investigate their ecology and role in human infection. Serological studies on these viruses, viz., Matruh & Bahig (Tete group), Burg El Arab & Matariya (Matariya group), to our knowledge were not carried out before.

Human sera (192) and different animals sera (774) were tested by complement fixation test (CFT) for antibodies against these four viruses. The animal sera included those of buffalo, camel, cow, pig, sheep, horse, dog, and rodents. The results showed that sera of human, camel, cow, horse, dog and rodents did not fix complement, even at a 1:4 dilution.

Table 1 shows the reaction of the other animal sera against the four viruses.

TABLE 1

Complement - fixing Antibodies to Tete & Matariya group Arboviruses

Animal species	Sera tested	Bahig virus		Matruh virus		Burg El Arabvirus		Matariya virus	
		No	%	No	%	No	%	No	%
Buffalo	95	8	8.4	11	11.5	12	12.6	7	7.3
Pig	93	1	1.1	-	-	-	-	16	17.2
Sheep	195	-	-	-	-	-	-	1	0.5

The sheep and pig sera reacted with a single antigen, viz., Bahig or Matariya, at a low titer of 1:4 or 1:8, whereas the buffalo sera exhibited multiple reactions with higher titers ranging from 1:8 to 1:32. The multiplicity of reactions by buffalo sera did not only reflect the crossing of Bahig-Matruh and Matariya-Burg El Arab complexes by CFT, but also six sera simultaneously positive for the two groups, i.e., the four antigens, were encountered. The specificity of these reactions should first be determined before considering their significance.

These serological data, although tentatively indicative of the scarcity of infection by these viruses, are by no means inclusive. Sera collected from localities where the viruses were isolated will later be tested. Most of these viral isolates were from fall migrating birds, suggesting Eastern Europe or Western Asia as the infection locale, and thus the viruses do not, probably, have a foothold in Egypt as yet.

II. Antigenic relationship of West Nile to Langat virus.

In a previous publication we have questioned the specificity of Langat HI antibodies encountered in human (3%) and rodent sera (15.4%), since all those sera were simultaneously positive for West Nile (WN) virus. To elucidate the relationship between WN and Langat antibodies, immune & hyperimmune sera were prepared in 6-week-old mice against each of the two viruses. For preparing the immune serum, a single injection (0.2 ml) of 10% virus suspension was given intraperitoneally (i. p.). A course of three i.p. injections at 7-day intervals (each 0.2 ml of 10% virus suspension) was adopted for the preparation of the hyperimmune sera. One week after the single or the last injection, mice were exsanguinated, blood of each group pooled, serum separated and stored frozen.

HI and CF tests were carried out for the pre- and post-immunization sera against the two viral antigens. The pre-sera were negative and the results of the post-sera are summarized in table 2.

TABLE 2

Cross-reactivity of West Nile and Langat Viruses.

Antigen	West Nile Antisera		Langat Antisera	
	Immune	Hyperimmune	Immune	Hyperimmune
West Nile	160/8°	320/64	40/8	80/16
Langat	10/4	20/8	80/16	320/32

°HI/CF: reciprocal of serum end-point dilutions.

These data shows that:

1. For each homologous system, the repeated injections (hyper-immune sera) increase the HI and CF titers which were already demonstrable from the single injection (immune sera).

2. Cross-reaction by HI and CF tests occurs in both directions, and it is more pronounced with the hyperimmune sera.

Taking these results into consideration, it seems that the Langat HI antibodies previously reported are probably cross-reactions from the endemic WN infection.

(Medhat A. Darwish and Imam Z. Imam)

Tick cell cultures

Several cell cultures were successfully prepared from developing adult tissues of Haemaphysalis spinigera, Haemaphysalis obesa and Rhipicephalus sanguineus in L-15 medium, supplemented with 10 percent tryptose phosphate broth and 10 percent foetal calf serum. The technique of Varma et al. 1975 (J. Med. Ent., 11: 698) was simplified and used for preparing the cultures. These were successfully subcultured and are now being serially passaged in this laboratory. Studies on susceptibility of these cell cultures to various arboviruses are in progress.

(P.Y. Guru, V. Dhanda and N.P. Gupta)

Laboratory evaluation of some repellents against larval trombiculid mites

An apparatus described by Bertram et al. 1967 (J. Roy. Army med. Cps., 113: 1-8) was modified and used for assessing repellency of certain chemicals against trombiculid mites. Five preparations, viz. dibutylphthalate, dimethylphthalate, N,N-diethyl-m-toluamide (Deet), a combination of 'Deet' and dimethylphthalate (a proprietary preparation), and pyrethrum, impregnated on filter paper and nylon/cotton fabric, were tested against Leptotrombidium deliense and L. akamushi. It was observed that the combination of 'Deet' and dimethylphthalate was more effective than others.

(S.M. Kulkarni)

REPORT FROM THE ARBOVIRUS RESEARCH UNIT
UNIVERSITY OF CALIFORNIA INTERNATIONAL CENTER
FOR MEDICAL RESEARCH
HOOPER FOUNDATION, SAN FRANCISCO
AND
THE UNIVERSITY OF MALAYA, KUALA LUMPUR

At the end of 1974, an analysis of mosquito catches in the Gunong Besout Forest Reserve jungle dengue study site led to the conclusion that *Aedes (Finlaya) 'niveus'* was the important jungle canopy vector of dengue virus. The analysis was based on comparisons of ground and canopy collections, species attracted to and feeding on monkeys, and species taken in the monkey-baited traps at the times the sentinel monkeys were shown to have been infected by dengue virus. These studies had already shown that only the sentinel monkeys held in the high canopy of the forest acquired dengue infections, while sentinel monkeys restricted to ground level did not. Since the analysis showed that canopy-dwelling *A. 'niveus'*, which prefers monkey hosts, was best correlated with the occurrence of dengue in the sentinel monkeys, program modifications were made in 1975 to improve collection of that species. This resulted in a great reduction in the total number of mosquitoes collected but a significant increase in the number of the target species taken alive.

During 1975, a group B virus was isolated from a pool of *A. 'niveus'* mosquitoes collected the previous year in the high canopy bait trap. The virus has been successfully reisolated from the original mosquito suspension as well as from the blood of an experimental monkey inoculated with the virus. Mice surviving inoculation with the virus have resisted lethal challenge with a mouse-adapted strain of dengue, indicating that the isolate is a strain of dengue virus. Final identification of the virus is in progress at the time of writing. If it is confirmed as a strain of dengue, it will represent the first isolation from a jungle mosquito*.

During 1975, an additional strain of dengue virus was isolated from the blood of a sentinel monkey bringing the total number of monkey isolates to five, all from forest canopy.

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* Dengue virus had been isolated previously only from two other species of mosquitoes, *A. aegypti* and *A. albopictus*, both associated with man. The first isolations in nature from both species were made by Dr. Rudnick in earlier studies.

Professor A. Ralph Barr of the School of Public Health, University of California, Los Angeles spent all of 1975 at the Institute for Medical Research, through the auspices of the UC ICMR. His studies of Malaysian mosquitoes were designed in part to assist the Arbovirus Research Unit, especially in attempts to colonize suspect forest vectors of dengue. He succeeded in establishing a method for the hatching of *Aedes 'niveus'* eggs, which we anticipate will lead to the laboratory colonization of the species.

The identification of a strain (P72-162) of Lanjan virus from *Haemaphysalis* ticks increases to five the number of isolates of that virus made by us from ticks in Malaysian forests. Little is known of the ecology of Lanjan virus. Therefore, the identification of P71-1362 strain from the blood of a wild *Macaca nemestrina* monkey in the southern part of the Gunong Besout Forest Reserve is of particular interest. It is the first Lanjan isolate from a vertebrate. The monkey (GB-57) was captured four times over a period of about six weeks during the months of September and October 1971. The virus was isolated from the blood sample taken on the second capture. The last two samples have not yet been tested for the development of Lanjan antibody.

Four agents were isolated from bat ticks (*Argas pusillus*) collected in Kuala Pilah *Scotophilus temminckii* roosts. We had previously isolated Keterah virus from the same species of tick as well as from the blood of a *S. temminckii* bat collected in north-eastern Malaysia. P75-331 was isolated from a pool of nymphs, P75-332 from a pool of adult females, and P75-333 and P75-336R from pools of mixed adult males and females.

A virus (P75-294) was isolated from a pool of unfed *Boophilus microplus* larvae hatched in the laboratory. If this agent proves to be a strain of Seletar virus, which we have described previously from field-collected *B. microplus* ticks, it will indicate that it is transovarially transmitted and that it is a true arbovirus. This is of special interest since *B. microplus* is a one-host tick. Transovarial transmission would normally, therefore, be necessary for transfer of the virus from host to host.

(A. Rudnick, A.B. Knudsen & H.G. Wallace)

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

The following summary reports the mosquito, virus isolation and cattle serological studies not previously reported from the North-West of Australia in the Ord River study site.

ENTOMOLOGY

Kununurra, the largest town in the Ord Valley, is the focus for very large populations of birds and mosquitoes. The irrigation areas are presently not important as mosquito breeding areas because of the excessive use of insecticides. The Ord River Dam (Lake Argyle) does not now support high mosquito or bird populations. However, this may change as the ecosystem stabilizes. The mosquito fauna of the Ord Valley is dominated by *Culex annulirostris* and the proportions of the major species collected in three subdivisions of the Ord Valley are given in Table 1. A check list of mosquitoes known in the Ord Valley is available to those interested.

VIRUS ISOLATIONS

One hundred and thirty viruses lethal for infant mice have been isolated from 485 pools made from 23,872 mosquitoes collected in the study site. One hundred and eleven of the virus isolates came from pools of *Culex annulirostris*. A few isolates have been identified as strains of MVE, Kunjin, Wongal and Sindbis.

SEROLOGY

Earlier reports have been made for group A and group B antibody in human and avian sera. We report here only the results with cattle sera using H-I tests with MVE, Sindbis and Ross River viruses. Table 2 shows the results of testing 880 cattle sera collected from cattle stations of a wide area of the North-West. In the study site area (with its higher human population density at Kununurra) 80% of cattle were positive for MVE. In areas more remote from the study site only 37% of cattle were positive for MVE.

(N.F. Stanley, M.P. Alpers, P.F. Liehne, C.G. Liehne, N.P. Hamilton, K.H. Chan, S. Paul).

TABLE 1 : The proportions of the major species collected in the 3 subdivisions of the Ord Valley

AREA :	*LAKE ARGYLE*	*DIVERSION DAM*	*LOWER ORD*
Total Number Adults Caught:	*172*	*47,249*	*714*
SPECIES:			
Culex annulirostris	79%	78.3%	61.6%
Culex fatigans		7.2%	
Culex australicus			8.1%
Aedes normanensis		1.3%	12.0%
Aedes vigilax			15.6%
Aedes tremulus	11%		
Aedeomyia catasticta		9.8%	
TOTALS	90%	96.6%	97.3%

TABLE 2.

	Number Tested	% positive by H-I tests with MVE	Sindbis	Ross River
Cattle*				
Kimberley	564	37	3	2
Kununurra	316	80	4	2

REPORT FROM THE VETERINARY RESEARCH STATION, GLENFIELD, NEW SOUTH WALES AND THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH, AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA, AUSTRALIA

Arthropod-borne viruses and nervous disease in horses

In the first months of 1971 and 1974, there were abnormally large numbers of reports of clinical nervous disease in horses in New South Wales. Several arthropod-borne viruses, including Murray Valley Encephalitis (MVE) and Ross River Virus (RRV) were known to be active at both times. Sera were examined for MVE and RRV HI antibodies and 14 animals were considered recent infections with MVE or related viruses and another 4 horses were thought to have been recently infected with RRV or a related virus (Table 1). Horses were considered recent infections if there had been a significant change in titre between bleeds or if sera were consistently reduced in titre two-fold or greater by 2-mercaptoethanol (2ME). Another horse, No 5, was included because it was only 7 months old.

Neutralisation tests were performed on the sera of 7 horses (Nos 1, 2, 4, 5, 7, 9 and 12) and results suggested that the infecting virus was MVE.

None of the horses considered MVE infections died and 10 were available for a convalescent rebleed. However, of the 4 horses thought to have been infected with RRV, 2 (Nos 16 and 17) died.

RRV as a possible cause of joint and muscle disease in horses

RRV is thought to be the agent of epidemic polyarthritis in humans. In February 1971, 2 horses with muscle stiffness and joint

swelling had high RRV HI titres. In April 1975 another horse, from the same district, experienced sudden stiffness in all limbs, muscle pain in the hind quarters and abdomen and swelling of the left shoulder joint. The acute serum of this animal had a RRV HI titre of 5120 which was reduced to 640 by 2ME.

(G.P. Gard and I.D. Marshall)

REPORT FROM THE VETERINARY RESEARCH STATION, GLENFIELD, NEW SOUTH WALES, AUSTRALIA 2167

Infection of feral pigs with RRV and with Flaviviruses

During a study of the ecology of the feral pig, large numbers of sera were available and were examined for MVE and RRV antibodies. It was anticipated that RRV antibodies would be demonstrable each year and that MVE antibody would be present only in those pigs alive in the first months of 1971 and 1974 when the virus was known to be active in N.S.W.

The results of MVE and RRV serology of feral pigs from north west N.S.W. are presented in Tables 2 and 3. MVE HI antibody was detectable in most sera, including all of 27 pigs shot in March 1974 and surprisingly in a high proportion of animals shot in 1972 and 1973. There was a low incidence of RRV HI antibody in most groups.

Plaque reduction neutralization tests were performed on selected sera (Table 3) and the results suggest that (a) pigs shot in 1971 and 1972 had been infected with MVE (b) pigs shot in 1976 probably had Kunjin antibody and (c) pigs shot in March 1974 had been infected with more than one Flavivirus, although titres tended to be higher to MVE. Sera of pigs shot in June 1973 were not available for neutralization testing but the fact that 74% of 42 pigs collected in June 1973 had MVE HI antibody (Table 2) suggests that a Flavivirus was also active in the summer of this year. Thirteen sera of MVE HI titre < 10 were included in these neutralization tests. Twelve had MVE N titres of 16 or less and 11 had Kunjin titres of 16 or less.

(G.P. Gard, J.R. Giles, R.J. Dwyer-Gray, G.M. Woodroofe)

TABLE I

RESULTS OF MVE AND RRV HI TESTS ON SERA OF HORSES WITH CLINICAL NERVOUS DISEASE

Horse	Date Bled	MVE HI Titre		RRV HI Titre	
		Untreated	2ME	Untreated	2ME
1	16.4.71	480	ND*	<10	ND
	17.5.71	120	ND	<10	ND
2	17.1.74	80	20	960	640
	17.2.74	640	640	320	320
3	17.1.74	320	80	120	120
	21.2.74	640	640	120	120
4	18.1.74	640	160	120	120
	6.2.74	240	240	120	120
5	11.2.74	640	480	<10	<10
	28.2.74	480	480	<10	<10
6	2.2.74	320	60	<10	<10
7	28.1.74	640	40	640	480
	19.2.74	640	640	480	480
8	30.1.74	320	80	<10	<10
9	5.2.74	1280	160	<10	<10
	17.2.74	640	640	<10	<10
10	16.2.74	240	120	<10	<10
	6.3.74	120	120	<10	<10
11	22.2.74	40	30	320	320
	8.3.74	240	240	320	320
12	22.2.74	40	30	1280	960
	8.3.74	320	240	960	960
13	12.3.74	320	80	<10	<10
14	28.3.74	480	120	<10	<10
15	8.3.74	480	320	2560	40
16	14.3.74	40	40	640	20
17	8.3.74	<10	<10	1280	320
18	20.5.74	15	15	640	120

*ND NOT DONE

TABLE 2

RESULTS OF MVE AND RRV HI TESTS ON FERAL PIG SERA

Date Collected	Number Examined	MVE HI Titre				RRV HI Titre			
		<10	10-20	>20	%>20	<10	10-20	>20	%>20
2.71	4	3	1	0	0	4	0	0	0
6.71	36	0	5	31	86	14	9	13	36
8.71	6	1	1	4	67	4	2	0	0
9.71	42	8	7	27	64	22	14	6	14
11.71	17	8	2	7	41	11	5	1	6
12.71	53	20	4	29	57	44	6	3	6
6.72	63	4	8	51	81	52	4	7	11
9.72	41	8	7	26	63	37	4	0	0
2.73	35	11	6	18	51	32	2	1	3
6.73	42	4	7	31	74	11	16	15	36
3.74	27	0	0	27	100	4	6	17	63
5.74	2	1	0	1	50	2	0	0	0
11.74	22	0	3	19	86	15	1	6	27
1.76	21	7	1	13	62	3	4	14	67
Totals	411	75	52	284	69	255	73	83	20

TABLE 3

RESULTS OF MVE AND KUNJIN N TESTS ON SELECTED FERAL PIG SERA

Pig	Date Collected	Age (mths)	MVE HI Titre	MVE N Titre	Kunjin N Titre
270	12.71	6	30	1024	32
276	12.71	6	60	512	<16
191	6.72	15	120	2048	64
194	6.72	15	60	2048	16
199	6.72	14	30	>2048	32
209	6.72	10	60	>2048	<16
227	9.72	14	60	128	<16
255	9.72	16	160	>2048	16-32
164	2.73	18	240	>2048	16
166	2.73	18	120	>2048	16
389	2.73	20	120	2048	64
393	2.73	20	120	>2048	64
401	2.73	20	60	>2048	16
625	3.74	10	>20	512	512
628	3.74	6	>20	512	256
629	3.74	6	>20	>2048	512
631	3.74	6	>20	2048	256
633	3.74	14	>20	>2048	256
602	1.76	17	120	16	>2048
609	1.76	16	80	16	512
620	1.76	16	120	32	>2048

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

The dengue viruses have been shown to multiply in a variety of cell culture systems, especially monkey kidney and hamster kidney cells. However, the rates of viral multiplication therein are not necessarily sufficient, compared with other kinds of arboviruses. We examined established cell line cultures for their capacity of supporting the growth of DEN-1 virus. Forty-seven cell lines were tested, which were supplied by the Flow Laboratories, U. S. A., and the Dainippon Pharmaceutical Co., Japan. Virus inoculum was a homogenate of brains from mice infected with DEN-1 Mochizuki strain. At intervals after the beginning of incubation at 37 C of the infected cultures, portions of the culture fluid were taken and the viral titers were measured by plaque counting on BHK-21 cell monolayer cultures under methylcellulose overlay medium. Of the data so far obtained, the following points are perhaps worthy of comment:

(1) Human diploid WI-38 cells could support the growth of DEN-1 virus. This may be utilized for production of anti-dengue vaccine, if any dengue-vaccine(particularly "live vaccine") be considered for human use.

(2) IMR (human neuroblastoma origin) and J-111 (human leukemic leucocyte origin) supported the growth of DEN-1 virus fairly well. The viral growth rates in both systems were better than that in BHK-21 cells which we have been routinely using in our dengue works.

(3) Negative data were obtained as for some cell lines which had been reported by other investigators to be capable of supporting the growth of dengue viruses. No reason(s) for such descrepancy have been clear. It may be likely, however, that cell clones used in different laboratories are not necessarily identical in their particular properties such as viral permissiveness. The same statement may be made as to the positive data we obtained.

The growth patterns of DEN-1 virus in the IMR and J-111 cells, including electron microscopic pictures, are being studied. (K. Shiraki, T. Matsumura)

(S. Hotta)

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

Human Japanese encephalitis cases in 1975 in Japan.

Totally 27 cases were confirmed, all in serological diagnosis. Among 27 cases, 11 were male and 16 were female. Number of death were 6, i.e. mortality was 22.2%. Most of the cases were reported from southern part of Japan,i.e. 13 from Kyushu and Okinawa and 12 from Shikoku. Age distribution is: 3 in 0 - 9 years, 0 in 10 -29 years, 1 in 30-39 years, 1 in 40-49 years, 1 in 50-59 years, 13 in 60-69 years and 8 in more than 70 years.

The first case occured 14th July and the last case 9th September being peak of incidence in August.

Identification of new viruses isolated from bats in Japan.

To elucidate a possible role of bats for overwintering of arboviruses in Japan, wild bats were captured and virus isolation was attempted at Oita Prefecture, Kyushu District.

Four hundred ninty six bats of 3 species were examined in 1971 and in 1972. Eight strains of virus were isolated from Miniopterus schreibersii Temminck and one strain from Rhinolophus cornutus Temminck.

The former 8 strains were almost identical and found to be a member of Flavivirus. However, it was found not to be identical to any known member of this group. A special attention should be made on seroepidemiological survey of Flavivirus in Japan since it has a close antigenic similarity to Japanese encephalitis virus. This strain is named as YOKOSE virus.

A strain isolated from R. cornutus was thought to belong to rhabdovirus group with bullet shape of the virion shown under the electron microscope. No antigenic relation was so far observed to rabies virus. No cross reaction was noted in CF with polyvalent serum against Hart Park, Flanders, Kern Kanyon, Klamath and Mt. Elgon bat viruses.

(A. Oya, S. Yabe, E. Arslanagic and M. Morita)

REPORT FROM THE NATIONAL INSTITUTE OF HEALTH AND TEIKYO UNIVERSITY, TOKYO JAPAN

Isolation of viruses from bats in Japan

A number different agents, such as rhabdovirus, arenavirus and so on, have been isolated from bats, but the majority, on immunological evidence, are in the flavivirus group. Sulkin et al. (1970) have published on the isolation of a total of 45 strains of Japanese encephalitis (JE) virus from bats (2.3%) collected all over Japan during the period of 1963-1965; and Cross et al. (1971) have reported isolation of a JE virus strain from bats in Taiwan in 1969-70. We have attempted to isolate viruses from 838 additional bats, some of which were collected in the same season with Sulkin et al. and the remainder from the period of 1966-1968. Nine strains were isolated (1.3%). Two of the isolates (TOK-1350 and TOK-1362) were from Vespertilio supernas collected in Fukushima Prefecture (northern part of Japan); they killed suckling mice (sm) within 3-5 days after intracerebral (i.cr.) inoculation.

Two other strains were also isolated from Ves. supernas collected at dwellings in Fukushima Pref. One strain was from Miniopterus shreiberi collected in a cave in a cave in Shizuoka Pref. (middle part of Japan); one strain was from the same species of bat collected in culvert in Miyazaki Pref.; and another one from Myotis macrodactylus collected in a mine in Kagoshima Prefecture (the last 2 prefectures being in the southern part of Japan). Four strains were isolated repeatedly from blood or spleen-kidney suspension.

Table 1 shows the biological properties of the 9 new isolates. All of the strains killed sm but not adult mice after i.cr. injection. The

$\log_{10}$ LD50 of the virus in sm brain ranged from 5.0 to 7.3 and the survival time after inoculation ranged from 4 to 9 days. All strains passed through a 0.22μ Millipore filter. One strain (KY-5) was ether resistant; another one (TOK-292) was equivocal; and the remaining 5 strains (KY-663, TOK-949, VS-820, VeJ-822 and TOK-1280) were ether sensitive. Two strains (KY-5 and TOK-292) were sodium deoxycholate (SDC) resistant; the other one (KY-663) was equivocal; and the remaining 3 strains, TOK-949, VeJ-822 and TOK-1280, were SDC sensitive (one strain, VS-820, has not yet been tested). No haemagglutinating activity was detected in sucrose - acetone extracted antigen even after protamin or supersonic treatment, but SA antigens of 4 strains (KY-663, TOK-292, TOK-949 and VS-820) were found to have CF activity. The remaining 3 strains (KY-5, VeJ-822, and TOK-1280) had no CF activity.

Immune serums were prepared against 7 of the isolates and JEV and Apoi viruses, and HI tests were undertaken against 3 alphaviruses (western equine encephalitis, Sindbis and Semliki Forest), 8 flaviviruses (dengue type 1, yellow fever (YF)-17D, JE-Nakayama-NIH, Rio Bravo, Powasson, Modoc, Negishi and Apoi) and Bunyamwera virus. Table 2 shows that the alphaviruses and Bunyamwera virus did not react by HI with the antisera to the new isolates, while the flaviviruses, especially YF-17D and Rio Bravo viruses, did react. The anti-JEV serum showed a little cross reaction (1:40). As a whole it was impressive that the 7 new isolates were immunologically related with the flavivirus group. They do not appear to be JE virus. Four strains (KY-5, TOK-292, KY-663, TOK-949) were sent to Sulkin's laboratory for further identification; those workers obtained results similar to ours. Tentatively, we have called the 7 bat strains KY-5, KY-663, TOK-292, TOK-949, TOK-1280, VS-820 and VeJ-822 until identifications

can be completed. Isolates VS-820 and TOK-949 react identically by cross CF test, probably due to their being collected at the same place.

(Masami Kitaoka, M.D., Consultant, National Institute of Health, Tokyo; and Teiji Miura, M.D., Dept. of Hygiene, School of Medicine, Teikyo University)

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Table 1

Biological properties of new isolates

Strain Name	Pathogenity for Sm	Days after inoculation	Filtrability 0.22μ	Ether resistant	SDC resistant	HA activity (SA-antigen)	CF-Activity
KY-5	5.9	6-9	+	+0.08	+0.05	-	-
KY-663	7.3	4-5	+	-5.9	-1.0	-	64
TOK-292	6.3	7-9	+	-1.7	+0.4	-	128
TOK-949	6.5	8-9	+	-3.8	-2.4	-	64
VS-820	5.6	8-9	n.t.	-5.1	n.t.	-	108
VeJ-822	5.3	5-8	+	-3.9	-2.7	-	-
TOK-1280	5.0	4-7	+	-4.8	-2.9	-	-
TOK-1350		3-4	n.t.	n.t.	n.t.	n.t.	n.t.
TOK-1362		4-5	n.t.	n.t.	n.t.	n.t.	n.t.

Sm = Suckling mice. Virus contents logarithmic value LD_{50}

Days = Death of disease after i.cr. inoculation.

SA = Sucrose acetone extract antigen. HA = Hemagglutinating

CF = Compliment fixing. n.t. = not tested

Table 2

Identification of new isolates with Flaviviruses by HI test

Antigen / Immune Serum	Alphavirus			Flavivirus								
	WEE	Sindbis	Semliki Forest	Dengue 1	YF-17D	JEV-Nakayama	Reo Bravo	Powasson	Modoc	Negishi	Apoi	Bunyamwera
KY-5	0	0	0	80	1280	±	320	±	±	±	10	0
KY-663	0	0	0	0	10	0	10	0'	0	0	0	0
TOK-292	0	0'	0	10	10	0	40	0	0	0	0	0
TOK-949	0	0	0	±	1280	±	80	0'	0'	0'	10	0
TOK-1280	0	0'	0	10	1280	40	640	0'	10	10	20	±
VS-820	0	0	0	10	160	0	80	0	0'	0'	0	0
VeJ-822	0	±	0	10	1280	±	160	±	±	10	±	±
JEV	0	±	0	20	1280	320	1280	0'	40	10	40	0'
Apoi	0	0'	0	0'	20	±	320	0'	±	±	20	0

0' = spur

Two strains, VS-820 and VeJ-822, and three strains, KY-5, TOK-949 and TOK-1280 seem to be closely related.

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

The use of Toxorhynchites mosquitoes to detect and propagate dengue viruses

The isolation of dengue viruses has been greatly facilitated by using parenterally inoculated Aedes albopictus or Aedes aegypti mosquitoes as laboratory hosts. While this procedure has an important advantage in sensitivity over the use of cell cultures or mice for similar purposes, it is not without disadvantages. One of the disadvantages is the relatively small quantity of inoculum which can be injected into a single insect without high mortality. Another is the toxicity of some undiluted human sera for these species of mosquitoes, especially for males. Also, the establishment of laboratory colonies of these two Aedes, or related species, would not be desirable in certain tropical areas where they were not found naturally.

In an attempt to overcome these difficulties we explored the use of mosquitoes of the genus Toxorhynchites for the isolation and propagation of dengue viruses. Toxorhynchites is a genus of unusually large and hardy mosquitoes which consists of about 60 species distributed largely in tropical areas. The adults do not feed on blood and the larvae, which are found in small containers, are predacious, feeding on larvae of other mosquito species. Because of these characteristics, there has been considerable interest in the use of Toxorhynchites to control deleterious species of mosquitoes which breed in small containers.

We tested two species of Toxorhynchites which have been introduced into Hawaii, T. amboinensis and T. brevipalpis. Both can be reared easily in small cages in the laboratory. Both sexes of T. amboinensis were as susceptible to infection with with each of the four prototype strains of dengue virus as was A. albopictus. Both sexes of T. brevipalpis also appeared as susceptible as A. albopictus in so far as they were tested. As judged by measurement of

complement-fixing antigen infected Toxorhynchites apparently contain at least as much virus as infected A. albopictus. Both Toxorhynchites species can be injected with 5 to 10 times the amount of inoculum used for A. albopictus without excessive mortality. Obviously, no special safety precautions are necessary when working with female Toxorhynchites. We also have found that Japanese encephalitis and yellow fever (17D strain) viruses replicate in Toxorhynchites, suggesting that this genus also may prove useful for the study of other arthropod-borne viruses.

(Leon Rosen)

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

After intrathoracic injection of wild-caught Aedes communis mosquitoes collected in the Yukon Territory, infectivity increments were detected in salivary glands and thoraces following incubation at 0, 13 and 23°C for 7 to 14 days, after injection with 0.1 mouse LD_{50} or greater doses of an unpassaged California encephalitis (CE) isolate (snowshoe hare subtype) from wild-caught adult A. communis (74-Y-234) and an isolate from larval mosquitoes (75-L-10) which received one suckling mouse passage. Immunoperoxidase staining of the cytoplasm of acinar cells of infected salivary glands was observed by light microscopy more regularly after incubation at 13 and 23°C than at 0°C, in mosquitoes which received 1.0 mouse LD_{50} or higher inocula. Virions 45 to 53 nm total diameter, which were surrounded by electron-dense peroxidase particles, were observed intracytoplasmically in infected mosquito salivary glands, following treatment with peroxidase-conjugated CE antiserum after incubation at 13°C for 21 days with the 75-L-10 strain, and after 13°C for 13 days for the 74-Y-234 strain. Another arctic Bunyavirus, Northway (NOR) and a subtropical Flavivirus, Murray Valley encephalitis (MVE), also replicated in salivary glands of A. communis after 7 to 14 days incubation at 0, 13, 23°C, but immunoperoxidase reactions were observed more regularly in salivary glands of mosquitoes after incubation at higher temperatures. The minimum infectivity dose of NOR for mosquitoes was 10 mouse LD_{50} and MVE 0.03 mouse LD_{50}.

After laboratory-bred A. aegypti mosquitoes imbibed infective blood containing the 75-L-10 and 74-Y-234 strains of CE virus, infectivity and immunoperoxidase reactions were observed in salivary glands after 13 and 20 days incubation at 13 and 21°C and the 74-Y-234 strain was transmitted after 13 days incubation at 21°C.

(D.M. McLean)

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

Surveillance for mosquito-borne encephalitis in California during the 1975 season was again remarkable in revealing an unusually low level of activity for western encephalitis (WE) and St. Louis encephalitis (SLE) viruses. For the second year in a row, and only the second year since record-keeping began in the 1940's, there were no indigenous cases of WE or SLE detected. Two cases of SLE occurred in persons who acquired infection out-of-state (one in Illinois, one in Texas). No equine cases of WE were laboratory-confirmed, although many clinically suspected cases were reported to the Department. A total of 1,002 mosquito pools were tested in suckling mice, from the usual sampling sites throughout the state, but surprisingly no virus isolates were made from California by this Laboratory (one isolate of Turlock virus was made from a pool of Culex tarsalis from Mohave County, Arizona). Intensive, local mosquito virus surveys by Dr. W.C. Reeves' and Dr. Telford Work's research groups did reveal foci of California group virus, Hart Park virus, and Turlock virus in Northern California; and of California group viruses, WE and SLE viruses in Imperial County, respectively. In summary, this was an unusually quiet surveillance year in contrast to the large epidemics of WE and SLE in midwestern, southern and eastern U.S.

As usual, Colorado tick fever (CTF) was a relatively common diagnosis: 18 confirmed human cases during 1975, the third highest year on record. Fluorescent antibody staining of peripheral blood smears continues to be an accurate and rapid diagnostic method. Electron microscopic studies have documented further that virus infection of early-stage hematopoietic cells, with persistence of virus into

circulating blood cells, is the mechanism for the persistent viremia in CTF.

Work has been completed on preparing Seed Virus Reagents and Immune mouse ascitic fluids, under contract with the Research Resources Branch, NIAID. The viruses include: Rio Bravo, Yellow Fever, SLE, West Nile, Tembusu, Bussuquara, Powassan, JBE, Semliki Forest, RSSE, Piry, Mucambo, VEE (Florida), Dengue 1, Dengue 4, Bocas, Gumbo Limbo, and LCM. A study reporting the serologic cross-reactions by indrect fluorescent antibody and plaque-reduction neutralization tests, as compared with standard HAI and CF tests, for the group B arboviruses will be reported subsequently. The ascitic fluids are good sources for preparing FA conjugates, for the identification of viral isolates. In contrast to the relatively broad cross-reactions among related viruses when tested by the indirect FA method, direct FA staining usually yields quite specific results.

A study of lymphocytic choriomeningitis (LCM) among house mice (Mus musculus) in the San Francisco Bay area was conducted, following a renewal of our interest in this well-known disease because of the large nationwide epizootic (1973-74) of pet hamster-associated human cases (58 cases in California). Of 107 mice tested from various study sites, 8 mice were found to be positive. A test for LCM antibody by the IFA method, using an organ pool suspension of snap-trapped mice instead of serum, and an anti-mouse FA conjugate prepared in this Laboratory, was a convenient method for detecting carrier mice without the difficulty of live-trapping them to obtain blood samples. Similar methods were used to study the large hamster epizootic previously. No recurrence of the pet hamster epizootic has been noted, but occasional Mus musculus-associated human cases continue to occur, and probably are often unsuspected and undiagnosed.

(R.W. Emmons)

REPORT FROM THE ENVIRONMENTAL AND ECOLOGY BRANCH, DUGWAY PROVING GROUND, DUGWAY, UTAH

As part of the arbovirus surveillance program in Utah, mosquitoes were collected during August and September 1975 from three locations in western Utah and at three locations near St. George in southern Utah. Specimens from light traps were pooled by species, date of collection and location and assayed in suckling mice. A total of 8,507 western Utah mosquitoes were assayed as 97 pools and 8 California group viruses were isolated. All 8 isolates were from Aedes dorsalis, 7 from Blue Lake and 1 from Callao. The southern Utah collections included 6,359 mosquitoes which were segregated into 91 pools and assayed. Four Western encephalitis (WEE) viruses were isolated including 3 from Culex tarsalis and 1 from Culex spp. abdomens. Three of the WEE viruses were from Bloomington and 1 was from Washington Field. Specimens which exhibited recent engorgement were submitted to the U.S. Public Health Service in Ft. Collins, Colorado, for precipitin studies to determine the source of the blood meal.

(G.T. Crane)

Phlebotomus Fever Group Viruses From a Pack Rat (Neotoma micropus) in South Texas.

Three virus strains were isolated from diluted whole blood taken from pack rats (N. micropus) captured near Brownsville, Texas. HIAF's prepared against isolates TBM3-24 (collected 12-5-73), TBM3-204 (12-18-73) and TBM4-719 (3-23-74) were used to cross-test these three isolates by CF and N tests in SM. By both tests, all three strains were indistinguishable. Screening of all three antigens by CF for reactions with reagents representing more than 200 arboviruses at YARU revealed reactions only with Chagres and Bujaru of the Phlebotomus Fever (PHL) group. To confirm the apparent relationship to the PHL group, HI tests were done with TBM3-204 antigen and 31 grouping HIAF's; inhibition of hemagglutinin (pH optimum 6.1 - 6.2/room temperature) was noted only with PHL grouping HIAF. TBM3-204 antigen and HIAF were tested by CF with five PHL group viruses (Table 1). These tests confirmed reactivity only with CHG and BUJ antigens and TBM3-204 HIAF.

Dr. Robert B. Tesh, Pacific Research Section, NIH, Honolulu, Hawaii kindly tested another strain, TBM4-719, by PRN tests with HIAF's for 23 different PHL viruses (Table 2); no neutralization of TBM4-719 was detected with antisera to any PHL virus other than the homologous. In addition, CHG and BUJ viruses were not neutralized by TBM4-719 HIAF.

It appears that these isolates from south Texas represent a new PHL group virus for which we shall suggest the name Rio Grande virus.

This is the first isolation of a PHL virus in North America. Phlebotomus fever viruses have been isolated in southern Europe at latitudes 25° north of Brownsville, Texas.

(C.H. Calisher, W.D. Sudia, D. Muth, R. Mc Lean, G. Smith and J. Lazuick)

TABLE 1. CF test results comparing TBM3-204 virus from Neotoma micropus collected in Texas with Phlebotomus Fever group viruses.

Antigens	TBM3-204	Chagres	Bujaru	Candiru	Naples	Sicilian
TBM3-204	≥128	0	0	0	0	0
Chagres	32	≥128				
Bujaru	4		16			
Candiru	0			64		
Naples	0				≥128	
Sicilian	0					32
Anhanga	0					
Arumowot	0					
Karimabad	0					
Itaporanga	0					
Icoaraci	0					
BeAn 100049	0					
Punta Toro	0					
Salehabad	0					
Sud An 754-61	0					

TABLE 2. Results of plaque reduction neutralization tests with Strain TBM4-719 virus (R. Tesh, Pacific Research Station)

Hyperimmune serum or ascitic fluid	Homologous titer	Strain TBM4-719
Frijoles	2,048 *	<16
Caimito	256	<16
Nique	128	<16
Aguacate	512	<16
Chilibre	1,024	<16
Cacao	512	<16
(Co Ar 3319)	128	<16
Punta Toro	8,192	<16
Chagres	1,024	<16
Icoaraci	32,000	<16
Candiru	512	<16
Itaporanga	4,096	<16
(Be An 100049)	128	<16
Pacui	4,096	<16
Anhanga	128	<16
Bujaru	<16	<16
Arumowot	2,048	<16
Sicilian	≥1,024	<16
Naples	256	<16
(Sud An 754-61)	64	<16
Karimabad	256	<16
Salehabad	32	<16
Gordil	256	<16
(TBM4-719)	512	512

* Reciprocal of highest dilution giving ≥90% plaque inhibition.

Group B Arboviruses from a Cotton Rat (Sigmodon hispidus) Captured in Texas.

A virus isolate, 71V-1251, was obtained from whole blood (collected from the retroorbital plexus) of an adult female cotton rat (S. hispidus) live-trapped 7-30-71 at Brownsville, Texas.

Another strain, MA-387-72, isolated by Dr. A. Lewis, Encephalitis Research Center, Tampa, Fla. from a liver-spleen-kidney pool of an adult male cotton rat collected 7-16-72 by Dr. R. H. Kokernot, Texas Tech. University, Lubbock, Texas was submitted to us for identification. The rat from which MA-384-72 was isolated had been captured near Presidio, Texas.

After isolation and passage in SM, the AST decreased from 6-9 to 4 days. No plaques were observed in DE or Vero cells inoculated with third SM passage material of either strain.

Preliminary HA and HI tests showed that both strains were group B arboviruses.

CF tests (Table 1) performed by D. Muth at YARU indicated a close relationship of MA-387-72 to Modoc, Cowbone Ridge, Jutiapa and perhaps SLE but not to the other group B arboviruses tested. The relationship was most close with JUT virus. Further CF tests (Table 2) with both strains confirm and extend these results. By virus-dilution N tests in SM, MA-387-72 is most closely related to Modoc (Table 3). These results suggest that the viruses CBR, JUT and MOD form an antigenic subgroup of the group B arboviruses. These viruses have mammalian hosts; none has been isolated from an arthropod vector and so may be transmitted by another agency.

(C.H. Calisher, D.B. Francy, D. Muth, and J. Lazuick)

TABLE 1: CF test reactions of MA-387-72 virus from *Sigmodon hispidus* (Texas) with Group B viruses *

Antigens	Antibody											
	MA-387-72	MOD	CBR	JUT	BSQ	D2	ILH	SLE	YF	POW	MML	RB
MA-387-72	≥1024	16	16	128	0	0	0	16	0	0	0	0
Modoc	32	128										
Cowbone Ridge	16		128	16								
Jutiapa	512		16	256								
Bussuquara	64				256							
Dengue 2	0					≥256						
Ilheus	16						32					
St. Louis	32							≥256				
Yellow Fever	0								≥256			
Powassan	0									16		
MML	16										128	
Rio Bravo	8											16

* Composite of 2 CF tests.

TABLE 2. Cross CF tests with MA-387-72 and certain group B arboviruses

Antigen	Hyperimmune Ascitic Fluid to: MA-387-72	71V-1251	MOD	CBR	JUT
MA-387-72	≥1024	128	16	a)	128
71V-1251	64	512	-	-	-
Modoc	32	-	128	-	-
Cowbone Ridge	16	-	-	128	16
Jutiapa	256	-	-	16	256

a) - Not Tested.

TABLE 3. Cross N tests with MA-387-72 and certain group B arboviruses a)

Virus	HIAF MA-387-72	CBR	RB	MOD	MML	SLE	POW
MA-387-72	4.7	2.0	1.3	2.4	0.7	0.5	0.0
Cowbone Ridge	1.9	≥2.0					
Rio Bravo	2.0		4.4				
Modoc	≥3.0			≥3.0			
MML	3.0				4.3		
SLE	0.6					3.3	
POW	0.1						≥3.1

a) Results given as Log Neutralization Indices.

Summary of TC-83 vaccine studies

The purpose of this work was to determine homologous and heterologous VEE N antibody titers in persons previously vaccinated with TC-83. Clinical illness in persons immunized with TC-83 and infected with heterologous antigenic types (IE, III) have been reported (K.M. Johnson and D.H. Martin, Adv. Vet. Sci. Comp. Med. 18:80-116, 1974). All Vector-Borne Diseases Division personnel had received TC-83 vaccine at variable intervals prior to testing. One individual (Campos) had a naturally acquired VEE IB infection in 1971. Sera were tested by the PRNT technic in DECC. Results (Tables 4-5) indicate that antibody titers to TC-83 virus correlated quantitatively with titers of antibody to epidemic-epizootic variants IA, IB and IC, but that N antibodies to enzootic variants were absent or of low titer.

(C.H. Calisher and C. Fillis)

TABLE 4. Results of neutralization tests with VBDD personnel sera

Last Name	MBV-D[a]	TC-83	VEE Type							
			I, subtype					II	III	IV
			A	B	C	D	E			
Armbrister	4	≥640	40	40	80	-[b]	-	-	-	-
Balza	4	≥640	160	80	320	40	40	40	20	-
Fillis	2	≥640	320	160	≥640	40	20	20	40	20
Karabatsos	84	≥640	160	80	320	-	-	20	-	-
Mann	46	≥640	160	80	320	40	40	80	20	-
Reibling	6	≥640	160	80	320	40	40	40	-	-
Trent	144	≥640	160	320	320	40	160	160	40	-
Wolff	20	≥640	320	320	≥640	80	20	20	20	-
Wyrick	22	≥640	80	40	320	20	-	20	-	-
Bailey	4	320	40	20	80	-	-	-	-	-
Bolin	19	160	20	-	40	-	20	-	-	-
Calisher	72	320	80	40	160	-	20	20	20	-
Heidig	22	160	-	20	40	-	-	20	-	-
Jakob	19	160	20	-	-	-	-	-	-	-
Kler	19	160	20	20	40	-	-	-	-	-
Mathews	19	160	40	20	40	-	-	-	-	-
Pegg	22	160	40	40	80	20	-	-	-	-
Walker	6	≥640	40	20	40	-	-	20	-	-
Wiggett	47	≥640	20	-	40	-	-	-	-	-
Campos		≥640	≥640	≥640	≥640	320	320	160	160	80

a) = No. of months between last VEE vaccination and bleeding for these tests.

b) = <1:20

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TABLE 5. Results of neutralization tests with VBDD personnel sera

Last Name	MBV-B	TC-83	VEE Type							
			I, subtype					II	III	IV
			A	B	C	D	E			
Bowen	37	80	20	20	40	-	20	-	-	-
Carter	12	80	20	-	20	-	-	-	-	-
Combers	20	80	20	20	80	-	-	-	-	-
Cropp	19	20	-	-	20	-	-	-	-	-
Francy	42	40	-	-	-	-	-	-	-	-
Gravdahl	48	80	20	20	80	-	-	-	-	-
Gus	45	40	-	-	-	-	-	-	-	-
Hayes	29	40	-	-	-	-	-	-	-	-
McLean	45	20	-	-	-	-	-	-	-	-
Meek	22	80	-	-	40	-	-	-	-	-
Monath	8	20	-	-	-	-	-	-	-	-
Montoya	36	80	20	20	20	-	-	-	-	-
Muth	45	40	-	-	-	-	-	-	-	-
Poland	19	80	-	-	40	-	-	-	-	-
Robinson	19	40	-	-	-	-	-	-	-	-
Rush	44	80	20	-	20	-	-	-	-	-
Shriner	34	20	-	-	-	-	-	-	-	-
Smith	46	20	20	-	-	-	-	-	-	-
Strasser	11	80	-	-	-	-	-	-	-	-
Trimble	?	80	-	-	20	-	-	-	-	-
Vorndam	4	160	-	-	20	-	-	-	-	-

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REPORT FROM THE SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION,

MICROBIOLOGY AND INFECTIOUS DISEASES,

SAN ANTONIO, TEXAS

Togavirus-like viruses in tissues of New World Monkeys.

Concern for the possible importation of virus-carrying simians has prompted surveillance of various available species for the presence of viruses. Monitoring procedures include: serologic testing, virus isolations and electron microscopic examination of tissues. Seropositives, while present, do not provide data on "time" of infection and, therefore, do not necessarily imply a current infection. The recognition of togavirus-like viruses in tissues derived from cebus (C. appella) and marmoset (Callithrix jacchus) monkeys by electron microscopy suggests that monkeys and apes (and probably other animals as well) are frequently persistently infected with different viruses acquired in the wild as a consequence of animal contacts or in captivity from human or other animal contacts. As this was a retrospective study, virus isolations from the tissues concerned were not performed. These observations suggest the need to develop programs defining microbial (viral) base line data on imported animals in order to familiarize investigators with endogenous and exogenous viruses of "normal" animals.

(S.S. Kalter)

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

The following is our report of Arthropod-Borne Virus activity in Texas for July-December, 1975.

Mosquito Surveillance
July-December

Number of mosquito pools tested----------884
Number of mosquito pools positive----------9
Number of mosquito pools negative----------875
Number of mosquitoes tested----------16,199

Isolates	Mosquito Species	Location
Hartpark	C. quinquefasciatus	Cameron Co.
Hartpark	C. quinquefasciatus	Hidalgo Co.
Hartpark	C. quinquefasciatus	Hidalgo Co.
Hartpark	C. quinquefasciatus	Hidalgo Co.
WEE	C. tarsalis	El Paso
Hartpark	C. tarsalis	Wichita Falls
WEE	C. tarsalis	El Paso
SLE	C. quinquefasciatus	Dallas
Hartpark	C. tarsalis	Travis Co.

(Charles E. Sweet)

REPORT FROM THE DEPARTMENTS OF VETERINARY SCIENCE AND OF ENTOMOLOGY, UNIVERSITY OF WISCONSIN - MADISON

LaCrosse Virus Studies.

Work with LaCrosse (LAC) Virus variants continues. Homologous and heterologous plaque reduction neutralization of LAC prototype virus and plaque-purified LAC-large (LAC-l) and small (LAC-s) plaque variants and their antiserum were compared. No significant differences were found. Thermal stability of the prototype, LAC-1 and LAC-s were also compared. Rates of thermal inactivation were similar; at 39°C greater than 10^5 PFU were lost by 60 hrs. All three virus preparations were remarkably stable at 17°C; there was less than a 10-fold loss of infectivity at 119 hrs, suggesting that LAC virus could persist for several days at tree-hole temperatures. Further studies on survival of virus suspensions under tree hole conditions are planned.

During the past summer season, sentinel chipmunks and squirrels were maintained in an enzootic LAC virus area. Six squirrels and four chipmunks were infected, and LAC virus was recovered from their blood. No significant differences in mean plaque size were observed. A range of plaque sizes occurred in virus isolated from both species but the smaller plaque types predominated. These observations are in contrast to the rapid selection for the small plaque type in squirrels and for predominantly large plaque types in chipmunks infected with prototype LAC in the laboratory.

We had assumed that mouse or mosquito passage does not influence LAC virus plaque size. LAC prototype plaque size was unchanged from suckling mouse passage 3 (the earliest passage level available) through 6. However, mean plaque size of LAC virus assayed directly from the blood of two

viremic sentinel squirrels and one viremic sentinel chipmunk was significantly larger than the same viruses following one passage in suckling mice, indicating that one mouse passage can result in selection for a different virus population. Mean plaque size of field isolates of LAC were unchanged following one passage in Aedes triseriatus.

W. Hansen, T. Ksiazek, T. Yuill

Bocas Virus Studies.

Two colonies of hibernating little brown bats, Myotis lucifugus, were collected from abandoned iron mines in April, 1971, in Wisconsin. Interest in sampling centered on possible recovery of one or more viruses from this insectivorous bat. A pool of organs (brain, blood and brown fat) was made from each bat, triturated and inoculated intracerebrally into suckling mice. Of 88 bats sampled from one mine, seven isolates of Bocas virus were made. Although Bocas virus was not isolated from bats collected on another mine, rabies was recovered from one organ pool.

No more than 3 of 5 suckling mice inoculated with bat tissue suspensions sickened or died. On inoculation of suckling mouse brain from sick or recently dead to other 3-5-day-old suckling mice, a 100% death pattern was produced. Infectivity titers of mouse brain after 1 or more passages ranged from $10^{2.6}$ to $10^{4.2}$ $SMLD_{50}$/0.03 ml. inoculated intracerebrally.

Identification of one of the isolates as Bocas virus was made by Dr. Robert E. Shope at YARU by complement fixation techniques employing anti-Wisconsin bat ascitic fluid against antigens of more than 100 arbovirus antigens.

The history and characteristics of Bocas virus are recorded in the International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates, 1975, pp. 158-159.

R. Anslow, D. Watts, J. Spalatin and R. Hanson

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

Forty-five confirmed cases of arboviral encephalitis were diagnosed in Wisconsin during 1975, including 1 Western, 7 St. Louis, and 37 California encephalitis.

La Crosse arbovirus studies are focused on detection and measurement of virus activity and the natural cycle in forests where LAC virus overwinters by transovarial transmission in diapausing eggs and emerges throughout each season in Aedes triseriatus from basal tree-holes, old tires, and similar water holding containers in these deciduous forested hillsides.

The role of the vertebrate hosts, chipmunks and squirrels, is under study by comparison of antibody prevalence and acquisition rates with the distribution of virus in larvae, pupae and adult Aedes triseriatus.

Studies of the distribution of LAC virus in Aedes triseriatus are continuing in the laboratory with direct fluorescent antibody (FA) technique visualizing viral antigen in the tissues of smeared or dissected individuals.

Vertical transmission rates of LAC virus are so far similar in transovarially infected males (31%) and females (39%), with widespread distribution of virus, prior to emergence, in gut, salivary, and gonadal tissues.

Horizontal transmission of LAC virus has been observed from transovarially infected Aedes triseriatus males to females. So far this has been repeated in 4 of 7 mating cage trials averaging 14 males (F_3 generation from a recent field collection) and 50 females from a long established laboratory strain. The overall infection rate in inseminated females surviving for 18 days was 4 of 104 (3.8%), and in 1 of 92 (1.1%) of those without detectable sperm in their spermathecae. The means of this virus transfer is under study with induced insemination and other limited contact trials to determine more about the possible oral and/or venereal routes of transmission which might supplement horizontal transfer of LAC virus thru viremic mammals in nature.

Horizontal transfer of LAC virus through blood meals from viremic mammals is also under study in the laboratory. Two groups of Aedes triseriatus females (F_1 progeny field strain) were infected by feeding on virus in blood, one with and the other without a previous blood meal. The group with the previous blood meal had a higher infection rate and a shorter time to disseminated infection than the group without a previous blood meal. Time to disseminated infection was highly variable in both groups of this non-laboratory selected population.

(Wayne Thompson and Barry Beaty)

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE*, DEPARTMENT OF MEDICAL MICROBIOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF TORONTO, CANADA

Laboratory investigations on sera from an outbreak of encephalitis in humans in southern Ontario

During July, August and September, 1975, cases of encephalitis occurred among patients in the Windsor-Essex and Niagara regions of southern Ontario. Investigations were carried out to determine whether arboviruses might be responsible for the outbreak. Initially, sera from 27 patients were tested by haemagglutination-inhibition (HI) with antigens prepared from the following viruses: Eastern Equine Encephalitis, Western Equine Encephalitis, St. Louis Encephalitis, California Encephalitis and Powassan. The same sera were also tested by complement fixation (CF) with St. Louis and California Encephalitis antigens.

None of the sera reacted with California, Western or Eastern antigens but three gave low level reactions with Powassan. Fourteen sera reacted in both HI and CF with St. Louis and two additional sera were positive with St. Louis by CF only. Many of the sera that were positive by CF had high titres in the 64 - 512+ range. One patient showed a rise in titre to St. Louis antigen.

By the end of the year 476 patients from different parts of the Province of Ontario had been investigated. The results are shown in the Table. None of the patients' sera reacted with EEE or WEE. Thirty-three patients' sera reacted with California by HI. None of these sera were positive for California by CF and a rising titre was not demonstrated by HI in any of the patients who were positive for California. Twelve sera gave reactions with Powassan. These are considered to be cross-reacting antibodies which appeared following St. Louis infection. Eighty-two sera gave positive reactions with St. Louis. Using accepted criteria for diagnosis 65 patients were considered to have St. Louis Encephalitis. The remaining 17 positive sera were of low titre and may be false-positive reactions or evidence of past Group B virus infection.

* A Laboratory operating in conjunction with the Laboratory Centre for Disease Control - Department of National Health and Welfare, Ottawa.

There were five fatal cases. Three were investigated and St. Louis virus was isolated from the brain of one fatal case. Three cases, including the one from whom virus was isolated, showed typical histopathological changes of viral encephalitis.

St. Louis antibody has been detected in birds and domestic chickens in the epidemic area. A small number of mosquito pools was tested for virus with negative results.

SLE virus was first isolated in Canada from *Culex tarsalis* mosquitoes collected in Weyburn, Saskatchewan in 1971. Antibody surveys provided evidence for the past presence of SLE virus in Manitoba, Alberta and British Columbia. The outbreak in Ontario is the first recognized outbreak of SLE in Canada and may reflect a northern extension of the large SLE outbreak that occurred in the United States during the past year. This is the first recorded ourbreak of arbovirus disease in humans in the Province of Ontario.

TABLE

PATIENTS FROM THE PROVINCE OF ONTARIO WITH SUSPECT ENCEPHALITIS INVESTIGATED FOR EVIDENCE OF ARBOVIRUS INFECTION

ANTIGEN	HI	CF
	POS / TEST	POS / TEST
Eastern equine	0 / 476	-
Western equine	0 / 476	-
California	33 / 476	0 / 33
Powassan	12 / 476	-
St. Louis	82 / 476	65 / 82

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

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

Arbovirus Summary - 1975

California encephalitis (CE) group viruses and Powassan virus continued to be endemic in various parts of the state (Tables 1 and 2). CE virus activity was again apparent in the Capital District area by the occurrence of one human case of CE, serum conversions in two sentinel rabbits (Arthropod-borne Virus Information Exchange No. 29, September '75) and isolation of CE virus from Aedes species.

In three patients with encephalitis, an etiology of Powassan virus was confirmed. A dog of the family where the fatal case of encephalitis occurred (Table 2, case No. 2) had hemagglutinating (1:20) and neutralizing (NI 2.3) antibody to Powassan virus.

Eastern equine encephalitis (EEE) was again detected in Oswego County when four equine deaths occurred in July and August. EEE virus was isolated from the brain tissue of two of these animals, and two others, from which no isolate was obtained, had high-titered HI antibody to the virus.

For the first time in New York State, infections with St. Louis encephalitis (SLE) virus were confirmed in seven patients (Table 2). Six of these patients resided in the Western part of the state. The resident of Westchester County with SLE had returned 2 days prior to his onset from Ohio and presumably contracted the disease there.

Cache Valley and Flanders were the most frequently encountered arboviruses without established role as human pathogens in New York State (Table 1).

(Margaret A. Grayson, John P. Woodall* and Rudolf Deibel)

*Present address: San Juan Labs., Bureau of Labs. CDC, GPO Box 4532, San Juan, Puerto Rico 00936

Table 1

Arboviruses Isolated from Mosquitoes in New York State, 1975

Virus	No. of Strains	County	Month	Species	Infection Rate
California encephalitis complex	1	Essex	June	Ae. communis	1:1230
	1	Suffolk	Aug.	Ae. sollicitans	1:169
	1	Schuyler	June-July	Ae. stimulans	1:785
	1	Schuyler	Aug.	Ae. vexans	1:1759
	1	Albany	Aug.	Ae. spp.	--
	2	Suffolk	Aug.-Sept.	Mixed mosquitoes	--
Cache Valley	1	Suffolk	Sept.	Ae. sollicitans	1:588
	2	Monroe	Aug.	Ae. stimulans	1:84
	1	Monroe	Aug.	Ae. vexans	1:737
	1	Chemung	Aug.	An. walkeri	1:1707
	1	Monroe	Aug.	Anopheles spp.	--
	1	Albany	Sept.	Culex pipiens	1:22
Flanders	2	Chemung	July	Coq. perturbans	1:4525
	1	Chemung	June	Culex pipiens	1:450
	6	Chemung	July	Culex pipiens	1:1039
	3	Chemung	Aug.	Culex pipiens	1:1976
	1	Monroe	Aug.	Culex pipiens	1:143
	19	Schuyler	July-Aug.	Culex pipiens	1:358
	1	Schenectady	July	Culex spp	--
	1	Oswego	Aug.	Culiseta melanura	1:44
	1	Chemung	Aug.	Culiseta minnesotae	1:420
	7	Chemung	July-Aug.	Culiseta morsitans	1:262
	1	Oswego	July	Culiseta spp.	--
	3	Suffolk	Aug	Mixed mosquitoes	--
	1	Suffolk	Sept.	Mixed mosquitoes	--

Table 2

Human Arbovirus Infections, New York State, 1975

Cases: 1-9 confirmed diagnostic

10-19 presumptive diagnostic

Case # County	Age (Years)	Sex	1975 Date of Onset	Diagnosis	Agent Indicated	Days after Onset	Antibody Titer CF	HI	NI
1 Oswego	8 mos.	F	8.2	Meningitis	California encephalitis group	3	<4	20	No sera left
						30	8	20	
2 Oneida	82	M	6.13	Meningo-encephalitis, died. Included in Sept. '75 report #29.	Powassan Powassan virus isolated from brain	7	4	40	No serum left
3 New York City	6	M	7.1	Meningo-encephalitis, tick acquired in Oswego Co. 6.26	Powassan	10	<4	20	Pos.
						57	8	80	Pos.
4 Herkimer	10	M	9.9	Encephalitis	Powassan	8	<4	<10	No sera left
						30	<4	80	
5 Erie	25	F	8.6	Meningitis	St. Louis encephalitis	1	<4	10	No sera left
						28	ND	80	
						55	16	80	
6 Seneca	66	F	8.15	Encephalitis	St. Louis encephalitis	5	ND	160	Pos.
						19	4	320	Pos.
						28	16	160	2.5
7 Erie	53	F	8.20	Encephalitis	St. Louis encephalitis	2	ND	20	No sera
						11	<4	80	
						35	8	80	

Human Arbovirus Infections, New York State, 1975 (continued)
Cases: 1-9 confirmed diagnostic
10-19 presumptive diagnostic

Case # County	Age (Years)	Sex	1975 Date of Onset	Diagnosis	Agent Indicated	Days after Onset	Antibody Titer CF	HI	NI
8 Westchester	17	F	9.16	Meningitis, arrived from Ohio 9.14	St. Louis encephalitis	1	<4	10	3.6
						9	8	80	3.9
						14	8	80	4.0
9 Niagara	50	F	9.1	Meningitis	St. Louis encephalitis	16	<4	20	3.3
						40	<4	80	4.1
10 Niagara	20	F	8.24	Encephalitis	St. Louis encephalitis	14	8	40	3.3
						24	8	40	3.9
						39	8	20	4.4
11 Erie	27	F	9.4	Encephalitis	St. Louis encephalitis	19	8	80	3.6
						50	4	40	No serum left
12 Monroe	25	M	8.31	Encephalitis	California encephalitis group	3	ND	10	4.0
13 Suffolk	28	F	8.17	Meningitis	California encephalitis group	3	ND	10	3.5
14 Erie	33	M	Not given	Erythema multiforme	California encephalitis group	Coll. 9.29	ND	10	4.2
15 Suffolk	50	F	9.25	Encephalitis	California encephalitis group	5	ND	10	4.0
16 Erie	17	M	9.22	Meningitis	California encephalitis group	16	<4	40	No serum left
						101	ND	ND	4.1

Human Arbovirus Infections, New York State, 1975 (continued)
Cases: 1-9 confirmed diagnostic
10-19 presumptive diagnostic

Case # County	Age (Years)	Sex	1975 Date of Onset	Diagnosis	Agent Indicated	Days after Onset	Antibody Titer CF	HI	NI
17 Broome	26	F	10.18	Encephalitis	California encephalitis group	7	ND	10	4.3
						11	ND	10	3.4
18 Seneca	7	F	10.8	Meningitis	California encephalitis group	3	ND	10	3.6
						25	ND	20	3.1
19 Albany	28	F	Not given	Meningitis	California encephalitis group	Coll. 11.3	ND	20	3.8

CF: complement-fixation
HI: hemagglutination-inhibition
NI: neutralizing index $\log_{10}$
Pos: reacting in screen neutralization test
ND: not done

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

As a base line for comparisons of chemical and physical properties of virulent and benign VE viruses, selected hamster-virulent (69Z1) and benign strains (BeAr35645 (Pixuna) and TC83) were cloned in primary hamster embryonic cell cultures and analyzed for defective interfering particles. These particles were not found, and the virulent and benign characteristics of the strains were maintained after cloning.

Percentages of cultured primary hamster embryonic cells productively infected with the one hamster-virulent and two -benign strains of VE virus were similar (1 of 3.6, 1 of 2.6 and 1 of 1.1 cells).

Polypeptide compositions of a virulent strain (69Z1) and a benign strain (TC83 vaccine) were also similar and showed four separable polypeptides of about 56,000, 52,000, 50,000 and 24,000 daltons. For the TC83 strain, the first three polypeptides were associated with viral envelopes and were glycosylated, whereas the 24,000 dalton polypeptide was not glycosylated. Polypeptides of another benign strain (BeAr35645, Pixuna) were like the above two viruses except that the 56,000 dalton polypeptide was absent. In extracts of primary hamster embryonic cells from cultures infected with TC83 strain, an additional polypeptide was found with a molecular weight of about 80,000 daltons.

There were no significant differences in phospholipid compositions between virulent strain 69Z1 and benign strain TC83 vaccine. Pixuna strain seemed to have slightly more phosphatidyl-choline and less phosphatidyl-ethanolamine.

Vector competence as a possible virulence marker of VE virus was explored further with three epizootic South American strains and colonized Aedes aegypti mosquitoes. Transmission rates ranged from 55 to 86%.

Temperature sensitive mutants of hamster-virulent, enzootic strain of VE virus (68U201) have been obtained by treatment of virus with nitrosoguanidine, a potent alkylating mutagen.

Experiments with primary cultures of hamster bone marrow cells suggested that hamster-virulent and -benign strains multiply in small percentages of cells, and that if cell destruction is occuring, it is camouflaged by the sizes of the total populations and by spontaneous deterioration of cells in cultures.

A hamster-virulent strain (63U2) and a benign strain (TC83 vaccine) adsorb to hamster peritoneal macrophages and grow well in macrophage cultures, but adsorption of benign strain BeAr35645 (Pixuna) could not be detected, and growth either did not occur or was much less than growth of the other two strains.

Although Pixuna and TC83 strains are normally avirulent

for mice inoculated subcutaneously, they killed 40 and 20% of athymic nude mice.

In search of natural sources of equine-virulent VE virus in Central America, young horses have been monitored as sentinels along the Pacific coast of Nicaragua. During 1973-1974, no unequivocal instance of antibody development occurred. Strains of VE virus isolated from enzootic foci in Guatemala during 1969-1971 were examined for virions capable of producing small plaques in Vero African green monkey kidney cell cultures. Since this characteristic had been shown to be associated with equine-virulent, epizootic Central American VE virus, three cloned strains and one isolate that produced uniformly small plaques were tested for horse pathogenicity by subcutaneous inoculation. The uniformly small plaque strain from 1969, isolated at an enzootic habitat approximately 35 km northwest of the epicenter of the 1969 outbreak in Guatemala, was virulent for horses since it uniformly produced fever and viremias of significant magnitude and caused encephalitis in two of four horses. Two cloned strains also produced fever and encephalitis, and one strain killed a nursing colt following inoculation. However viremia titers were lower than usually found with epizootic strains, and thus it seemed that the three cloned viruses had virulences like enzootic isolates even though they possessed the characteristic of small plaques in Vero cell cultures.

Short-incubation, virus-dilution hemagglutination-inhibition tests of strains of VE virus from the Americas using early rooster antibodies made to them and hemagglutinins made in primary chicken embryonic cell cultures revealed two interrelated antigenic clusters with one strain linking the two groups. Since some results are at variance with the currently-used subtype classification of VE virus based on antibodies from spiny rats and Vero cell culture antigens, these studies are being continued.

In 1973, chimpanzees were inoculated as part of a collaborative project, with dengue virus types 1-4. Viremias occurred and antibodies developed, but no disease was detected. The chimpanzees were again inoculated in March 1975 with dengue virus type 2, and no overt disease or febrile or hematologic manifestations occurred. Viremias and antibody responses are being measured.

A strain of virus (70U39) isolated several times from sentinel hamsters exposed during 1970 and 1972 on the Pacific coast of Guatemala was found by Dr. R. Shope and associates at Yale University to be related to Minatitlan virus originally isolated in Mexico. Further neutralization tests have shown that 70U39 is Minatitlan virus.

(W.F. Scherer)

REPORT FROM THE DIVISION OF LABORATORIES AND EPIDEMIOLOGY, STATE OF NEW JERSEY DEPARTMENT OF HEALTH, TRENTON, NEW JERSEY

The following tabulations show our findings concerning arboviruses and arboviral disease in New Jersey for the third and fourth quarters of 1975 (July 1 through September 30, 1975 and October 1 through December 31, 1975, respectively).

(Martin Goldfield)

Summary: 1975 Mosquito Pool Isolates

Number of Mosquitoes Collected: 54,041

Number of Pools Tested: 1,322

		Virus Isolates	
Species	No. of Isolates	EE	WE
A. cana.	1	1	-
A. tris.	1	1	-
A. vex.	1	-	1
C. mel.	41	25	16
C. pip.	1	-	1
C. sal.	3	1	2
C. terr.	1	1	-
Totals	49	29	20

Group	Virus and No. of Strains EE	WE	Isolated from	Collected in	Month	Year
		1	C. salinarius	Bass River	July	1975
A		3	C. melanura	Bass River	July	1975
A	1		C. melanura	Bass River	August	1975
A	8		C. melanura	Burlington County	August	1975
A		1	C. melanura	Burlington County	August	1975
A		1	A. vexans	Burlington County	August	1975
A		1	C. pipiens	Hudson & Bergen	August	1975
A	2		C. melanura	Ocean County	August	1975
A		1	C. melanura	Ocean County	August	1975
A	1		A. canadensis	West Windsor	August	1975
A	1		A. triseriatus	West Windsor	August	1975
A	2		C. melanura	Woodbine	August	1975
A		1	C. melanura	Woodbine	August	1975
A	2		C. melanura	Bass River	September	1975
A	1		C. salinarius	Bass River	September	1975
A	1		C. territans	Bass River	September	1975
A	4		C. melanura	Burlington County	September	1975
A		5	C. melanura	Burlington County	September	1975
A	1		C. melanura	Ocean County	September	1975
A		1	C. melanura	Ocean County	September	1975
A	3		C. melanura	Woodbine	September	1975
A		2	C. melanura	Woodbine	September	1975
A		1	C. salinarius	Woodbine	September	1975
Totals	27	18				

Isolations from Arthropods in New Jersey

Group	Virus and No. of Strains		Isolated from	Collected in	Month	Year
	EE	WE				
A	2		C. melanura	Woodbine	October	1975
A		2	C. melanura	Woodbine	October	1975
Totals	2	2				

Isolations from Avians in New Jersey

Location	Species	Date Specimen Collected	Isolation Data		
			Brain	Blood	Virus
Brigantine	Catbird	8-12-75		X	WE
Brigantine	Blue Jay	8-14-75		X	EE
Brigantine	Catbird	8-15-75		X	WE
Belleplain	Blue Grosbeak	8-18-75		X	WE
Belleplain	N. Water Thrush	8-18-75	X	No Blood	WE
Mercer County Park	Unknown Warbler	8-19-75		X	EE
Belleplain	Catbird	8-19-75		X	WE
Belleplain	Cardinal	8-20-75		X	WE
Belleplain	Catbird	8-20-75		X	WE
Belleplain	Catbird	8-20-75		X	EE
Brigantine	Catbird	8-26-75	X	No Blood	WE
Belleplain	Ovenbird	8-27-75		X	EE
Mercer County Park	Catbird	8-28-75		X	WE
Belleplain	Ovenbird	9-2-75		X	EE
Belleplain	Verry	9-3-75		X	EE
Belleplain	Ovenbird	9-3-75		X	WE
Belleplain	Catbird	9-4-75	X	No Blood	EE
Belleplain	Ovenbird	9-10-75		X	EE
Brigantine	Catbird	9-11-75		X	WE
Totals	19	---	3	16	

Isolations from Animals in New Jersey

Location	Animal Species	Date Specimen Collected	Isolation Data	
			Specimen	Virus
Williamston	Pheasant	8-15-75	Brain and Amniotic fluid	EE
Forked River	Pheasant	8-20-75 8-26-75	Brain	EE
Titusville	Chukkar	9-4-75	Brain suspension Allantoic fluid	EE
Franklinville	Diamond Dove	9-4-75	Brain suspension Allantoic fluid	EE
Chesterfield	Pheasant	9-19-75	Brain	EE
New Egypt	Pheasant	9-15-75	Egg fluid	EE
Trenton	Pheasant	9-17-75	Brain suspension	EE
Fort Dix	Pheasant	8-1-75	Brain	EE*

*Diagnosed by Dr. Tudor at Rutgers.

ISOLATION AND/OR SEROLOGY OF ANIMALS IN NEW JERSEY — 3rd Quarter 1975

Location	Animal Species	Date Specimen Collected	Isolation Data		Serology Data				Pathology
			Organs	Blood	HI		CF	Neut	
Salem, Salem County	Equine	8 - 3 - 75	No Brain Submitted	Negative	B1	320	<4	ND	No Brain Submitted
Alloway Salem County	Equine	8 - 7 - 75	Brain - EE	Negative	B1	160	<4	ND	Positive
Williamstown Gloucester County	Equine	8 - 11 - 75	Brain - Neg.	Negative	B1 B2	640 1280	<4 <4	ND ND	Positive
Monroeville Salem County	Equine	8 - 18 - 75	Brain - EE	Negative	B1	40	<4	ND	Positive
Quinton Salem County	Equine	8 - 17 - 75	No Brain Submitted	Negative	B1	640	<4	ND	No Brain Submitted
Franklinville Gloucester County	Equine	8 - 19 - 75	No Brain Submitted	Negative	B1	5120	>32	ND	No Brain Submitted
Williamstown Gloucester County	Equine	8 - 20 - 75	Brain - EE	Negative	B1 B2	160 320	<4 <4	ND ND	Positive
Shilo Cumberland County	Equine	8 - 10 - 75	No Brain Submitted	Negative	B1 B2 B3 B4	1280 2560 2560 >10,240	<8 <4 <4 >32	ND ND ND ND	No Brain Submitted
Tabernacle Burlington County	Equine	8 - 20 - 75	Brain - EE	No Blood Submitted	—		—	—	Positive
Atco C Camden County	Equine	8 - 21 - 75	Brain - EE	No Blood Submitted	—		—	—	Positive
Pennsgrove Salem County	Equine	8 - 22 - 75	No Brain Submitted	Negative	B1	2560	ND	ND	No Brain Submitted
Williamstown Gloucester County	Equine	8 - 21 - 75	Brain - EE	No Blood Submitted	—		—	--	Positive
Atco Camden County	Equine	8 - 20 - 75	No Brain Submitted	Negative	B1	320	ND	ND	No Brain Submitted
Franklinville Gloucester County	Equine	8 - 26 - 75	Brain - EE	Negative	B1 B2	640 2560	ND ND	ND ND	Positive
Jackson Ocean County	Equine	8 - 27 - 75	No Brain Submitted	Negative	B1 B2	2560 640	128 128	ND ND	No Brain Submitted
Milton, Delaware*	Equine	9 - 2 - 75	Brain - Neg.	Negative	B1 B2	640 1280	<4 A/C	ND ND	Positive

*Included is one horse from the State of Delaware

Location	Animal Species	Date Specimen Collected	Isolation Data		Serology Data				
			Organs	Blood		HI	CF	Neut	
Center Square Gloucester County	Equine	9 - 2 - 75	Brain - EE	Negative	B1	1280	<4	ND	Positive
Blue Anchor Camden County	Equine	9 - 2 - 75	Brain - EE	Unsatisfactory		-	-	-	Positive
Quinton Salem County	Equine	8 -30 - 75	Brain - EE	Negative	B1	20	<4	ND	Positive
Whiting Ocean County	Equine	9 - 1 - 75	No Brain Submitted	Negative	B1 B2	160 640	<4 <4	ND ND	No Brain Submitted
Whiting Ocean County	Equine	9 - 1 - 75	Brain - EE	Negative	B1 B2	160 640	8 8	ND ND	Positive
Medford Burlington County	Equine	8 -30- 75	No Brain Submitted	Negative	B1	640	A/C	ND	No Brain Submitted
Jackson Ocean County	Equine	9 - 3 - 75	Brain - EE	Negative	B1 B2	160 640	A/C A/C	ND ND	Positive
Monroeville Salem County	Equine	9 - 3 - 75	Brain - EE	Negative	B1	2560	<4	ND	Positive
Monroeville Salem County	Equine	9 - 5 - 75	Brain-Neg.	Negative	B1	10	ND	ND	Positive
Berlin Camden County	Equine	9 - 6 - 75	No Brain Submitted	Negative	B1	5120	16	ND	No Brain Submitted
Elmer Salem County	Equine	9 - 9 - 75	Brain - EE	Negative	B1	1280	<4	ND	Positive
Pemberton Burlington County	Equine	9 -14 - 75	Brain -Neg.	Negative	B1	10	<4	ND	Positive
Pennsgrove Salem County	Equine	9 -15 - 75	No Brain Submitted	Negative	B1	320	<4	ND	No Brain Submitted
Jackson Ocean County	Equine	9 -17- 75	No Brain Submitted	Negative	B1	640	ND	ND	No Brain Submitted
Salem Salem County	Equine	9 -22 - 75	No Brain Submitted	Negative	B1	1280	8	ND	No Brain Submitted

Isolation and/or Serology Animals in New Jersey

Location	Animal Species	Date Specimen Collected	Isolation Data		Serology Data			Pathology
			Organs	Blood	HI	CF	Neut	
Hancocks Bridge Salem County	Equine	10-2-75	Brain-EE	Negative	B_1 160	8	ND	Positive
Richland Cumberland County	Equine	10-5-75	No Brain Submitted	Negative	B_1 1280	ND	ND	No Brain Submitted
Glassboro Gloucester County	Equine	10-10-75	Brain-EE	No Blood Submitted	-------	--	--	Positive
Tuckerton Ocean County	Equine	10-10-75	No Brain Submitted	Negative	B_1 1280 B_2 5120	16 64	ND ND	No Brain Submitted
Mt. Laurel Camden County	Equine	10-28-75	Brain-EE	Negative	B_1 160 B_2 320	ND ND	ND ND	Positive

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

Chromatographic separation of Venezuelan and Western encephalitis virus subtypes.

We have recently learned that hydroxylapatite chromatography is a useful tool for distinguishing between epizootic and enzootic Venezuelan encephalitis (VEE) viruses of antigenic subtype I. We have also successfully applied this technique to separate plaque variants from virus populations which initially appeared to be homogeneous with respect to plaque morphology.

VEE virus strains listed in the table were grown in BHK-21 cells and intrinsically labeled with ^{32}P as described previously (1). Columns were prepared using a slurry of commercially obtained hydroxylapatite, (Bio-Rad HTP, lot#14607). The slurry, in 0.05M Na_2HPO_4/NaH_2PO_4 buffer, pH 7.0 (starting buffer), was poured into a column 0.9 cm in diameter, and washed with starting buffer until a constant bed volume of 10.0 ml was obtained. The ^{32}P-labeled virus preparations were diluted in starting buffer to contain approximately 10^9 pfu in 0.5 ml, the volume applied to the column. After the virus inoculum suspensions had entered the column, a linear gradient of elution buffer, 0.05 to 0.50M phosphate, pH 7.00, was applied. The total elution volume was 100 ml, collected in twenty 5-ml fractions. To assay ^{32}P activity, 0.2 ml of each fraction was dissolved in scintillation fluid as described (1). For these preliminary studies, only those fractions containing peak ^{32}P activities were assayed for pfu on duck embryo cell monolayers under medium containing 1% agarose.

Figure 1 depicts the elution profiles for the 8 VEE stains tested, and the table itemizes the phosphate molarities and plaque morphologies of the elution peaks. The elution peaks for the epizootic VEE strains

Phosphate molarity and plaque morphology of elution peaks

VEE subtype	VEE strain	Phosphate molarity (plaque morphology)[1] Major peak	Minor peak
I-A	Trinidad	0.33 (L)	0.10 (L)
I-B	69Z1	0.418 (S)	0.08 (L)
I-B	PTF-39	0.417 (S)	0.10 (L)
I-C	P676	0.430 (S)	0.05 (L)
I-D	3880	0.05 (L)	0.168 (MIX)
UNCLASSIFIED	Tumaco	0.05 (L)	0.400 (MIX)
I-E	68U201	0.05 (L)	"tails" (MIX)
I-D	Magangue	0.359 (MIX)	0.078 (L)

[1] L = large plaques > 5 mm in diameter after 48 hr incubation.
S = small plaques < 2 mm in diameter after 48 hr incubation.
MIX = mixture of L & S plaques in approximately equal proportions.

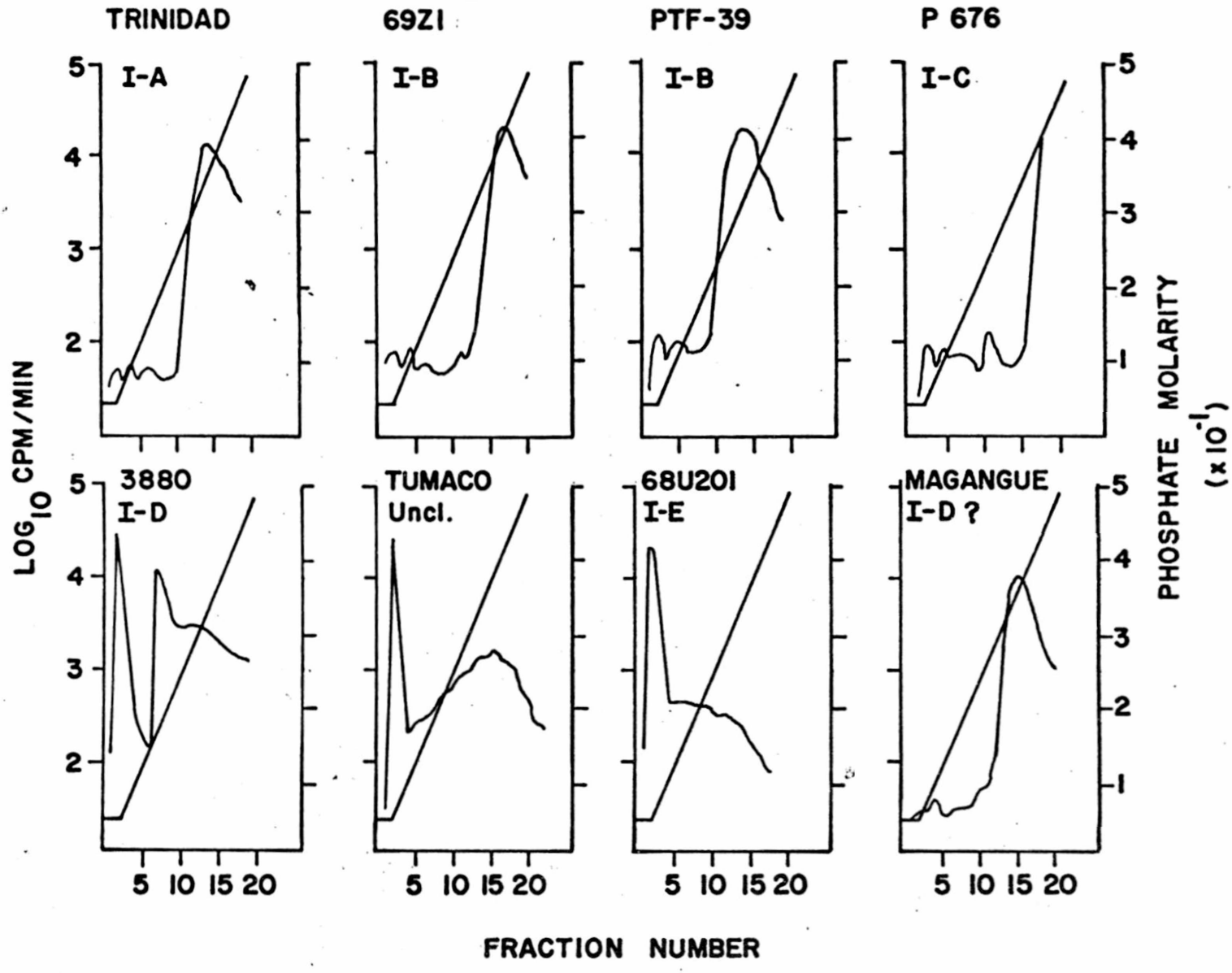
TRINIDAD
69ZI
PTF-39
P 676
I-A
I-B
I-B
I-C
3880
I-D
TUMACO
Uncl.
68U201
I-E
MAGANGUE
I-D ?
LOG10 CPM/MIN
PHOSPHATE MOLARITY
(x10-1)
FRACTION NUMBER
5 10 15 20

FIGURE 1

from subtypes IA, IB, and IC corresponded to relatively high phosphate molarities. Minor peaks at low phosphate molarities were also detected for strains 69Z1, PTF-39, and P676; large plaque virus was isolated from the minor but not the major peaks. However for the Trinidad strain, the viruses isolated from both the major and minor peaks were uniformly large plaque. In contrast to the epizootic viruses, enzootic strains yielded more erratic elution profiles. For 3 of the strains tested (3880, Tumaco, and 68U201), the major peak occurred immediately after the void volume, at 0.05M, and was uniformly large plaque. However, for these 3 viruses there was a minor peak, or "tail," which yielded a mixture of large and small plaques. The fourth strain, Magangue, while apparently most closely related to the I-D strains (2), yielded a pattern more closely related to the epizootic strains, although the elution peak contained a mixture of plaque types. Since Magangue virus produced relatively high viremias in horses (2), similar to epizootic strains, the similar elution patterns obtained for Magangue and the epizootic strains may reflect similar surface properties of these strains which in turn may be related to their ability to maintain high level viremias in horses.

The elution profiles depicted in figure 1 were reproducible, provided that identical chromatographic procedures were employed for each run. We do not know if the conditions employed are the optimal conditions required to resolve all virus subpopulations in the stock strains tested. Hydroxylapatite preparations differ in their ability to resolve virus peaks. Also critical is the volume elution buffer relative to bed volume, the pH of the buffer, and the steepness of the gradient.

Western encephalitis strains could also be differentiated using hydroxylapatite chromatography. A virulent strain of WEE, 72V4768, and an avirulent strain, CM4-146, (isolated from Oeciacus vicarius, a cliff swallow "bug") were obtained in first Vero cell passage from Dr. Tom Monath and Dr. Charles Calisher, CDC, Ft. Collins. To label these viruses with ^{32}P, they were passed once in BHK-21 cells. Like the first Vero passage virus stocks, the ^{32}P-labeled 72V4768 produced uniformly large (8-10 mm) plaques and CM4-146 produced uniformly minute (<1.0 mm) plaques on duck cells. The ^{32}P-labeled virus stocks were chromatographed, figure 2. For strain 72V4768, two peaks were obtained corresponding to 0.05 and 0.18M phosphate. The first peak contained only large plaque (8-10 mm) virus, the second a mixture of large and intermediate (3-5 mm) plaques. For strain CM4-146, the major elution peak correspond to 0.40M phosphate and contained only minute plaques (1.0 mm); however, early fractions contained large (8-10 mm) plaques, and fraction 10 contained intermediate plaques. To determine if chromatography could also detect a subpopulation of large plaque virus in unpassaged, avirulent WEE isolates, Dr. Calisher sent us the original unpassaged isolates CM4-146, 4-977, and 4-975. We applied 0.5 ml of each isolate, diluted 1:10 in starting buffer, to newly packed columns, and titrated each fraction. By this technique we recovered large plaque virus from strains CM4-146 and 4-977, but not from 4-975, and an intermediate plaque from 4-977. We are presently testing these isolates for virulence, pH-HA optima, and reversion frequency, and attempting to calculate the ratio of large-to-

minute plaques in these populations. However, it appears that the high reversion rate of uncloned avirulent WEE "bug" isolates (3) may be related to the existence of a large plaque subpopulation in the original virus population as it was isolated from the bug.

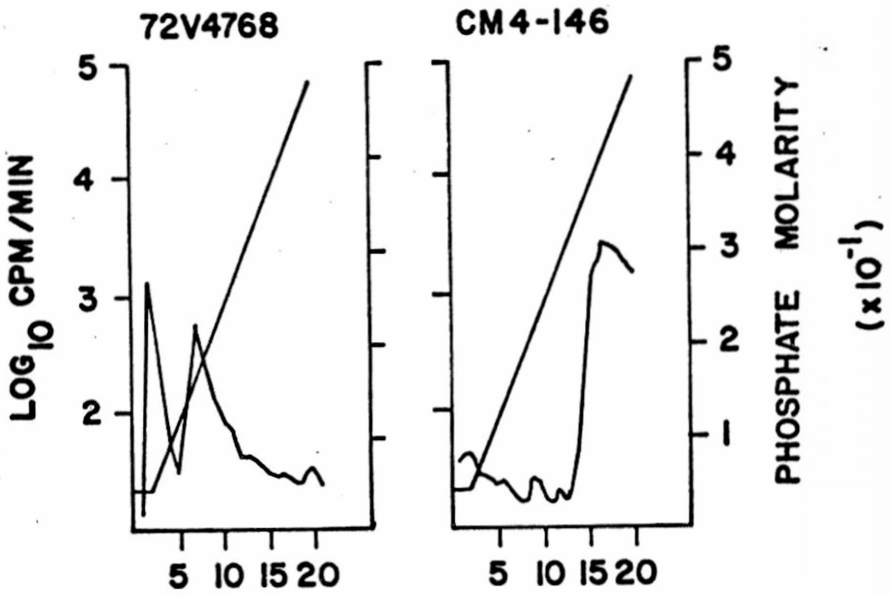

FIGURE 2.

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(Peter B. Jahrling)

Observations on the response of monkeys to intranasal challenge with Japanese encephalitis virus

The clinical course of infection and lethal response were determined for 2 species of monkeys after intranasal (IN) inoculation of the Peking strain of Japanese encephalitis (JE) virus. Clinical signs, rectal temperatures, total and differential blood leukocyte counts, hematocrits, and pathologic lesions were determined following challenge.

Two rhesus (Macaca mulatta) and 2 cynomolgus (M. fasicularis) monkeys were chemically restrained with ketamine hydrochloride and placed in dorsal recumbency. Each monkey was administered IN 0.5 ml of a suckling mouse brain suspension of JE virus containing 3×10^7 plaque-forming units (pfu). Half the inoculum (0.25 ml) was instilled dropwise with a tuberculin syringe into each nostril. All monkeys developed signs of JE virus encephalitis. The time of onset and clinical signs observed in the monkeys following IN challenge were similar (Fig. 1). Clinical signs and the number positive/total included fever (4/4), depression (4/4), anorexia (4/4), tremors (3/4), paralysis (4/4), and coma (4/4). One cynomolgus monkey developed a fever on day 2 postinfection which subsided and did not recur until day 5, after which time both monkeys were febrile. One rhesus died on day 11 and a second, on day 12, whereas 1 cynomolgus monkey died on day 11 and the other was sacrificed on day 12 when moribund. Leukocytosis accompanied by a moderate increase in mean neutrophil count was noted midway during the course of infection.

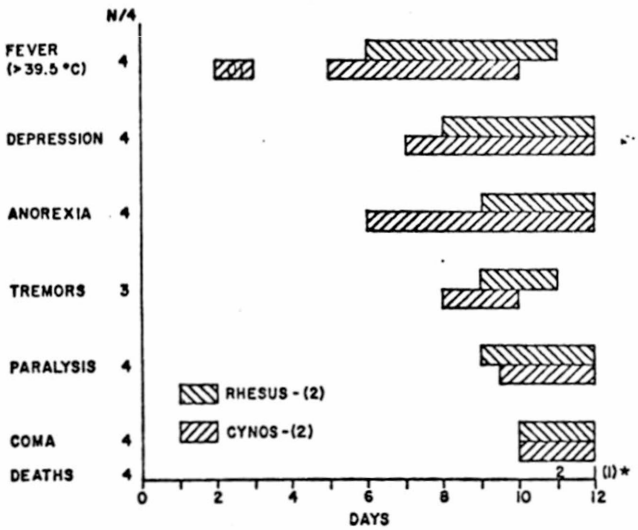

FIGURE 1. CLINICAL SIGNS OF JE VIRUS-INFECTED MONKEYS
*One monkey was sacrificed.

Necropsies were performed on all monkeys. Gross observations revealed only moderate emaciation and dehydration. Characteristic microscopic lesions of JE virus infections were observed in the central nervous system of all monkeys. The most significant microscopic changes were noted in the brain stem and spinal cord, with lesser involvement of the cerebellum and cerebrum. Although the number of monkeys comprising the study was small, no species-related differences in response to a large challenge dose of JE virus was evident.

In a subsequent experiment, an IN MLD_{50} equivalent to 2.5×10^4 pfu of the Peking strain of JE virus was determined with 16 cynomolgus monkeys. A graded dose response manifested by clinical signs and mortality was observed (Table 1).

Table 1. JE Virus Monkey IN MLD_{50} Determination

Virus Dose (pfu)	Febrile By Day	Dead/Total (Day of death)
4×10^6	6	2/2 (14, 14)
4×10^5	7	3/3 (15, 15, 17)
4×10^4	9	1/3 (13)
4×10^3	7	1/3 (18)
4×10^2	-	0/3
4×10^1	-	0/2

All of the 5 monkeys in the 2 highest virus-dose groups died, whereas only 2 of 6 in the intermediate dose groups, and none of the 5 in the lowest dose groups, developed clinical signs of encephalitis or died following IN challenge.

(Donald G. Harrington, Michael R. Elwell, and Duane E. Hilmas)

The use of attenuated Venezuelan equine encephalomyelitis (VEE) vaccines in man: A review

Attenuated VEE vaccines have been available since 1959 for the immunization of at-risk laboratory and field workers. This article summarizes the efficacy and limitations of these vaccines in over 5000 inoculated persons. Six different passage levels of attenuated VEE vaccine have been produced and studied at USAMRIID. Most information is available concerning human responses to the 83rd passage which constitutes the vaccine that has been utilized in most laboratories. The TC-83 vaccine elicits demonstrable neutralizing antibody responses in 84 to 94% of vaccinees; this variability may relate to the vaccine lot and/or the group of individuals receiving the vaccine. Three lines of evidence suggest that attenuated VEE vaccines provide immunity to VEE infections in man: (1) resistance to challenge with Trinidad strain VEE virus after vaccination with TC-80 (McKinney et al., Am. J. Trop. Med. Hyg. 12:597, 1963); (2) lack of detectable antibody response to revaccination performed one year after primary vaccination with TC-83; and (3) a virtual disappearance of clinically apparent, laboratory-acquired virulent VEE infections after the initiation of routine vaccination with attenuated VEE vaccines at Ft. Detrick.

Evidence from USAMRIID and several collaborting laboratories suggest that the protection afforded humans by TC-83 vaccine is directed chiefly against those VEE virus strains (I-A, I-B, I-C) which are closely related to the vaccine subtype (I-A), and that protection against more distantly related strains (e.g. I-D, I-E, III) may be less solid. High neutralizing antibody titers to VEE strains I-A, I-B, I-C persisted for at least 6-9 years in 93-100% of 20 vaccinees, but only low titers persisted to more distantly related VEE subtypes in 0-50% of these vaccinees. Antibody titers (HI and PRN) to TC-83 virus in a previously vaccinated individual may be boosted by infection with a more distantly related VEE virus subtype, but boosted poorly or not at all by vaccination with combined inactivated Eastern and Western equine encephalomyelitis vaccines.

Reactions to vaccination, characterized by febrile, influenza-like illnesses, occur in 15-37% of TC-83 vaccinees; severity sufficient to cause absence from work occurs in 10-15%. Minor local reactions to vaccination were reported by less than 5% of vaccinees. There is no evidence for specific viscero-or neurotropism of TC-83 vaccine. Previously reported electrocardiographic changes of volunteers after vaccination (Alevizatos et al., Am. J. Trop. Med. Hyg. 16: 762, 1967) have been shown to be identical to changes observed in the electrocardiograms of unvaccinated controls. Allergic reactions to vaccination have not been identified.

A close relationship exists between immunogenicity and reactogenicity in individual vaccinees. Significantly higher HI and PRN antibody titers were observed in vaccinees who developed febrile responses to vaccination than in afebrile vaccinees.

Individual cases have been recorded that suggest a temporal relationship between VEE vaccination and (1) abortion, (2) teratogenesis, (3) glomerulonephritis and (4) Parkinson's disease. A causal role of VEE vaccination has not been established in these cases, but we strongly recommend that women of child-bearing age be vaccinated only during their menstrual period. The vaccine has not been evaluated in children or the elderly. Attenuated TC-83 vaccine should continue to be used to immunize adults at-risk to infection with VEE virus subtypes.

(Donald S. Burke and Robert Edelman)

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

Our studies of the ecology of Keystone (KEY) and Jamestown Canyon (JC) viruses in the coastal plains of Maryland have focused on the role of vertebrates and mosquitoes in the natural cycle of these viruses. We have also attempted to identify the mosquito species involved in the transmission of St. Louis encephalitis virus to humans in Prince George County, Maryland during Sept. of 1975.

Jamestown Canyon Virus

Whitetail deer fawns developed a viremia following subcutaneous (SC) inoculation with 2.0 ml of an inoculum that contained $10^{5.0}$ $SMLD_{50}$/1.0 ml of a local strain of JC virus (Table 1). Virus was first detected in blood of 3 deer on day-1 post inoculation (PI) and by day 3 all deer except no.188 had developed a viremia. The duration of viremia ranged from 2 to 4 days and virus titers ranged from a trace to $10^{5.0}$ $SMLD_{50}$/1.0 ml. Attempts to recover virus from blood taken on days 5 & 7 were unsuccessful. All deer were shown to be free of serum neutralizing antibody of JC and KEY viruses prior to the inoculation date.

On day 30 PI, 3 of the deer that received JC virus were inoculated SC with 2.0 ml of an inoculum that contained $10^{6.6}$ $SMLD_{50}$/1.0 ml of a local strain of KEY virus. The remaining 3 deer were inoculated on day 80 PI, with a comparable dose of KEY virus the objective being to determine if deer infected with JC virus could subsequently serve as amplifying host of KEY virus. No evidence of virus was detected in blood taken on days 1 thru 5 and on day 7 PI for either group of deer. Further studies are being conducted to determine the immune response profile of deer inoculated with these viruses. In addition, studies to establish the viremia and immune response of gray squirrels and cottontail rabbits after inoculation with JC virus are underway.

Studies aimed at establishing the vector competence for selected mosquito species are in progress. The mosquito, Aedes canadensis failed to become infected after ingesting $10^{3.0}$ $SMLD_{50}$/1.0 ml of JC virus in defribinated blood. On increasing the virus dose to $10^{4.5}$ $SMLD_{50}$/1.0 ml 1 of 9 and 1 of 8 mosquitoes became infected after a 14 and 21 day incubation period, respectively. Transmission of a lethal dose of virus was not accomplished by either infected mosquito. After mosquitoes ingested a virus-blood mixture containing $10^{6.0}$ $SMLD_{50}$/1.0 ml, a substantial increase in the infection rate occurred and transmission of virus to suckling mice was accomplished by mosquitoes after an incubation period of at least 9 days.

Attempts to demonstrate vertical transmission of JC virus by A. canadensis were unsuccessful. Virus was not recovered from 21 pools that consisted of 502 eggs laid by 15 infected mosquitoes. In addition, negative results were obtained on assaying 19 pools that contained 170

fourth stage larvae that were reared from eggs laid by 10 infected mosquitoes. No evidence of virus was detected in a total of 122 pools or 1250 field collected A. canadensis larvae.

Keystone virus

Whitetail deer fawns failed to develop a viremia after SC inoculation with 2.0 ml of an inoculum that contained $10^{4.5}SMLD_{50}$/1.0 ml of a local strain of KEY virus. Serum specimens taken from deer before the inoculation date were found to be free of detectable neutralizing antibodies to KEY and JC viruses. On day 80 PI these deer were inoculated SC with an inoculum that contained $10^{5.6}SMLD_{50}$/1.0 ml of JC virus. Attempts to recover virus from blood of deer on days 1 thru 5 and on day 7 PI were unsuccessful, suggesting that deer were protected by antibody developed to KEY virus. Studies regarding the latter are in progress.

The magnitude and duration of viremia in gray squirrels after SC inoculation with 0.5 ml of an inoculum that contained $10^{6.0}SMLD_{50}$/1.0 ml of KEY virus are presented in Table 2. Blood specimens taken on day 1 PI failed to yield virus at 10^{-1} dilution of the inoculum. A trace of virus was recovered from 5 of 9 squirrels on day 2 PI and by day 3 PI all squirrels except one exhibited virus blood levels that ranged from $10^{3.0}$ to $10^{4.6}SMLD_{50}$/1.0 ml. Virus titers increased on day 4 PI for most squirrels and then decreased considerably on day 5 PI.

The high prevalence rate of tularemia and KEY virus antibody in field captured cottontail rabbits have hampered studies aimed at determining their response to inoculation with KEY virus. During July of 1975, 6 rabbits were inoculated SC with 0.5 ml of an inoculum that contained $10^{5.5}SMLD_{50}$/1.0 ml of KEY virus. Attempts to recover virus from blood specimens taken from 3 of 6 surviving rabbits over a 4 day period were unsuccessful. Francisella tularensis was identified at the most likely cause of death in 2 of the 3 rabbits that died during the study. The 3 surviving rabbits were sacrificed on day 5 PI. The failure to recover KEY virus may have resulted from preinfection with this or other California group viruses as serological reactors were detected in serum taken prior to the inoculation date.

A second group of 6 cottontail rabbits were inoculated in December 1975 with 0.5 ml of an inoculum that contained $10^{4.8}SMLD_{50}$/1.0 ml of KEY virus. Three of the rabbits were found to possess serum neutralizing antibody to KEY virus which probably rendered them refractory to infection. The seronegative rabbits developed a viremia that was first detected in trace amounts on day 2 PI. On day 3 PI the remaining rabbits had developed a viremia. The duration of viremia ranged from 1 to 4 days and virus titers ranged from trace levels to $10^{4.7}SMLD_{50}$/1.0 ml. Additional studies are needed to fully assess the course of KEY virus infection in cottontail rabbits.

Laboratory studies aimed at elucidating the role of selected mosquito species in the natural cycle of KEY virus are in progress. The mosquito,

Aedes atlanticus readily became infected after ingesting defibrinated blood that contained $10^{5.2}$SMLD$_{50}$/1.0 ml and $10^{6.0}$SMLD$_{50}$/1.0 ml of a local strain of KEY virus (Table 3). Attempts to demonstrate transmission of the virus to suckling mice, however have been unsuccessful. Preliminary studies suggest that an alternative indicator system will be required to detect virus transmission. The virus content of individual A. atlanticus mosquitoes following ingesting of a virus-blood mixture that contained $10^{5.2}$SMLD$_{50}$/1.0 ml were indicative of virus multiplication. Virus titers determined immediately after mosquitoes ingested virus averaged $10^{4.0}$SMLD$_{50}$/1.0 ml. A slight decrease in virus content occurred on day 3 as indicated by an average titer of $10^{3.6}$SMLD$_{50}$/1.0 ml. On day 5 and 7 virus titer had increased ot an average of $10^{4.6}$ and $10^{5.0}$SMLD$_{50}$/1.0 ml. and, persisted at comparable levels on days 14 and 21 post feeding. Additional studies are underway to establish virus transmission rates and the threshold level of infection for A. atlanticus and KEY virus.

Experiments involving Aedes canadensis and KEY virus show this mosquito to become infected on ingesting KEY virus in chicken defribinated blood. Infection rates, however were much lower than that obtained for A. atlanticus. Attempts to demonstrate virus transmission with this species using mortality of suckling mice as an indicator system were unsuccessful. The amount of KEY virus recovered from individual A. canadensis mosquitoes after ingesting a virus blood mixture containing $10^{5.2}$SMLD$_{50}$/1.0 ml was very low. Virus titers determined immediately after this mosquito ingested virus averaged $10^{3.9}$SMLD$_{50}$/1.0 ml. Subsequent average titers were $10^{2.6}$, $10^{2.3}$, $10^{2.5}$, $10^{3.2}$, $10^{2.5}$ and $10^{3.0}$SMLD$_{50}$/1.0 ml for days 2, 4, 7, 14, 21, 34 post feeding, respectively.

St. Louis Encephalitis Virus*

There were 8 confirmed human cases of St. Louis encephalitis (SLE) virus infection during August, September and October in Prince George County, Maryland. Confirmation of cases were based on either a 4-fold change in hemagglutination inhibition titer to SLE virus antigen, or a single such titer equal to or greater than 1:80.

Preliminary studies aimed at identifying vectors of SLE virus indicate that 2 pools of Culex species and 1 pool of Aedes species yielded isolates of the virus. The minimum field infection rate was 1/163 and 1/436 for Culex species and Aedes species, respectively.

* Collaborative study, R.M. Altman and G. Mallek, Maryland Department of Agriculture, Division of Plant Industries Pest Management Section, College Park, MD 20742

(D.M. Watts, D.E. Hayes, R.R. Pinger, J.W. Taylor, C.L. Bailey, B.F. Eldridge, J.M. Dalrymple, F.H. Top, and P.K. Russell).

TABLE 1. DURATION AND MAGNITUDE OF VIREMIA IN WHITETAIL DEER FAWNS FOLLOWING SUBCUTANEOUS INOCULATION WITH $10^{5.0}SMLD_{50}$/1.0 ml OF JAMESTOWN CANYON VIRUS

Deer Number	Days Post Inoculation 1	2	3	4	5	7
180	--[1]	3.4[2]	2.9	1.8	-	-
195	3.2	5.0	-	-	-	-
188	-	-	-	-	-	-
185	-	-	2.3	2.2	-	-
108	T[3]	4.1	3.9	2.6	-	-
109	2.4	3.9	3.4	T	-	-
189 (control)	-	ND[4]	-	ND	-	ND
182 (control)	-	ND	-	ND	-	ND

[1] Virus not recovered from 1:5 dilution of blood

[2] $Log_{10}SMLD_{50}$/1.0 ml

[3] Trace of virus

[4] Not done

TABLE 2. MAGNITUDE & DURATION OF VIREMIA IN GRAY SQUIRREL AFTER SUBCUTANEOUS INOCULATION WITH $10^{4.5}$ $SMLD_{50}$/0.03 ml OF KEY VIRUS

Gray Squirrel No.	Days Post Inoculation				
	1	2	3	4	5
1	- [1]	-	3.4 [2]	2.9	-
2	-	-	3.8	3.9	2.5
3	-	-	3.0	3.5	3.1
4	-	+[3]	3.0	3.5	-
5	-	+	4.6	4.1	+
6	-	+	+	4.0	3.0
7	-	-	3.2	3.4	-
8	-	+	3.1	3.0	-
9	-	+	-	-	-
10 (Controls)	-	-	-	-	-
11 (Controls)	-	-	-	-	-

[1] Virus not detected at 10^{-1} dilution

[2] $Log_{10}SMLD_{50}$/1.0 ml

[3] Trace of virus, less than half of mice died at 10^{-1} dilution

TABLE 3. INFECTION AND TRANSMISSION RATES FOR AEDES ATLANTICUS[1/] AFTER INGESTING KEYSTONE VIRUS[2/] IN DEFRIBINATED BLOOD THROUGH A LAMB SKIN MEMBRANE

Virus Dose ($SMLD_{50}$/1.0 ml)	Incubation (Days)	Infection Rates
$10^{5.2}$	3	4/5(80%)
	5	5/5(100%)
	7	5/5(100%)
	14	7/7(100%)
	21	4/5(80%)
$10^{6.0}$	3	3/3(100%)
	5	5/5(100%)
	7	ND
	14	4/4(100%)
	21	3/3(100%)
Controls (A. atlanticus		0/20

1/ A. atlanticus reared from field collected larvae.

2/ KEY virus, 4th BHK-21 (clone-13) passage

REPORT FROM THE LIFE SCIENCES DIVISION, MELOY LABORATORIES, SPRINGFIELD, VIRGINIA

Laboratory primates are frequently employed in studies of dengue viruses. The data presented here from such a study demonstrate the existence of covert immunological sensitization to dengue viruses in primates. This phenomenon poses a potentially significant problem to immunological studies in laboratory primates. In view of the possible role of immunological sensitization in dengue hemorrhagic fever, our findings in primates may also represent a consideration for investigators studying the immunology of dengue viruses in man.

Two seronegative rhesus monkeys were inoculated subcutaneously with about $10^{2.0}$ infectious units of a DEN-3 virus (H87 isolate) which had received three suckling mouse brain and seven hamster kidney (HK) cell culture passages. The virus had also been treated with the mutagen N-methyl-N'-nitro-N-nitrosoguanide. Animals were bled daily on days 3 through 7, 14, and 21. All attempts to detect viremia by inoculation of HK and LLC-MK_2 cultures and suckling mice were negative. These sera were also found to contain no detectable HI antibody.

On day 52, these two monkeys plus a third control animal received 10^7 infectious units of DEN-3 virus in its 23rd mouse brain passage and bled as previously described. All attempts to detect viremia were again unsuccessful. Table 1 shows that the control monkey (No. 12) exhibited a slowly-developing, low-titered HI antibody response usually considered to be of the primary type. In contrast, the two previously infected animals (Nos. 6 and 7) manifested a high-titered rapid HI antibody response which temporally and quantitatively resembled secondary-type responses in dengue-infected primates.

These data strongly suggest that immunological sensitization can occur in primates without the detection of viremia or a primary antibody response. Extrapolated to man, these findings may partially explain the apparent secondary-type antibody responses observed in dengue outbreaks in "immunological virgin" populations.

(A.S. Lubiniecki, and G.C. Tarr of the Department of Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.)

Table 1. Serum HI Antibody Titers to DEN-3 Prior and Subsequent to Vaccination and Challenge[a]

Monkey No.	-7	0	7	14	21	52	59	66
6	<10	<10	<10	<10	<10	<10	160	320
7	<10	<10	<10	<10	<10	<10	160	40
12	<10	ND[b]	ND	ND	ND	<10	<10	10

[a]Monkey 6 and 7 were vaccinated on day 0; all three monkeys received P_{23} mouse brain material on day 52.

[b]Not done.

REPORT FROM THE BUREAU OF LABORATORIES, STATE OF FLORIDA DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES, JACKSONVILLE, FLORIDA

As part of the general virus diagnostic services provided to the medical community of Florida, we tested 571 patients' sera against a battery of antigens associated with central nervous system diseases during the period from July 1975 - December 1975. Two cases of EEE were detected, one in a 47-year-old female and one in a 68-year-old male, from the north-eastern and north-central parts of Florida, respectively. Both patients are living with brain damage. The patients with SLE were ill in Chicago (1) and in the Bahama (3) and then came to Florida where they were hospitalized. Where histories could be obtained, the patients with constant Group B titers had previously resided in known endemic areas in the Caribbean.

See accompanying table.

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

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

July 1975 — December 1975

Species	Number of Sera	Reactors
Human	571	2 EEE 4 SLE° 13 Group B°°
Horses	55	20 EEE 3 WEE
FIELD SPECIMENS a. Small Mammals	88	6 SLE** 11 VEE*** 8 EEE
b. Wild Birds	124	9 SLE**
Chukars	4	1 WEE
TOTAL	842	77

* 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

° One (1) patient from Chicago, three (3) patients from the Bahamas.

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

** North Florida Counties.

*** Collected in the South Florida endemic area.

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

1975 Dengue epidemic in Puerto Rico

Following the 1969 dengue-2 epidemic in Puerto Rico, laboratory confirmed cases of dengue-2 infection occurred sporadically in the southwestern portion of the island during 1970 through mid-1975. This activity was believed to represent a long tail-off of the 1969 epidemic in a region which was relatively spared during the peak of epidemic activity in other parts of the island.(1,2). During January through August 1975, the number of confirmed dengue cases increased in comparison with previous years (61 cases in contrast to 6 cases during the same period of 1974).

Dengue fever was confirmed serologically in a resident of metropolitan San Juan whose illness began on September 18. This was the first documented evidence of dengue transmission in the metropolitan area since the 1969 epidemic and strongly suggested the potential for subsequent epidemic activity in an urban area with approximately one million inhabitants. Aedes aegypti population indices were high in San Juan as well as in other parts of the island, with larval house indices ranging from 10 to 35%.

Tropical storm Eloise (later Hurricane Eloise) struck Puerto Rico on September 16, 1975, and heavy rainfall during and after this storm further increased the A. aegypti population indices.

In mid-October, a cluster of suspected dengue cases was detected in Carolina, a large suburban community which is part of metropolitan San Juan. A house-to-house survey conducted on a systematic sample of 85 households in Carolina collected clinical, serologic, and entomologic data. The survey documented that (1) the attack rate for dengue-like illness was increasing in the community; (2) the dengue serotype responsible for the activity was type 2 as determined by virus isolation from four cases and identification by the complement fixation (CF) test; and (3) vector populations were relatively high (3.9 female A. aegypti collected per man-hour). Dengue activity subsequently increased dramatically in the San Juan metropolitan area, especially in Bayamón, a large suburban area with approximately 200,000 inhabitants.

As of February 14, 1975, 1285 cases of dengue-like illness had been detected by the dengue surveillance since September 1, and 132 of these have thus far been confirmed by seroconversion and/or by virus isolation. The 1285 cases are shown (Figure) by week of onset and by residence within or outside the San Juan metropolitan area.

Although 64.4% of the reported dengue-like illness has occurred in the San Juan metropolitan area, the distribution on the island is widespread. Of the 79 municipalities on the island, 42 have reported dengue-like illness through the surveillance system, and dengue has been confirmed in 14 of the 42 municipalities. A few dengue-like illnesses have been reported from 10 additional municipalities, but no clinical data or serological specimens were collected from these cases.

Currently, a total of 13 presumptive dengue viruses have been isolated from sera of acute cases. Four of these have been shown to be dengue-2 by the CF test, and definitive identification is pending on the other isolates.

To date three cases of dengue hemorrhagic fever (DHF) have been documented, and all three patients have recovered. Case 1, a 35-year-old male, experienced gross hematuria a week after the onset of dengue-like illness. Case 2, a 45-year-old male, 5 days after the onset of a dengue-like illness developed severe bleeding from the gums followed by widespread petechiae, ecchymoses, profound thrombocytopenia (4,000 platelets/mm^3), and positive tourniquet test. The most recent, case 3, a 14-year-old male, several days after the onset of a dengue-like illness developed petechiae on the palate, epistaxis, positive tourniquet test, and thrombocytopenia (88,000/mm^3). Serological studies by the hemagglutination inhibition (HI) test and/or the CF test have shown typical secondary type serological responses for cases 1 and 3.

Case 2, however, does not have a typical primary or secondary type serologic response. Although HI and CF antibody titers in the serum specimen taken 9 days after onset of illness are exceedingly high for dengue-2 ($\geq$20,480 for HI and 1,024 for CF), the titers for DN-1, DN-3, and DN-4, yellow fever, and St. Louis encephalitis are relatively very low and not typical of a broad secondary serologic response. Neutralizing antibody titers by the plaque reduction neutralization test (PRNT) also are extremely high for dengue-2 (>16,384) and relatively low (32) for dengue serotypes 1, 3, and 4. A similar neutralizing antibody pattern has been described (3) in a 5-year-old child on Niue Island in the Pacific who experienced hemorrhagic manifestations in association with an apparent dengue infection. This child, who had never been off the island and was born years after the previous dengue epidemic, was presumed to represent a primary dengue infection. The interpretation and significance of the antibody pattern observed in case 2 is not clear at the present time.

Two cases of suspected dengue shock syndrome (DSS), including a fatal illness, were not supported by laboratory evidence.

Comprehensive clinical, virological, and immunological studies were undertaken on a series of 55 patients with acute dengue-like illness who were seen at an outpatient clinic in Bayamón. These patients were examined every other day during the course of their illness, and again several weeks after recovery. In addition to detailed clinical history and physical examination, including tourniquet test, blood specimens were taken for complete blood count and differential, platelet count, coagulation time, prothrombin time (PT), and partial thromboplastin time (PTT). In addition, serum aliquots were placed in liquid nitrogen within one hour of collection for subsequent determination of sequential viremia levels (using the A. aegypti intrathoracic inoculation technique) and for complement system studies. These studies should yield valuable information on the pathogenesis of classical dengue and dengue hemorrhagic fever.

Three cycles of ultra low volume (ULV) aerial spraying with Malathion were carried out in the San Juan metropolitan area as follows: December 11 and 16, December 17 and 19, and December 30. Due to technical reasons, the latter application was ineffective and a final spraying was conducted on January 9, 1976. Additional mosquito control operations included (1) ULV ground application of Malathion from truck-mounted equipment in much of the San Juan metropolitan area and some nearby communities; (2) peridomestic larviciding by application of Malathion by pressurized hand sprayers in selected communities islandwide; (3) larviciding with Abate and Malathion in cemeteries islandwide; and (4) an islandwide effort via radio, television, newspaper releases, and pamphlets to reduce peridomestic breeding sites of A. aegypti.

Assessment of aerial spraying by mortality of caged mosquitoes and by adult mosquito collections in selected sites in the San Juan metropolitan area indicated satisfactory results for all but the third cycle of aerial spray application. These data and the sharply reduced incidence of dengue-like illness reported after aerial spray operations were completed indicate that ULV aerial spraying played a major role in interrupting the epidemic. In selected monitoring sites, however, adult population densities have now returned to pre-treatment levels. Persistent low-level activity is anticipated and epidemic activity may recur during the next rainy season.

Finally, studies were undertaken to estimate the economic impact of epidemic dengue on Puerto Rico. Large corporations are cooperating by providing absenteeism data, and in addition, individuals with confirmed dengue are being interviewed to determine type of employment, time lost from work, medical costs, and related information.

To date, spread of dengue activity to other islands in the Caribbean has not been documented.

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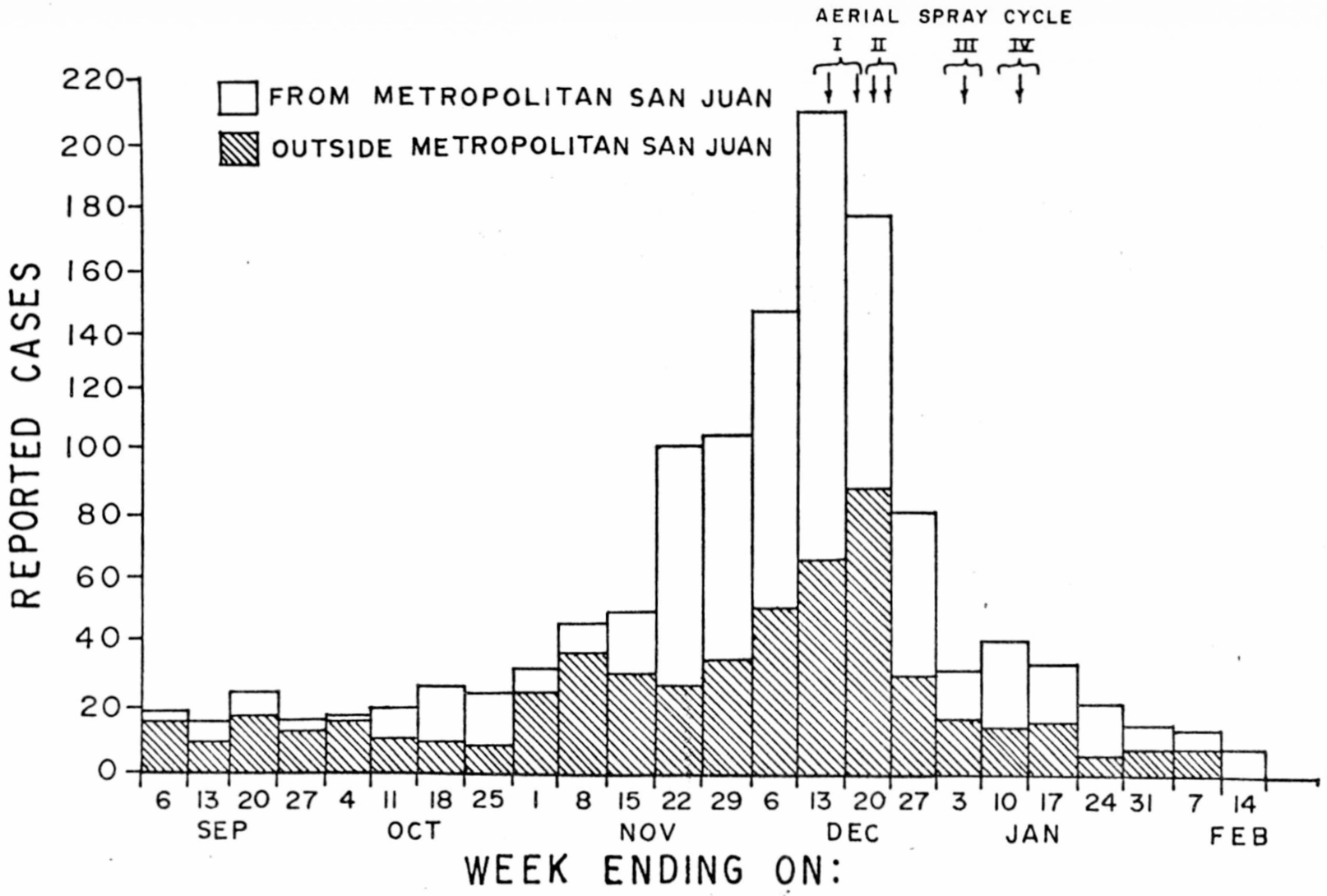
(B. L. Cline, C. G. Moore, R. López-Correa, G. E. Sather, E. Ruiz-Tibén, G. Kuno, J. P. Woodall)

CASES OF DENGUE-LIKE ILLNESS* IN PUERTO RICO

SEPTEMBER 1, 1975 - FEBRUARY 14, 1976



* DENGUE-LIKE ILLNESS DEFINED AS AN ACUTE FEBRILE ILLNESS ASSOCIATED WITH ONE OR MORE OF THE FOLLOWING SYMPTOMS OR SIGNS: SEVERE HEADACHE, OCULAR PAIN, BODY ACHES, AND A RASH.

REPORT FROM VIRUS DISEASES SECTION
GORGAS MEMORIAL LABORATORY, PANAMA CITY, PANAMA

I. Arbovirus Field Studies with Panamanian Sloths

Previous work at GML-MARU has shown that three-toed sloths (Bradypus infuscatus) are capable of mounting exceptionally long and high viremias when inoculated with yellow fever virus, and could potentially be amplifying hosts during YF jungle epizootics (Arthropod-borne Virus Information Exchange #27).

In September 1974 a collaborative GML-Smithsonian Institution project was designed to detect yellow fever viremia and seroconversion in sloths of the Cerro Azul area of Panama, which at that time was threatened by jungle yellow fever. Radio transmitters were attached to 32 Bradypus and 4 two-toed sloths (Choloepus hoffmanni); the animals were released in the forest and periodically recaptured and bled.

Four virus strains were isolated from 114 blood samples tested. Two of these have been identified as Changuinola group agents, one is a possibly unregistered member of the Phlebotomus fever group, and one is a member of the Simbu group. Seroconversion to the virus isolated was detected in the animals yielding the Changuinola and Phlebotomus fever group agents; the animal infected by the Simbu group agent died before recapture. In addition, eight other Bradypus and one Choloepus seroconverted to one or more of the agents isolated during the September 1974-August 1975 study period. Phlebotomus fever group virus activity occurred only at the end of the rainy season (November to December); Changuinola infections occurred in the dry season (January to May) and at the beginning of the rainy season (May and July); Simbu group infections were recorded in December and July. Yellow fever activity was not detected.

A serological survey of wild sloths and howler monkeys in two areas of Panama affected by yellow fever (Maje and El Llano-Carti) showed no YF antibody in 33 adult sloths, in contrast with a 60% positive rate in monkeys. The samples were taken 4-7 months after the disappearance of yellow fever from the two localities.

Antibody surveys of four different Panamanian localities (Maje, El Llano-Carti, Barro Colorado Island, and Aguacate) revealed that antibodies against St. Louis encephalitis virus and the agents isolated in Cerro Azul are probably widespread in Panamanian sloths and most commonly found in two-toed sloths. These surveys are still in progress.

(C. Seymour and P. H. Peralta)

II. St. Louis Encephalitis (SLE) Virus in Cormorants

In July and August of 1973, epizootic death occurred in adult olivaceous cormorants (Phalacrocorax olivaceus) in Panama. SLE virus was isolated from 3 of 5 moribund cormorants tested. One isolation was made from a sick bird which landed on the roof of GML, in the center of Panama City; the others were from offshore islands in the Pacific ocean. These observations suggested that cormorants might transport SLE virus many miles during their daily feeding flights, and perhaps serve as sources of new foci of virus activity if their viremia levels were high enough and long enough to infect vector mosquitoes; also, if SLE virus were pathogenic for cormorants, epizootic death could serve as an index of high levels of SLE virus activity. Viremia level and pathogenicity were tested in 1975 by inoculation experiments.

Four fledgling cormorants seronegative to SLE virus were maintained for two months in captivity until contour and flight feathers had completely grown in. They were inoculated subcutaneously with 2.3 $\log_{10}$ SMicLD$_{50}$ of first Vero cell passage SLE virus isolated from the viscera of a moribund cormorant in 1973. Whole blood samples taken following inoculation were titrated in suckling mice ic and in Vero cell tube cultures. Fluids from tubes showing CPE were passed into mice; brains of dead mice were tested for SLE-specific CF antigen to confirm viral etiology of death.

Table 1 shows that three of four birds became viremic. Detectable viremia lasted at least four days; peak titers were 4.0, 3.5, and 5.9 $\log_{10}$ SMic LD$_{50}$/ml. No birds showed any signs of illness. Bird D, which had the highest peak titer, was ill for unknown reasons when inoculated, but had returned to normal appearance by day 14. All viremic birds developed both HI and neutralizing antibody by day 14. Bird C developed neither detectable viremia nor antibody.

The results suggest that SLE virus per se does not kill cormorants. The epizootic deaths in 1973 may have been due to another cause, and SLE virus persisting from previous infections may have appeared spontaneously in the tissues and blood of moribund birds. Explant cultures of organs of two of the experimentally infected cormorants are presently being tested for SLE virus.

(C. Seymour, P. H. Peralta, P. Galindo)

TABLE 1. Daily viremia titers* of cormorants inoculated with SLE virus.

BIRD	SYSTEM	DAY POST INOCULATION 1	2	3	4	5	6	7	8	9	10	14	21
A	Vero	—**	3.5	3.0	—	—	—	—	—	—	—	—	—
	SMic	nt/	3.4	4.0	2.1	nt	1.7	—	nt	—	—	—	—
B	Vero	—	—	—	—	—	—	—	—	—	—	—	nt
	SMic	—	—	—	—	—	—	—	nt	nt	nt	nt	—
C	Vero	—	1.0	1.5	2.3	—	—	—	—	—	—	—	nt
	SMic	—	2.3	3.2	3.5	2.8	—	—	nt	nt	—	nt	—
D	Vero	—	—	—	—	0.7	1.3	4.5	4.0	0.8	1.5	—	nt
	SMic	—	—	nt	nt	2.4	4.0	5.9	5.5	4.3	nt	nt	—

* Expressed as $\log_{10}$ SMic LD_{50}/ml or $\log_{10}$ TC ID_{50}/ml.

** — indicates undiluted blood negative.

/ nt: not tested.

III. St. Louis Encephalitis Virus in Culex pipiens quinquefasciatus

There is evidence of the occurrence of human, wild animal, and mosquito St. Louis encephalitis (SLE) virus infections in various forested areas of Panama. In the capital, Panama City, however, no cases of SLE have been recognized in spite of the fact that the common vector in outbreaks of the central U.S., Culex pipiens quinquefasciatus, is present here throughout the year and is particularly abundant during the dry season. Furthermore, the virus has been isolated three times in one of the suburbs of the city (from ground dove Columbigallina talcapoti and sentinel suckling mice in 1962; from Deinocerites pseudes mosquitoes in 1964) and once in the heart of the city (from a cormorant in 1973). We therefore decided to evaluate the vector potential of the local C. pipiens quinquefasciatus for SLE virus as the first step in the investigation of human SLE in Panama City.

The mosquitoes used in these experiments were originally captured on the edge of Panama City and have now been colonized approximately five years. The strain of SLE virus used was isolated from a cormorant in Panama in 1973 and passed once in Vero cell culture. Female mosquitoes, 3-5 days old, fed on viremic weanling mice and were then maintained at 27°C for 16-30 days before being tested for virus in Vero cells. Before being harvested, mosquitoes which originally fed on the higher concentrations of virus were allowed to feed on individual 4-day-old suckling mice or 2-day-old chicks for a determination of transmission rates. The salivary glands of those which fed successfully and occasional others which did not, were dissected out and fixed in acetone for subsequent fluorescent antibody test.

The infectivity results are shown in Table 2. The threshold of infection occurred at approximately 2.5—2.8 $\log_{10}$ Vero $TCID_{50}$/ml. Transmission was successful 100% of the time after 21 days, at which time all salivary glands from infected mosquitoes demonstrated fluorescence in each of the six lobes. Earlier times were not tested. Both the infection and transmission rates of the Panamanian C. pipiens quinquefasciatus were of the same order of magnitude as those seen in U.S. strains as demonstrated by Chamberlain et al. (Am. J. Trop. Med. & Hyg. 69: 221-236, 1959) who used a strain of SLE virus isolated from Culex tarsalis in the U.S.A.

Therefore, the lack of reported SLE cases in Panama City cannot be attributed to poor vector competence of Panamanian C. pipiens quinquefasciatus. Presently a serological survey of humans from areas of the city where C. pipiens quinquefasciatus mosquitoes are prevalent is in progress to determine whether any human SLE virus infections are occurring. If results indicate substantial human infection, two studies are possible to explain the absence of observed clinical SLE illness in Panama. First, the virulence of Panamanian SLE strains could be compared with that of U.S. epidemic strains. Second, a thorough surveillance of hospitalized

aseptic meningitis and encephalitis cases, including virological studies of clinical specimens, could determine whether SLE illness actually is occurring but is not presently recognized.

(L. D. Kramer)

TABLE 2. SLE virus infection rates of Panamanian *Culex pipiens quinquefasciatus*

Virus titer of infective blood meal (log_{10}/ml)		Percentage of mosquitoes infected
Vero TC ID_{50}	$SMicLD_{50}$	
2.2 — 2.5	3.0	0 (0/43)*
2.8 — 3.4	3.3 — 3.7	21 (4/19)
4.0	3.9	38 (3/8)
5.0	5.0 — 5.7	96 (24/25)

*(Number of mosquitoes infected/number of mosquitoes which engorged.)

IV. Venezuelan encephalitis (VE) virus in herons

Eight juvenile seronegative striated herons (Butorides striatus) were inoculated subcutaneously with 2000 Vero cell pfu of epizootic VE virus, variety I B ; the Nicaraguan MF-8 strain was used after one passage in suckling mice and one in Vero cells. Blood samples were taken for eight days following inoculation; they were titrated simultaneously in Vero cells, suckling mice (0.02 ml ic) and weanling mice (0.02 ml ip), using 10^{-1} as the strongest dilution. Plasmas taken day 26 post-inoculation were tested for HI and neutralizing antibodies.

Table 3 summarizes the viremia results, which do not include one bird which died immediately after being bled on day 1, with a viremic titer of 3.8 $\log_{10}$ $SMicLD_{50}$. All other birds showed no signs of illness, and developed both HI and neutralizing antibodies.

Thirteen bloods were positive in both weanling and suckling mice. For these, SMic titers averaged 0.6 logs higher than corresponding WMip titers; extremes ranged from 1.1 logs higher by SMic than WMip, to 0.4 logs higher by WMip than SMic.

These low, short viremias suggest that striated herons may not be important in maintaining or spreading epizootic VE virus even during their annual dispersal flights. The highest WMip LD_{50} titer in any bird is virtually equal to the highest WMip enzootic VE virus titer reported in experimentally inoculated striated herons (Grayson, M.A., and P. Galindo. 1939. J.A.V.M.A. 155: 2141-2145).

(C. Seymour and P. Galindo)

TABLE 3. Viremia durations and peak titers of striated herons inoculated with epizootic VE virus

SYSTEM	Detectable Viremia Duration (Days)			Viremia Peak Titer ($\log_{10}$ LD_{50} per ml)		
	Min.	Max.	Mean	Min.	Max.	Mean
SMic	1	3	2.1	3.4	6.3	5.2
WMip	1	2	1.9	2.7	5.9	4.6
Vero pfu	0	2	1.4	<1.7	5.0	3.3

REPORT OF THE NATIONAL INSTITUTE OF HEALTH OF COLOMBIA

BOGOTA, COLOMBIA

OUTBREAK OF DENGUE FEVER IN CENTRAL COLOMBIA WITH SPECIAL REFERENCE TO THE TOWN OF ARMERO

Introduction

Until 1952 dengue, was endemic, although very seldom diagnosed, in regions of the Upper Magdalena Valley that were infested with Aedes aegypti. The overall prevalence of neutralizing antibody for dengue 2 in the area was 23 per cent and practically everybody had developed antibodies by the age of 50. Since the mosquito was commonly found along Colombia's Atlantic coast until about 1950, it is possible that endemic dengue also existed there until that time, as was the case in other Caribbean ports.

Between 1952 and 1960 A. aegypti was eradicated from all parts of Colombia, except the city of Cúcuta, adjacent to Venezuela, o country which has remained infested up to the present time. After eradication, vigilance of Aedes was limited to the port areas of Barranquilla, Santa Marta and Cartagena. Around 1969, the northern part of Colombia became infected with the mosquito, which by the end of 1972 had invaded an area of 58,000 km^2 with urban populations totalling 2,350,000 inhabitants. A serious outbreak of dengue 2 was observed in the reinfested area from mid-1971 to May 1972. On the basis of serological surveys, it has been estimated that no less than 450,000 cases occurred during the epidemic. No hemorrhagic cases or shock syndromes were detected, but it must be stated that no particular efforts to detect them were made.

Reinfestation of Central Colombia by the vector

Efforts to control A. aegypti in Northern Colombia have been made since July 1972. As a result, the degree of infestation is at present low in the coastal cities. However the measures taken did not prevent the mosquito to proceed South and invade Central Colombia and the Upper Magdalena Valley sometime between January 1972 and April 1975. Outbreaks of dengue began to be noticed in Central Colombia by mid-1975. The number of affected towns at present is not adequately known, but it appears to be rather large, since cases have been reported from at least 13 towns located between Armero and Neiva (Figure 1). Nor is known the magnitude of the epidemics in the different localities. They may reach significant

proportions, since the area was free from Aedes and dengue during the period 1952-1972, and in certain places it is densely populated, for instance in Ibague (pop. 205,000) or Neiva (pop. 121,000).

With the purpose of studying the incidence of the disease in one of the affected localities, and possibly in this way to make an educated guess of what might be happening in other places, the town of Armero was selected. Armero (altitude 352 meters; mean temperature 26°C) lies in the heart of a prosperous and large agricultural area where rice, cotton and beef are produced. Tropical forests are found only far from the place, at a distance of 180 kilometers. The town has 17,600 inhabitants who live in 3,100 houses concentrated in 197 blocks. Two cases of dengue were diagnosed there by one of us, (A. Morales) early in September 1975. Since very few patients consulted with the local doctors -the disease being diagnosed always as typhoid, influenza or rubella- we decided, among other studies, to make a house-to-house survey in order to establish whether febrile cases compatible with dengue were occurring or had occurred during the preceding twelve weeks. A census of the visited houses was made and a brief clinical history was obtained from each febrile patient, either acute or convalescent. A variable number of blood specimens were taken in each household from individuals either with or without febrile antecedents. In total, 420 houses were visited where 2,245 persons used to live. The houses were selected as follows : from all the 197 blocks in town, 142 were chosen at random and in each one of the selected blocks three houses, also at random, were studied (in 6 blocks only 2 houses per block were surveyed). A simultaneous entomological survey was carried out in the selected 420 houses. Both surveys lasted from September 17 until October 2, 1975. A summary of some of the most important findings is presented henceforth.

Entomological situation in Armero

From the 142 blocks thus studied, only one was free from A. aegypti. Seventy five per cent of the 420 houses were found to be infested with the mosquito. In 97 houses both adults and immature forms were found; in 121, only adults and in 43, only immature forms. The attack rate of females was studied on several occasions : it was never greater than two mosquitoes per man per hour.

Clinical and serological findings

Out of the 2,245 individuals who lived in the visited households, 540 (24 per cent) exhibited a history of previous or current fever compatible with the diagnosis of dengue. Of the remaining persons, a few complained of having experienced vague "febrile" episodes in the preceding weeks, but the large majority did not recall having been ill during the ninety days prior to our visit. During the survey, 650 blood specimens were obtained from the total sample of 2,245 persons. So far we

have made attempts at isolation of viruses with a number of "acute" specimens, which indicate, as shown below in detail, some infections with dengue 2. In addition, we have studied until now 462 sera by CF and HI with dengue 2 and dengue 3; moreover the majority of these sera have been tested with dengue 1, dengue 4, Ilheus, St. Louis, Yellow fever and Bussuquara. The analysis of the corresponding results by titer, age, sex and other variables, will be presented at a later date. In this report we merely present the data concerning the proportion of individuals exhibiting dengue antibodies, as shown in Table 1, which indicates positive correlation between the clinical and the serologic findings. In fact 91 per cent of late convalescents from illnesses compatible with the diagnosis of dengue, exhibited dengue antibodies. On the other hand, only 17 per cent of individuals with no history of disease during the preceeding weeks showed dengue antibodies. The majority of the positive cases in this category are adults who possibly had had some previous infection with any group B agent, including 17D. In the same category there are, however, some children with high titers for dengue 2, a fact which suggests a recent asymptomatic infection with the same virus.

Epidemic curve

Taking into consideration the date of onset of disease in the 540 cases with diagnosis compatible with dengue, it appears that the first case occurred on June 25, 1975 and that the outbreak reached its climax during September, as shown in Figure 2. The incidence of the disease between June 25 and October 2 was at least 24%. Possibly it was larger, since we visited the different houses only once; had we revisited on October 1 and 2 those households surveyed between September 17 and September 30, probably more cases would have been detected. Anyway, if we extrapolate that 24 per cent to the 17,600 inhabitants of Armero, we may estimate in 4,200 the number of dengue cases which should have occurred in the whole town, during the aforementioned period.

Virus isolations

Attempts at isolation of virus were made from patients found ill during the survey and from other febrile individuals who did not live in the selected houses and, therefore, did not belong to the survey. So far, 50 attempts have been made by inoculating the patient serum in LLC-MK2 cells. Plaque-forming agents were recovered from 11 cases. Three of these agents have been identified as dengue 2 virus. The remaining ones are under study. By inoculations of patient sera into baby mice and subsequent blind passages, we have been unable to produce overt disease in the mice. However some of the them have exhibited in their brains antigen, reacting with anti-dengue serum (kindly supplied by Dr. B.L. Cline) when immunofluorecent techniques were used. In addition, other inoculated mice have shown HI antibodies for dengue 2 and dengue 3 one month after inoculation.

Xenodiagnosis

Xenodiagnoses (using laboratory-reared A.aegypti) were performed on 34 patients. Using also immunofluorescent techniques, antigen reacting with dengue antibody was found in mosquitoes from 23 of the 29 xenodiagnoses thus far studied. Some of these "positive" xenodiagnoses corresponded to patients whose sera had not yielded viruses when inoculated into LLC-MK2 cells. The correlation between both procedures is shown in Table 2, indicative of the higher sensitivity of xenodiagnosis for detecting dengue.

Studies on paired sera and convalescent sera

Although we have not completed yet the study of 58 paired sera, it is important to mention that in the majority of the sera thus far studied, when dealing with apparently primary infections, the second specimen shows CF antibodies both for dengue 3 and dengue 2, the titer being higher to the latter. This is not exactly what we observed during the dengue outbreak of 1971-1972, when, also in primary infections, the majority of the cases exhibited CF antibodies only for dengue 2, when their second serum was studied. Moreover, there have been a few convalescents in Armero, also apparently primary infections, who exhibit CF antibodies higher for dengue 3 than for dengue 2 and no antibodies for other group B agents. We are studying this rather confusing situation since it indicates an immunological response broader than the one we were familiar with. One could speculate that the broad response could be due to the presence of a dengue 2 strain slightly different from the one isolated from the coast three years ago, even the presence of some dengue 3 infections (hence the necessity to identify all the isolated strains) or merely differences in the quality of the antigens used. Although we have been unable to compare the antigens utilized three years ago with the ones we are using now, our current antigens behave in a similar way to those prepared in well-known U.S. institutions, which we keep in our institution for our quality control.

Complications of dengue

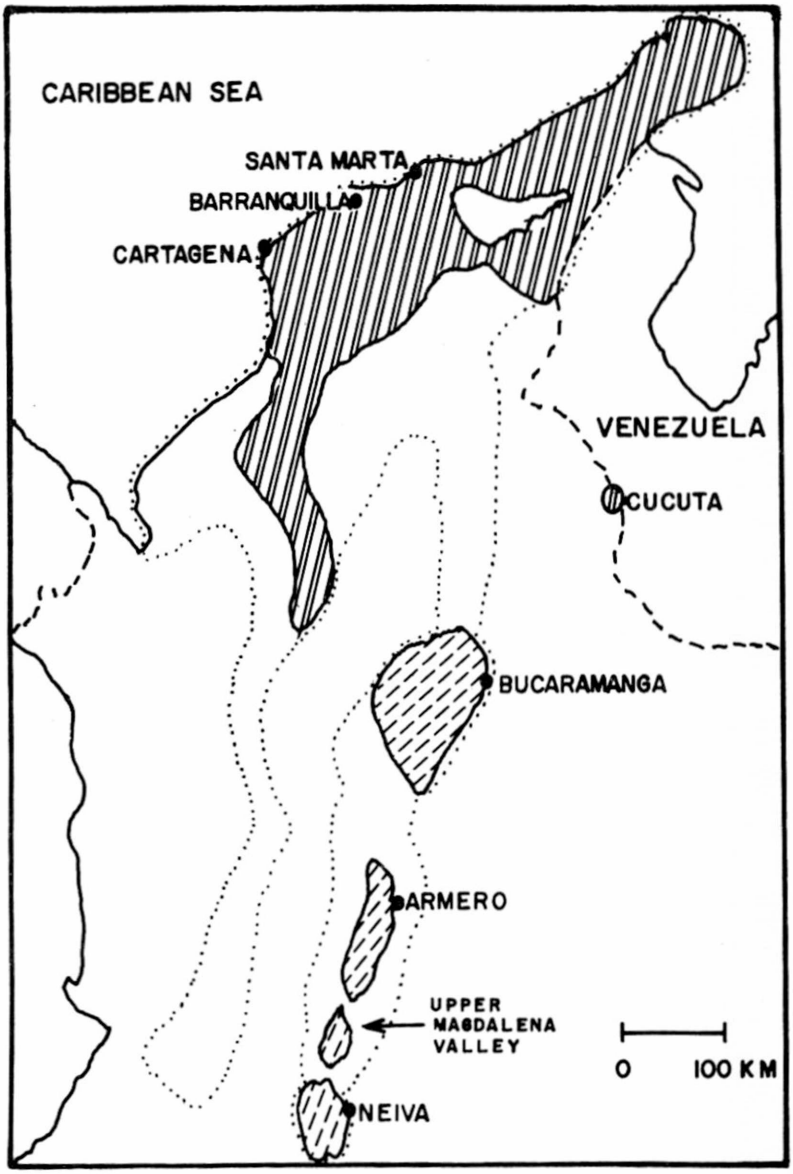
A systematic clinical study of new dengue cases, including the proper laboratory tests, has been started in the Upper Magdalena Valley in order to answer the question whether or not DHF or shock syndrome occurs in Colombia. It is a question we cannot answer at present. According to the available information we are able merely to say that such complications have never been reported from the country.

(Hernando Groot, Alberto Morales, Margarita Romero and Hernando Vidales)

Bogotá, 20 de febrero, 1976

FIGURE 1

REINFESTATION OF COLOMBIA BY *AEDES AEGYPTI*



AREAS WHERE THE MOSQUITO WAS PRESENT UNTIL 1949 AND FROM WHICH IT WAS ERADICATED DURING 1952–1960

AREA REINFESTED BETWEEN 1969 AND 1972

AREA REINFESTED SOMETIME BETWEEN 1972 AND MID-1975

Table 1

Dengue antibodies in 462 human residents from Armero, Colombia. Serum specimens obtained between Sep 17 and October 2, 1975, during an outbreak of Dengue Fever.

Category of donor	Total number of cases	Number of cases positive for dengue antibodies *	Percentage of cases with dengue antibodies
Acute febrile disease compatible with dengue when serum was obtained	51	16	31
Convalescent from febrile disease compatible with dengue (serum obtained 5-20 days after onset of disease)	74	65	88
Convalescent from febrile disease compatible with dengue (serum obtained 21 or more days after onset of disease)	107	97	91
History of previous undefined febrile disease	23	17	74
No history of dengue or any febrile episode during 90 days before day when blood was taken	207	36	17

* All sera were tested by CF and HI with dengue 2 and dengue 3. Sera with titers 1:8 or greater by CF with any one of the two antigens, or with titer 1:4 by CF accompanied by HI titer of at least 1:80 were considered positive.

FIGURE 2

ARMERO, COLOMBIA, JUNE 25-OCTOBER 2, 1975: DAILY OCCURRENCE OF 540 FEBRILE CASES WITH DIAGNOSIS COMPATIBLE WITH DENGUE

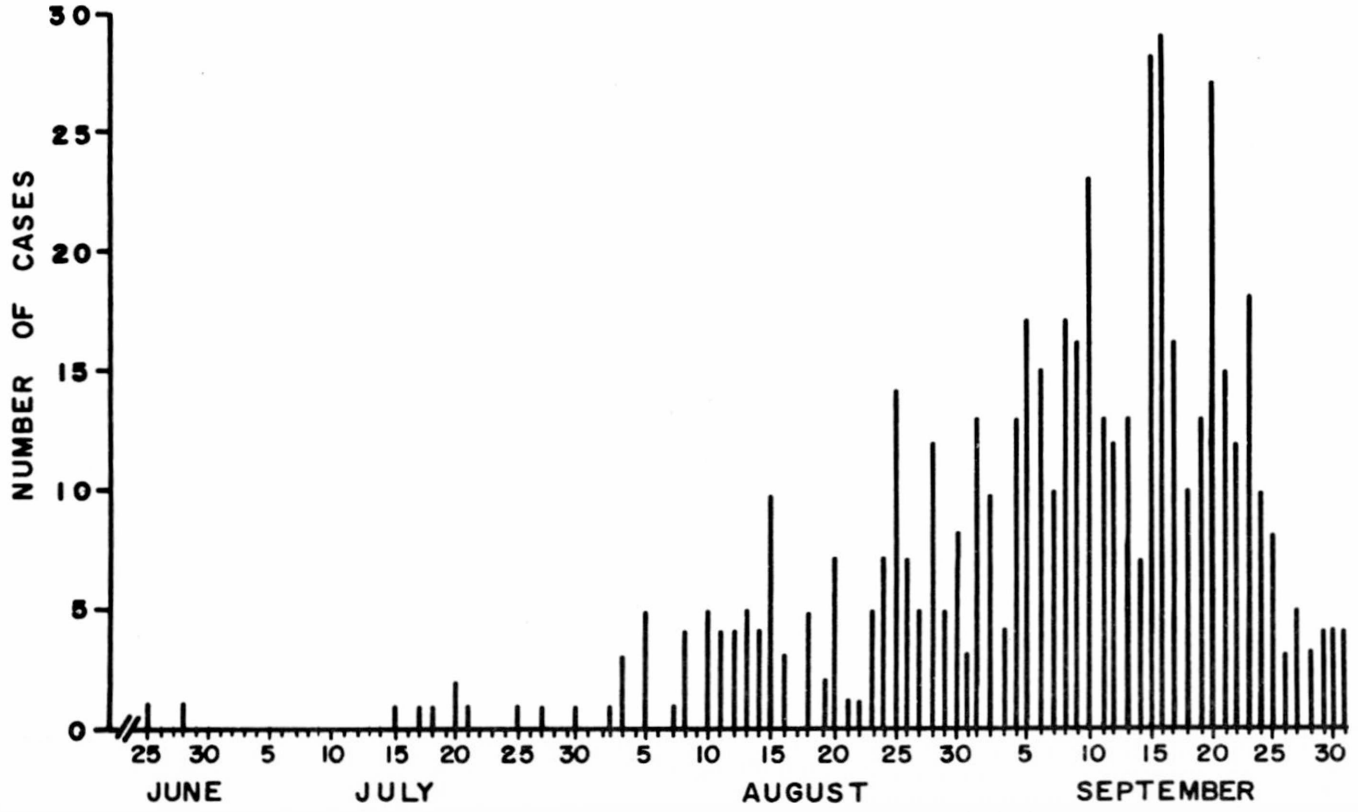


Table 2

Comparative results of xenodiagnoses performed on 29 febrile patients, presumably with dengue, and inoculations of their sera into LLC-MK2 cells cultures. (Xenodiagnosis and bleeding for obtention of serum were made simultaneously in each case).

		Xenodiagnosis		
		Positive [a]	Negative	Total
LLC - MK2 CELL CULTURE	Positive [b]	5	0	5
	Negative	7	3	10
	Under Study	11	3	14
	Total	23	6	29

[a] Positive : Presence in mosquito of antigen reacting with anti-dengue serum in immunofluorescent test.

[b] Positive : Development of plaque-forming agents, identified in 3 cases as Dengue 2, not yet identified in two cases.

REPORT FROM THE EVANDRO CHAGAS INSTITUTE, BELEM FSESP, BRAZILIAN MINISTRY OF HEALTH, BELEM, BRAZIL

Epidemic Oropouche virus disease.

1. Santarém area.

An explosive outbreak of human febrile illness caused by Oropouche vírus occurred in several localities of Santarém county in 1975. The county is located at the confluence of the Tapajós and Amazon rivers, in the Amazon region of Brazil.

The epidemic was first recognized in February 1975, in the village of Mojuí dos Campos, which is located some 20 Km south of the city of Santarém. This city and Belterra were affected in the following months, probably as a result of the spread of the epidemic which took place in and around Mojuí, mainly in the Palhal area. The epidemic was also recognized in the small village of Ater-do-Chão located about 15 Km from Santarém city, during the period July- August.

Altogether some 90.000 people live in these towns. The attack rate was estimated to be 20-30%, on the basis of the presence of HI antibodies in the sera of persons bled after the epidemic was over.

Serological studies carried out late in November showed no signs of an epidemic of Oropouche in 3 small towns (Óbidos, Alenquer and Monte Alegre) located immediately across the Amazon river, despite constant river traffic between these three towns and Santarém.

The major clinical symptoms consisted of fever, chills, headache, myalgia, arthralgia, dizziness and photophobia. These symptoms were present in 63% or more of the patients. No deaths could be attributed to the disease but several patients became severely ill, occasionally to the point of prostation. A significant number of people reported one or more episodes of recurrence of the symptoms. Most recurrences occurred 10 or more days after the initial onset. Others, however, informed us that the time between recurrence was only 1 or 2 days. The recurrence was characterized sometimes by the reappearance of the same symptoms present in the initial bout, though often with more intensity. At other times it consisted of only headache, myalgias and asthenia.

On the basis of virus isolations and serological conversions, 55 acute cases were diagnosed in Mojuí dos Campos and 26 in the Palhal area. Data of this kind are not available yet for the other localities affected by the epidemic.

There were no marked differences in the distribution of cases between the sexes.

More than 25.000 biting midges and over 10.000 mosquitoes were collected, most of them in the village of Vojuí and the Palhal area. Over 95% of the midges were Culicoides paraensis. Three strains of Oropouche vírus were isolated from Culicoides. One of these isolates came from engorged midges and in the case of the other two isolates the midges were not checked for the presence of blood meal. Four isolations of Be An 109303 vírus of the Guama group were obtained from Culicoides. No viral agent was recovered by inoculation of mosquitoes (most of them Culex p. quinquefasciatus) into mice.

Of the 602 wild and domestic mammals examined for the presence of Hl antibodies to Oropouche vírus only one rodent of the genus Proechimys was positive. All reptiles examined were negative. However, 34 (4.9%) out of the 681 wild birds and 12 (5.8%) out of the 206 domestic birds examined were positive for the presence of Hl antibodies to Oropouche vírus.

2. Itupiranga

The epidemic in the town of Itupiranga probably started in April of 1975 but it was not recognized until June, when laboratory confirmation of cases was obtained. This town has about 3.000 inhabitants and it is located on the left side of the Tocantins river, some 400 Km south of Belém, and over 600 Km from Santarém.

Both in Itupiranga and in Alter-do-Chão transmission of Oropouche vírus to baby mice and to young mice exposed to the bite of wild caught Culicoides was attempted, with negative results.

This finding, together with the low rate of isolation from Culicoides in these epidemics, the lack of isolates from Culex p. quinquefasciatus in these epidemics, and the low isolation rate from mosquitoes in past epidemics, indicates that the main vector or vectors of this disease are still unknown. Oral infection seems not to play a role in the transmission of the vírus, since the agent could not be isolated from the throat of dozens of viremic cases.

Francisco P. Pinheiro, G. Bensabath, Amélia A.T. da Rosa and Jorge F.T. Rosa.

Arbovirus-macrophage interaction in vitro

The intracellular mechanisms that enable macrophages to inactivate or otherwise restrict replication of virus remain obscure, but in some instances this function is the critical determinant of host susceptibility to infection by viruses.

The time course of extracellular infectious virus titers of arbo-infected peritoneal macrophages from mice 6 to 7 weeks old were followed in vitro.

Eastern equine encephalitis, Semliki forest virulent and avirulent, Middelburg, West Nile, yellow fever and western equine encephalitis viruses were used.

The most important results can be summarized as follows:

1. For all viruses tested, new synthesized infectious virus was found in the culture medium after 6 hrs incubation.

2. After 6 hrs incubation, infectious virus titers decrease according to thermal inactivation, except for EEE, SFV and SFA.

3. There is no correlation between the lethal effect in mice after I.P. infection, and the degree of multiplication in peritoneal macrophages.

4. Infected macrophages show very low viral yields in comparison with L_{929} cells, infected with the same multiplicity of infection.

5. A less pronounced multiplication of SFV and SFA occurs in macrophages from mice previously immunized with SFA.

6. Multiplication of SFV and SFA was slightly enhanced in proteose peptone stimulated macrophages.

7. In contrast with normal macrophages, SFA immunized macrophages show a clear cytopathogenic effect after inoculation with homologous and heterologous virus.

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

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

Isolation of further TBE strains of the tick species Ixodes ricinus in the Federal Republic of Germany.

In 1975 another 7 strains of TBE virus could be isolated from adults and nymphs of the tick Ixodes ricinus. The ticks (6085) were collected from sites in Baden-Württemberg and Bavaria. In these localities Central European Encephalitis could be identified as a human disease. The titers obtained after four IC passages in suckling mice were in the order of 8.5 to 9.4 log 10. With a human convalescent serum all strains showed a neutralizing reaction. For the locality of Karlsruhe a minimum-infection index (MII) of 2.00 and for Pfaffenhofen (60 km north of Munich) a MII of 1.07 could be determined.

Region and Country	No. of ticks	strain	No. of ticks/ pool	NI
Karlsruhe Baden-württemberg	499	K 23	25 N	⩾4.4
Pfaffenhofen Bavaria	5586	B 266	50 N	3.4
		B 287	50 N	⩾3.9
		B 325	50 N	⩾4.3
		B 358	50 N	⩾4.6
		B 365	10 ♀♀	3.8
		B 369	10 ♀♀	⩾4.3

(R. Ackermann, B. Rehse-Küpper, B. Abar and W. Klenk)

REPORT FROM ARBOVIRUS UNIT, VIRUS DEPARTMENT

ISTITUTO SUPERIORE DI SANITA', ROME, ITALY

Tick-borne encephalitis in Italy

Several cases of an acute disease of the central nervous system, causing often a severe encephalitis or meningo-encephalitis, are reported in different Italian regions. The available information on the clinical picture of the disease did not give any indication on the nature of the causative agent. In order to study a possible role of arboviruses as etiologic agents of meningo-encephalitis in Italy, we decided to study the cases which were occurring during 1975 in the province of Florence and were hospitalized at the Departments of Neurology and Infectious Diseases of the University of Florence. During 1975 we received samples from 29 patients: virus isolation was attempted from blood and/or cerebro-spinal fluid taken during the acute phase of the disease from 15 of them, serological tests were performed on single or paired sera obtained from 25 of them. The great majority of cases (22 out of 29) occurred during the period between May and September 1975.

No virus isolation was obtained up to now from blood and cerebro-spinal fluid after inoculation into suckling-mice; passages from two samples are still in progress. Acetone-extracted sera were examined by HI test against five arbovirus antigens (WEE, EEE, TBE, WN and Bhanja) (Table 1). Sera from three patients reacted with TBE antigen. Treatment with 2-mercaptoethanol lowered the HI titres of the acute samples in two out of the three cases, indicating a primary infection. The only serum available from the third positive patient was taken nearly three weeks after the onset of the disease and did not show a titre reduction after 2-ME treatment, possibly because of the long interval elapsed. Neutralizing antibodies were present in the acute serum samples of the two serologically confirmed cases of TBE infection.

The three cases were adults, non fatal and occurred respectively on June, July and November 1975. The major symptoms were fever, nuchal rigidity, flaccid paresis and respiratory insufficiency. The examination of the cerebro-spinal fluid showed pleocytosis, high protein content and positive Pandy reaction. The three patients have been living in Central Italy for the last years, no history of travelling is recorded. The two confirmed TBE cases occurred in small villages in the province of Florence where cultivated and wild areas are present.

For the first time TBE virus could be implicated as responsible for cases of human encephalitis in Italy, although serological evidence of circulation of TBE virus was previously demonstrated.

(Clinical examination and treatment of the patients were performed by Prof.L.Amaducci, Dept.of Neurology and Prof.P.Paci, Dept.of Infectious Diseases of the University of Florence).

Dr.P.Verani, Dr.M.Balducci, and Dr.M.C.Lopes

Table 1 - Serological tests with patients' serum samples against Hypr strain of TBE virus.

Patient	Date of sample collection	Days after onset	HI		CF	N
			before 2-ME	after 2-ME		
L.G.	June 18/75	8	640[+]	20	<8[+]	3.82[++]
	June 25/75	25	320	40	<8	n.d.
	July 17/75	37	320	160	8	n.d.
M.S.	July 6/75	-	320	20	n.d.	2.40
	Dec. 12/75	-	80	80	n.d.	n.d.
B.V.	Dec. 13/75	-	320	320	n.d.	n.d.

[+] - Reciprocal of serum dilution

[++] - NI_{50}

n.d.- not done

REPORT FROM THE CZECHOSLOVAK ACADEMY OF SCIENCES, INSTITUTE OF PARASITOLOGY, PRAGUE, CZECHOSLOVAKIA

Study of Lednice (Yaba 1) virus in Macaca mulatta monkeys

To explain the significance of Lednice (Yaba 1) virus for primates and at the same time indirectly for man, we followed the course of infection of Macaca mulatta monkeys clinically, virologically, and serologically.

In two experiments the monkeys were infected with strain 6118 of the virus by subcutaneous injection of 40 and 460 mouse LD_{50} per gramme of body weight. Behaviour of monkeys, their appetite, body temperature and weight, as well as the blood picture, virus and antibodies in the blood, and virus in the regional lymph nodes, were followed.

During 4 months of observation no apparent symptoms of disease or changes in behaviour of the monkeys were observed; also body temperature was within normal limits. The complete hematologic investigation carried out as long as 29 days p.i. revealed that no significant changes in the blood picture had occurred which would signal damage of any of the experimental animals during the experiment. Virological findings were also scanty: in no animal was viremia detected in the course of 15 days p.i. In spite of the fact that specific fluorescence of viral antigen was observed in regional lymph nodes 3 and 5 days p.i., we were unable to prove the presence of any virus capable of passaging in suckling mice. Antibody response (neutralizing antibodies detected in suckling mice) could be shown convincingly 7 days p.i. only in those animals that had received the higher dose of virus or after reinfection.

From the results obtained we conclude that the Macaca mulatta monkeys are not susceptible to the Lednice virus and that these animals apparently

do not play any role in spread of infection. However, the immunoresponse of monkeys does provide a possible explaination of the negative serologic findings in man: the infection of man in nature by the very small dose of Lednice virus transmitted by mosquitoes does not stimulate a detectable antibody response. Also, the very low minimal infection rate of mosquitoes with Lednice virus in nature limits exposure of man.

(D. Malkova, V. Danielova, L. Viktora, and J. Holubova)

*Isolation of Tahyna virus from field collected Culiseta annulata (Schrk.) larvae

797 larvae of the second to fourth instars were collected between July 3-5, 1974 from the puddles of the river bed on the edge of a forest near the village of Drnholec (southern Moravia). The larvae were taken to the laboratory in plastic flasks containing puddle water and separated under the microscope as being A. vexans (237 samples). A. cinereus (240 samples) and Culiseta annulata (320 samples). All larvae were of the first generation since due to unusually dry summer the hatching of mosquitoes was delayed. Their entomological determination was immediately followed by virologically processing.

From eleven baby mice (1-2 day old) inoculated i.c. with a mixture of suspensions taken from the first tubes designated LA 65 and LA 66 (Culiseta annulata) one mouse was eaten on the 10th day. Reisolation experiments were done by inoculation from a replicate set of up-to-now unopened tubes. From the baby mice inoculated i.c. with suspension LA 65 five mice died on the 10th day, one on the 11th day and one survived. In the next passage the mice died on the 5th and 6th days. In the third passage all inoculated mice died between the 2nd and 6th days. The isolate was identified as a strain of Tahyna virus.

*Isolation of Tahyna virus from the blood of sick children

In the summer months of 1974 we were regularly visiting several children's outpatient clinics and one children's ward in the district hospital in southern Moravia to take blood for virus isolation from all children having fever. Blood from each child was taken into two tubes containing heparin solution (5 I.U./1 ml) and into a third one for serological testing. Samples for virus isolation were immediately placed in a portable ice box at -2 to -4°C and kept there for 1 to 4 hours until the tubes could be put in a freezer at -65°C at the laboratory. The heparinized blood was inoculated i.c. into SPF (strain H) 1- to 2- day-old mice.

Case No. 1: H.L., a boy aged 4 years, became ill on 25 August, 1974 with an acute onset of fever (37.8°C), malaise and a mild pain in the throat. The next day the evening temperature of 38.5°C plus ear aches were recorded. On 27 August in the children's outpatient clinic the temperature of 37.8°C was recorded and conjunctivitis and pharyngitis was observed. Blood was taken for virus isolation. On the next day (for the last time) the temperature of 37.2°C with a mild cough was recorded. Tahyna virus was isolated from the patient's blood.

In the serum of the patient (diluted 1:4) taken on the day of virus isolation, neutralizing antibodies against 2.7 $\log_{10}$ of the isolated virus were not detected (in suckling mice) but in the serum taken two months later a dilution of serum 1:24 protected 50% of the mice against the dose mentioned above.

Case No. 2: K.R., a boy aged 7 years, became ill in the evening of 8 September with an acute onset of fever (38.8°C). The next day he was taken to the children's outpatient clinic where meningeal symptoms were observed and the boy collapsed. He was immediately sent to the children's hospital where fever of 38.2°C with sore throat and influenza-like symptoms were recorded. The ES rate was 28/60 and the leucocyte count was 4,900 per mm^3. Blood was taken for virus isolation. From 11 September he was without fever but on 16 September fever of 39.0°C for the last time was recorded. Tahyna virus was isolated from the patients blood.

In the serum (diluted 1:4) taken on the day of virus isolation, neutralizing antibodies against 2.0 $\log_{10}$ of the virus were not detected. In the serum taken 7 months later (in April) a dilution of serum 1:214 protected 50% of the mice against the test dose mentioned above.

(V. Bardos, J. Ryba, Z. Hubalek and M. Medek; V. Kania from the District Health Center, Breclav)

*Editor's note: This report was received too late for inclusion in Issue No. 29, due to mailing difficulties. The essential data have since been published in Acta Virologica No.5/1975.

REPORT FROM THE D.I. IVANOVSKY INSTITUTE OF VIROLOGY, MOSCOW, USSR; THE YALE ARBOVIRUS RESEARCH UNIT, NEW HAVEN, CONNECTICUT, USA; AND THE WHO VIRUS DISEASE UNIT, GENEVA, SWITZERLAND

Analysis of the results of the collaborative assay of the Chik, Den-2 and JE inactivated antigens

Collaborative studies of laboratory diagnosis of dengue (DEN) and chikungunya (CHIK) haemorrhagic fever in Southeast Asia and Western Pacific Regions were carried out during 1972-1974. The objectives of these studies were (1) to investigate the possibility of using inactivated noninfectious antigens for HI and CF tests; (2) to standardize the procedures of the two tests; (3) to ascertain whether the laboratories are ready to perform diagnostic studies. The study was initiated by Dr. Paul Bres (WHO).

Noninfectious CHIK, DEN-2 and JE antigens were prepared in the Arbovirus Department, the D.I. Ivanovsky Institute of Virology, USSR Acad. Med. Sci., Moscow (Prof. S.Ya Gaidamovich), and tested at Yale Arbovirus Research Unit (USA) by Dr. R.E. Shope and Dr. J. Casals. Coded sera were supplied by the Yale Arbovirus Research Unit, USA (Dr. R.E. Shope). The antigens and antisera were distributed to all laboratories in September, 1973. The assay results were received from 10 laboratories. Laboratories in Jakarta and Fiji did not participate. The only method used in both laboratories in Burma was the HI test.

METHODS

The protocol for the assay of the antigens or sera in HI and CF tests was worked out by Dr. P. Bres (WHO) and discussed with all the participants. Along with the methods recommended by the WHO, laboratories were to test the antigens and sera simultaneously by their routine methods if these differed from the technique described in the protocol. A micromethod was recommended; however a macromethod might be used as well. Laboratories, where the procedure of performing the tests differed from that recommended by the WHO, were instructed to carry out the assay by a routine method along with the WHO method.

Each laboratory received sets containing CHIK, JE and DEN-2 antigens. CHIK and JE antigens were supplemented with homologous immune ascitic fluid, one ampule each. The set of coded sera

contained sera numbers 1-21, one ampule each.

Antigens were prepared from infected suckling-mouse brain tissue by sucrose-acetone extraction, inactivated with betapropiolactone (BPL) and lyophilized. Descriptions of the antigens are presented in Table 1. All antigens were tested by Dr. R.E. Shope and Dr. J. Casals at the Yale Arbovirus Research Unit prior to the collaborative assay. The results of the titration completely coincided with those obtained in the laboratory of manufacture. The antigens had been sent to the WHO in April-May, 1972, where they were stored at -20°C until September, 1973. The antigens had been mailed at environmental temperature to collaborating laboratories where they were kept at 4°C until tested. Thus, from 1.5 to 2.5 years elapsed from antigen preparation to the assay.

Coded sera were prepared by Dr. R.E. Shope at the arbovirus laboratory, Yale University. There were 7 sera in all, 3 ampules each. The sera were coded as follows.

Serum	Ampule No.	
I	1,5,10	Immune ascitic fluid (IAF) to Dengue 2 virus
II	2,3,11	IAF to Japanese encephalitis virus (Nakayama strain)
III	6,15,21	IAF to a group A virus (chikungunya)
IV	16,18,20	Normal IAF
V	4,7,8	Human blood serum
VI	9,13,17	A mixture of IAF to Dengue 2 and group A virus
VII	12,14,19	A mixture of IAF to Japanese encephalitis and group A virus

RESULTS

Laboratories in Moscow, Poona, and Singapore employ a routine method which coincides with the method recommended by the WHO, therefore the assay was performed only by one method. Differences in routine techniques used in other laboratories are as follows:

Australia - a macromethod is used;

Rangoon - MRI and National Laboratory - a modified technique is used for preparing buffer solutions though their composition is the same;

Kuala Lumpur - V-plates are used instead of U-plates; sera for the HI test are treated with acetone;

Hong Kong - in the HI test sera are treated with acetone for 1 hour;

Korea - sera are treated with acetone, a macromethod is used.

Table 2 summarizes the results of antigen titration in the HA test. If laboratories failed to send the protocol of antigen titration, the titer was calculated by the results of the control titration of 8 U of antigens.

The results of the assay showed that the antigens retain potency for 2 1/2 years after preparation, with the titer of CHIK and JE antigens unchanged. DEN-2 antigen is less stable, a two-fold reduction in its titer was observed in 2 1/2 years. Dr. Mi Mi Khin, the Burma Medical Research Unit, Rangoon, carried out detailed studies in antigen stability over a 1-year observation period (personal communication). According to Dr. Khin's data, CHIK and JE antigens, kept dissolved at 4°C, showed the original titer at 3 weeks, with only a two-fold reduction in titer after a year whereas DEN-2 antigen completely lost its activity.

The results of antigen titrations in various laboratories coincided, sometimes the titer was one dilution higher or lower than that described by the manufacturer. In some instances the optimum pH also varied from the average acid or alkaline values by 0.2. Occasionally, the antigen titration done by the WHO method provided worse results than by routine methods (MRI, Rangoon; Australia) which fact is probably explained by less experience in using the WHO method.

HI and CF tests were to be performed within 3 days: screening on day 1, antibody titration on days 2 and 3. The results are presented in Tables 3, 4 and 5. The data obtained on day 2 are summarized according to the WHO method. To make the analysis procedure more convenient, identical samples of the serum were grouped according to the code. Thus, each serum bearing differentnumbers was repeated 3 times. This makes it possible to compare the reproducibility of the results of the assay of each serum in 3 samples.

Serum I. The serum reacted with antigens of dengue and JE in HI and CF tests, however, it reacted with the dengue antigen in a higher titer, therefore the reaction with the JE antigen is group specific. Rare positive results in HI and CF tests with CHIK antigen are apparently nonspecific.

Serum II. A high titer of antibodies to JE antigen in the HI test (above 160) and the CF test (above 128) was demonstrated. The reaction with dengue 2 virus in the HI test (up to 80) and in a low

titer by the CF test was group specific. The serum did not react with CHIK antigen inthe CF test. Rare positive reactions in a low titer in the HI test with CHIK virus were apparently explained by inhibitors.

Serum III. There was a high titer of antibodies to CHIK antigen in HI and CF tests. Antibodies to dengue 2 and JE virus were not found.

Serum IV. Control normal IAF. Positive results in the HI test in some samples (in a titer of 10-20) were possibly due to nonspecific inhibitors. Some positive results in the CF test with all antigens were accounted for by anticomplementarity of the serum.

Serum V. The serum reacted with JE and dengue antigens in the HI test, reacting with JE antigen in a higher titer. There was a weak reaction with both antigens in the CF test which may have been in part explained by anticomplementarity of the serum. The results of the assay indicate that in this serum there were antibodies against group B arboviruses.

Serum VI. The serum contained antibodies to all antigens tested. The highest titers of antibodies revealed in HI and CF tests were those to dengue virus. The titer of antibodies in the HI test with JE antigen was somewhat lower than with dengue virus and in the CF test it was considerably lower. With CHIK antigen, a higher titer of antibodies was observed in the HI test and a mean level in the CF test.

Serum VII. The serum contained antibodies to all antigens, but predominant were antibodies to JE antigen with which all samples showed highest titers in HI and CF test. With the Dengue antigen, the serum reacted in the HI test and it reacted very weakly in the CF test. With the CHIK antigen, the serum reacted in the HI test and it reacted distinctly in the CF test.

Thus, the results of the assay were correct and quite consistent for the IAFs and sera employed. However, it should be noted that absolute values of the titers of different samples of the same serum often differed both in different laboratories and within one laboratory. These differences were more manifest in cases with low antibody titers and less manifest in cases with high antibody titers.

There were some paradoxical results when extremely contradictory values were obtained with one of three samples of the same serum.

Differences in antibody titers positively reacting in the HI test in different laboratories may be associated with the use in

the HI reaction of 8 units in some laboratories (Moscow, Poona, MRI in Rangoon) and of 4 units of antigen in others (Singapore, Kuala Lumpur). It is likely that in some instances the difference in titers depends on varying qualities of kaolin which can partially absorb antibodies during treatment of sera.

The analysis of the results makes it possible to make the following conclusions:

1. Inactivated liophylized CHIK, JE and dengue 2 antigens retained their specific activity and stability of titer for over 2 years.

2. Differences in the procedures of tests performed according to a routine method and the method recommended by the WHO, except in the Hong Kong laboratory, are not critical and actually did not influence the results.

3. Absolute values of antibody titers in the HI test in different laboratories coincided if the titer of antibodies was high. When the titer of antibodies was low the differences in titers were considerable.

4. With the sera used, group specific reactions in the CF test were less marked.

5. Reproducibility of the results and preference for the HI test or the CF test for diagnosis is determined to a considerable degree by past experience. Most laboratories use the HI test and in Singapore the CF test is preferred.

6. For a more complete evaluation of inactivated antigens, it would be desirable to carry out examination of paired sera of patients simultaneously with live and inactivated antigens in several laboratories.

(S.Ya. Gaidamovich, R.E. Shope, J. Casals, P. Bres)

TABLE I DESCRIPTION OF INACTIVATED ANTIGENS

Antigen	Strain	Date of preparation	Series	Titer HA Test	Titer CF test	Optimum pH
Chikungunya	Ross	February, 1972	3	5120	620	
		February, 1972	4	2560	320	6.2 - 6.4
		February, 1972	5	2560	640	
Dengue-2	New Guinea	April, 1972	40	640	320	
		April, 1972	43	640	320	6.4 - 6.6
		April, 1972	46	160	320	
Japanese encephalitis	P-I	February, 1972	35	1280	320	6.2 - 6.4
		February, 1972	39	2560	320	

TABLE 2. ANTIGEN TITRATION IN THE HI TEST

Laboratory	Date	Chikungunya			Japanese encephalitis			Dengue-2		
		Series	Optimum pH	Titer	Series	Optimum pH	Titer	Series	Optimum pH	Titer
Moscow	11/73	3	6.2-6.4	5120	39	6.2-6.4	2560	46	6.4-6.6	640
Poona	after 3/74		6.4-6.6	3840		6.4	3840		6.6	960
Calcutta	after 3/74		6.2	2560		6.2	5120		6.4	640
Hong Kong	10/73		6.4	2560		6.2	2560		6.4	1280
Korea	after 3/74		6.4	640/320		6.4	1280/640		6.6	160
Rangoon, RMI	10/73	4	6.4	320/2560	39	6.2	1280/2560	46	6.4	160/640
Rangoon, Nat.Lab.	9/74	4	6.2-6.4	2560	39	6.2-6.4	2560	46	6.2-6.4	640
Australia	11/73		6.4	320/2560	39	6.2-6.0	320/5120		6.4	80/640
Kuala Lumpur	15/11/74	4	6.2-6.4	1280		6.0-6.2	2560	40	6.2	320
Singapore	4-5/12/74	4	6.2	2560	39	6.2	2560	40 and 46	6.2	640

Methods: WHO/Routine

TABLE 3. TITRATION OF SERA WITH ANTIGEN OF DENGUE-2 VIRUS

Serum (IAF)	No. ampules	Titer in HIT				Titer in CFT				
		10	10-20	40-80	160	4(8)	4-16	32-64	128	Anticomplementary
I	1			3	7			3	4	
	5			3	7			2	6	
	10	1		4	5			2	6	
II	2		2	7	1	7	1			
	3		4	5	1	7	1			
	11	1	4	5		7		1		
III	6	9	1			8				
	15	9			1	8				
	21	10				8				
IV	16	8			1	4		1	1	2
	18	8	1			3		2		3
	20	10				3			2	3
V	4		3	6	1	6	1			1
	7		4	6		4	2			2
	8		4	5	1	5	2			1
VI	9	1		5	4			4	4	
	13			5	5			5	3	
	17			3	6			5	3	
VII	12		5	4	1	7		1		
	14		4	6		7	1			
	19		3	7		5	2			1

TABLE 4. TITRATION OF SERA WITH ANTIGEN OF JAPANESE ENCEPHALITIS VIRUS

Serum (IAF)	No. ampules	Titer of HIT					Titer of CFT			Anticomplementary
		10	10-20	40-80	160	4(8)	4-16	32-64	128	
	1		1	5	4	4		3		
I	5		1	6	3	3	2	3		
	10	1	1	5	3	3	3	1	1	
	2				10				8	
II	3			1	9				8	
	11				10				8	
	6	9	1			7	1			
III	15	8	1		1	8				
	21	9	1			8				
	16	9			1	4	1		1	2
IV	18	9		1		4	1	1		2
	20	10				4	1	1		2
	4			7	3	7				1
V	7			7	3	4	2			2
	8			7	3	6	1			1
	9	1	1	5	3	3	4	1		
VI	13		3	5	2	4	3	1		
	17		2	4	3	5	3			
	12				10		1	7		
VII	14		1		9			8		
	19				10			8		

TABLE 5. TITRATION OF SERA WITH ANTIGEN OF CHIKUNGUNYA VIRUS

Serum (IAF)	No. ampules	Titer of HIT 10	10-20	40-80	160	Titer of CFT 4(8)	4-16	32-64	128	Anticomplementary
	1	9	1			7		1		
I	5	10				7	1			
	10	9			1	7	1			
	2	9	1			8				
II	3	10				8				
	11	7	2		1	8				
	6	1	1	3	5	1	3	4		
III	15	1		4	5	1	4	3		
	21			4	6	1	3	4		
	16	7	1		1	4		1	1	2
IV	18	6	3			4	1	1		2
	20	9			1	4	1	1		2
	4	6	4			7				1
V	7	4	5	1		7				1
	8	3	7			7				1
	9	1		8	1	1	6	1		
VI	13		2	6	2	2	5	1		
	17		3	4	2	2	4	1		
	12		3	5	2	2	5	1		
VII	14	1	3	5	1	2	4	2		
	19		2	5	3	2	5	1		