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Mary Ellen Gist, Secretary

COMMENTS FROM THE EDITOR

Readers will observe that the lead-off report in this issue of the Info-Exchange is the 1973 Annual Report on the Catalogue of Arthropod-borne and Selected Vertebrate Viruses of the World. Its inclusion in the Info-Exchange is a deviation from past procedure, as heretofore the annual reports of the Catalogue have been printed separately. However, in view of the fact that the Annual Report and the Info-Exchange are mailed to the same people, it seemed expedient to try putting the two together. We hope you like it. This comprehensive analysis reflects the painstaking care of Dr. T.O. Berge, Chairman of the Information Exchange Subcommittee and Editor.

Again congratulations are in order for the high quality of information submitted for inclusion in this issue. Much retyping effort was saved by those of you who observed the conventional Info-Exchange headings and spacings in the copy you submitted, as such material required mere simple paste-up to be camera ready. My secretary thanks you.

The deadline for submission of material intended for Info-Exchange Issue No. 28 is March 1, 1975. Please mark your calendar.

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ERRATUM

An error has been pointed out in the report of the Hormel Institute, p. 111 of Info-Exchange No. 26. The title at the top of the page should read, "Cultivation of high infectivity of Japanese encephalitis virus in BHK-21 shaker cultures employing reduced amounts of serum in Weymouth-oleate medium."

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1973 ANNUAL REPORT ON THE CATALOGUE OF ARTHROPOD-BORNE AND SELECTED VERTEBRATE VIRUSES OF THE WORLD*

By

THE SUBCOMMITTEE FOR EXCHANGE OF INFORMATION ON THE ARTHROPOD-BORNE VIRUSES

I. Objectives:

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

II. Materials and Methods:

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

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* The Catalogue is supported by the Research Resources Branch, Collaborative Research, of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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

Catalogues Distributed: Eight complete Catalogue sets and accumulated abstracts were issued to new participants in 1973. At the end of the year, 158 mailings of Catalogue material were being made, including 61 within the continental U.S.A. and 97 to foreign addresses. Distribution by continent was: Africa 14, Asia 19, Australasia (including Hawaii) 8, Europe 31, North America 69, and South America 18.

Abstracts and Current Information: 599 abstracts or references were collected, coded by subject matter, prepared on 3" x 5" slips, collated, and distributed to participants in the Information Exchange program. Of these, 378 were obtained from Biological Abstracts, 189 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 32 from personal communications or other sources. A total of 8,247 such references or units of information have been issued since the start of the program.

Recommended Antigenic Grouping: One new antigenic group, Thogoto, was added during the year to conform with the system of serological groupings recommended by the Yale Arbovirus Research Unit. The Thogoto virus is the only registered virus of this antigenic group.

Registration of "New" Viruses: Six viruses were initially registered in the Catalogue in 1973, as listed below.

Name of Virus	Year Isolated	Country	Initial Source	Antigenic Group
Chobar Gorge	1970	Nepal	*Ornithodoros* spp.	UNG.
Issyk-Kul	1970	USSR	*Nyctalus noctula*	UNG.
Northway	1972	USA	*Aedes* spp.	BUN
Okhotskiy	1969	USSR	*Ixodes putus*	KEM
Sokuluk	1970	USSR	*Vespertilio pipistrellus*	B
Zaliv Terpeniya	1969	USSR	*Ixodes putus*	UUK

Four viruses came from Asiatic USSR, one from Nepal, and one from Alaska. Three were first recovered from ticks, two from bats, and one from mosquitoes. All viruses were isolated between 1969 and 1972.

Virus Registration Withdrawn: The Abal virus C 5502 was withdrawn from the Catalogue during 1973 after it was found that it was indistinguishable from the Johnston Atoll virus by complement-fixation and neutralization tests. The addition of 6 viruses initially registered in 1973 and the withdrawal of one left the number of viruses registered in the Catalogue at the end of the year at 314.

Catalogue Revision: 79 registration cards for previously registered viruses were revised and reissued in 1973. About 24 registrations still remain to be revised. In addition, 99 registration cards were reprinted, some with relatively minor changes, as replacements for incorrectly printed cards.

Synopsis of Information in Catalogue: Owing to relatively minor changes or additions to much of the information summarized in the 1972 Annual Report, because of the small number of viruses registered in 1973, some of the tables included in the report for 1972 have been omitted here. Other tables have been changed somewhat in format and in manner of presenting data. While it is hoped that each figure and table is self-explanatory, the following discussion will be aimed at bringing out some of the salient points which can be found in the data summarized.

Figure 1. Year of Initial Isolation: The 6 viruses initially registered during 1973 have been added to this figure and the one withdrawn deleted, according to the year of discovery.

It can be seen that only 35 of the 314 registered viruses (11.1%) had been isolated in the first half of this century, with 86 per cent of all such catalogued viruses being initially discovered only in the past 20 years. The great proportionate increase in viruses recovered in nature began in 1954 with more than 50% of all registered viruses being first isolated since 1960. Allowing for the necessary time required for adequate study of recently isolated viruses to determine their antigenic distinctness from previously recognized agents (often 3 to 5 years), no trend toward a real decline in the reporting of new viruses can be seen.

As remarked in previous reports, the time of isolation probably bears little relation to the time of first existence of most of these viruses, but rather to the period of establishment and staffing of special arbovirus laboratories, and to the time when extensive general surveys began to determine the presence of unknown viruses in biting arthropods or naturally infected vertebrates.

Table 1. Alphabetical Listing of Registered Viruses: Table 1 serves as an alphabetical list of all viruses registered in the Catalogue at the end of 1973. Included after each virus name is a recommended abbreviation which is frequently employed for conservation of space when large numbers of viruses are being listed for special purposes. Antigenic groups to which viruses have been assigned are also shown in this table in condensed form. Where no antigenic group is indicated, the individual virus is unique in the sense that no serological (or immunological) relationship has yet been demonstrated linking it to any other known virus. In later tabulations, related viruses will be listed by antigenic groups as well as in other ways designed to suggest biological or morphological interrelationships.

Table 2. Antigenic Groups of Registered Viruses: It will be recalled that the originally described antigenic groups of arboviruses were designated A, B, and C, but in present practice, the first discovered virus of a newly recognized serogroup lends its name to the antigenic cluster. Viruses, to be assigned to an antigenic group, must be shown in some measure to be serologically related to, but clearly distinguishable from, a previously isolated virus.

Table 2 lists alphabetically the antigenic groups established for viruses registered in the Catalogue through 1973, and the number of registered viruses assigned to each group. Where only one virus is shown in a group, the registered virus has been shown to be related to one or more other viruses which have not been registered in the Catalogue. African horsesickness, Bluetongue and probably Epizootic hemorrhagic disease of deer have been designated as groups because there are a number of antigenically distinct strains belonging to each, although only one of each is registered.

In other groups, the degree of relationship between individual members of a serogroup may frequently be less close. A subcommittee of the American Committee on Arthropod-borne Viruses, known widely by the acronym, SIRACA (Subcommittee on Interrelationships Among Catalogued Arboviruses, Chairman: Jordi Casals), has been delegated general responsibility for the determination of whether antigenically related viruses are independent members of a serological group, or if they should be considered strains of a single virus.

Largely through the work of the Yale Arbovirus Research Unit (YARU) and the associated WHO Reference Centre for Arboviruses, at least 45 distinct antigenic groups have been designated and a supergroup conceived. Less than 20 percent of all registered viruses remain in an ungrouped category, i. e., have not been shown to be serologically related to any previously described viruses or to each other.

In some instances, a few of the member viruses within one antigenic group have been found to show small but reproducible cross complement fixation reactions with certain members of other groups, i. e., intergroup reactions. 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 belong to the supergroup but cannot be assigned to any of the individual groups. Taken collectively, the 84 viruses placed in the Bunyamwera Supergroup comprise more than one fourth of all viruses registered in the Catalogue.

The largest single antigenic group is Group B (51 viruses) followed by Group A (20 viruses). Six other groups contain more than 10 member viruses each (Bunyamwera, C, California, Simbu, Kemerovo, Phlebotomus); all other groups are below 10 in number.

Table 3. Initial Isolations by Decade with Country of Origin: While Figure 1 shows the total number of viruses isolated in each given year beginning with 1902, Table 2 lists the initial isolation of specific registered viruses by the decade of discovery and according to the continent or sub-continent and country from which each was first recognized. Because of the large number of virus names involved, recommended abbreviations are employed. These abbreviations and the associated complete names of the respective viruses may be found in the alphabetical list of registered viruses, 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 a specific search 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 far larger number 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. Many of the factors associated with the discovery of registered viruses have been summarized and discussed in previous reports and need not be considered further here.

Tables 4 through 23 list viruses primarily by antigenic groups; but an attempt has also been made, where practicable, to further group viruses by actual or suspected principal arthropod vector and/or by taxonomic status. It should be emphasized that categorization other than by antigenic grouping is based in part on tabulations suggested by YARU Annual Report, and in part is interpretive on the part of the editor; it is in no way authoritative. In each table is shown in condensed form information on isolation from arthropod vectors and vertebrate hosts, broad geographic (continental) area of virus occurrence, production of disease in man in nature or by laboratory infection, evaluation of arthropod-borne status, and proved or possible taxonomic status based on morphological or physico-chemical characterization of representative viruses of some serogroups. Much of the information upon which the classification by genera is here introduced has been provided by Jordi Casals and the SIRACA, by Frederick A. Murphy and by Robert E. Shope.

Table 4. 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 (SEAS rating 20 or 21. The criteria employed by the Subcommittee on Evaluation of Arthropod-borne Status (SEAS) for classifying catalogued viruses in this regard have been set forth in the Arthropod-borne Information Exchange newsletter Issue No. 23 (October 1972) and reviewed in Issue No. 26 (March 1974)).

Representative Group A arboviruses have been recovered on every continent and from many classes of vertebrates including man. All appear to be associated primarily with mosquito vectors, although some have also been recovered from naturally infected mites, small biting flies or midges and Triatoma. About half have been shown to produce disease in man, some 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, SIRACA considers that six immunological sub-sets exist, namely EEE, VEE, WEE, Semliki, Middelburg, and Ndumu. These are termed "complexes" although several consist of single viruses (or types), having no known close relatives (Arbovirus Infoexchange No. 21, Nov. 1970, p. 9-11). Other complexes consist of several closely related viruses which can be further subdivided into subtypes and/or varieties, although further critical serological evaluation is indicated.

In the present international taxonomic schema, Group A viruses form a genus Alphavirus in the 'family' Togaviridae as designated by the International Committee on Nomenclature of Viruses (ICNV). (Melnick, J.C., 1972, Prog. Med. Virol., 14:321)

Tables 5, 6, and 7. Group B viruses: The family Togaviridae also includes the large and important set of serologically related Group B viruses, all provisionally placed 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 principal vectors or other properties.

About one half (26) of the 51 registered viruses assigned to Group B appear to be transmitted naturally by mosquito vectors and have seldom been isolated from other blood-sucking arthropods. These are listed in Table 5 and again, with the exception of several insufficiently studied members, 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 and have been isolated on all continents, although only one representative (WN) has been reported from Europe.

The second important sub-set of Group B viruses include those which are tick-borne in nature (Table 6). These include 12 arboviruses, some of which are serologically very closely related or indistinguishable, but are said to be clearly differentiated on the basis of clinical, epidemiological and ecological markers. Members of the tick-borne Group B complex have not been reported from Africa, Australasia, or South America. They have not been recovered from mosquitoes or most other arthropods other than ticks, nor have isolations been reported from marsupials or sentinel animals. Human illness has been commonly reported, both in nature and as a result of laboratory exposure.

The remaining 13 viruses in Antigenic Group B (Table 7) have never been isolated from wild-caught arthropods or from sentinel animals and their arthropod-borne status is,therefore, in doubt. Six viruses have been recovered only from rodents, 5 from bats, and one each from man and domestic birds (turkey). None has been recovered in Australasia, Europe, or South America.

Tables 8, 9, 10, 11, 12, and 13. Bunyamwera Supergroup: In these tables are listed viruses placed in 10 serogroups plus 6 unassigned viruses, all of which have been assembled in the large Bunyamwera supergroup as discussed earlier (see p. 5). Different antigenic groups listed in a single table are done so for convenience; no significance should be attached to their juxtaposition other than alphabetical arrangement. In addition to their antigenic inter-relatedness, 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 (Murphy et al, 1973, Intervirol. 1:297-316). The family name Bunyaviridae has been proposed, with all member viruses of the Supergroup classified as the Bunyamwera genus.

Table 8. Bunyamwera Group: The Bunyamwera group consists of 17 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. Within the group, 5 complexes can be discerned (Arbovirus Infoexchange No. 22, June 1972, pp. 3-5), the members of which are more closely related antigenically to each other than to viruses falling in other complexes (sets) within the group. According to this view, the 5 sets can be given as follows, although more neutralization test data are said to be required to establish more firmly the degree of cross-relationships between individual viruses:

1. Bunyamwera (Bunyamwera, Germiston, and Ilesha).
2. Cache Valley (Cache Valley, Batai-Calovo, Lokern, and Main Drain. Maguari, Tensaw, and Tlacotalpan are considered to be varieties of Cache Valley Virus).
3. Wyeomyia (Wyeomyia, and Sororoca).
4. Kairi
5. Guaroa

Other later-registered Bunyamwera group viruses (Anhembi, Northway) were not included in the examination of data on viruses registered at the time of the SIRACA meeting in March 1971.

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. Twelve of 17 viruses are rated as arthropod-borne, with data on the remaining 5 inadequate to make a firm judgement.

Representatives of the BUN group have not yet been reported from Australasia, with one from Asia, 2 from Europe, 3 from Africa, 6 from South America, and 8 from North America. They have not been shown to be significant from the standpoint of production of disease in man or lower animals.

Table 9. Bwamba and C Groups: The Bwamba group consists of 2 serologically related, mosquito-associated arboviruses reported only from Africa. Bwamba 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 arthropod-borne or probably arthropod-borne viruses. Nine of the 11 have been associated with sporadic human febrile illnesses, and most have been shown to infect rodents or marsupials in nature.

Following examination of the available serological data, the SIRACA concluded that Group C arboviruses fell into 3 sub-sets or complexes, namely, Caraparu, Marituba, and Oriboca (Arbovirus Infoexchange No. 17, July 1968, pp. 5-7 and No. 21, Nov. 1970, pp 9-11). The Caraparu complex was found to contain the Caraparu, Apeu, and Madrid viruses, with Ossa considered a subtype of Caraparu. The Marituba complex consisted of Marituba (subtypes Marituba, Murutuca, and Restan) and Nepuyo (subtypes Nepuyo and Gumbo Limbo) viruses. The Oriboca and Itaqui viruses were placed in the Oriboca complex.

Table 10. California and Capim Groups: The 11 registered members of the California group are again primarily associated with mosquito vectors and most of them (9/11) have been reported from the western hemisphere. European representatives include the Inkoo and Tahyna viruses with the latter occurring also in Africa (Lumbo strain). 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. Two members have been recovered from naturally infected rodents and one from bats. Four members have been implicated in human illness, but this may not give a true picture since establishment of specific viral etiology of disease induced by closely related viruses could not ordinarily be accomplished without isolation and detailed serologic identification of the causative virus in each case.

Available data on 9 of the 11 California group viruses listed in Table 10 have been examined by SIRACA; the Bocas and Inkoo viruses had not been registered at the time of the meetings of this group (March 1969 and June 1970) (Arbovirus Infoexchange No. 20, Mar. 1970, pp. 6-8, and No. 21, Nov. 1970, pp. 9-11). As with the Group C viruses, 3 complexes were suggested in the California group: California, Trivittatus, and Melao. The California complex consisted of the California encephalitis (CE) virus, with California encephalitis, Jamestown Canyon, Keystone, LaCrosse, San Angelo, and Tahyna viruses said to be subtypes, all of which were distinguishable from each other. Jamestown Canyon and Jerry Slough were nearly indistinguishable serologically. The Trivittatus and Melao complexes each consisted of a single virus.

Five of the 6 viruses listed in the Capim group were isolated from mosquitoes and 5 from sentinel animals. Three have been isolated from rodents and one also from a marsupial; none has been implicated in human disease. Capim group viruses have not been reported from the eastern hemisphere.

Table 11. Guama, Koongol, and Patois Groups: Guama group viruses consist of 6 members limited to North and South America in distribution. Five of the 6 have been isolated from mosquitoes and the 6th from sentinel mice exposed in nature. Five have been recovered from rodents, 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 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.

Table 12. Simbu Group: Twelve of the 15 members of the Simbu group have been isolated either from culicine mosquitoes or from culicoides, or both. Of those not yet recovered from 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 (pigs, goats, sheep, cattle), one from monkeys, 3 from birds, one from hares and rabbits, one from sloths. Two viruses have been associated with disease in man. Rodents, bats, and marsupials have not been shown to act as reservoir hosts.

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

Two viruses are considered to be proved arboviruses, 2 as probable arboviruses, and the remaining 11 as possible arboviruses.

Table 13. Tete Group and Unassigned (SBU) Viruses: Little is known of the 4 Tete group viruses; they have been recovered only from wild-caught birds in Europe, Africa, and Asia. The Bahig and Matruh viruses have been isolated from migratory birds in Egypt and Cyprus; they are indistinguishable by CF but can be differentiated readily by cross-HI tests. All 4 are regarded as possible arboviruses, but definitive information is lacking.

Of the 6 unrelated, unassigned viruses belonging in the Bunyamwera supergroup, 5 have been isolated from culicine mosquitoes but no other arthropods; one from birds, and 3 from sentinel animals. Two have been reported only from the North American continent, 3 from South America only, and one from Africa alone. One is rated as a proved arbovirus, one as a probable arbovirus, and 4 as possible arboviruses.

Table 14. Phlebotomus-Fever Group: The PHL group consists of 13 viruses which have been linked serologically to each other by cross-reactions in one or more test systems: hemagglutination-inhibition, complement fixation, plaque reduction neutralization, or agar gel precipitation tests. Only 7 of the agents, however, have been isolated from phlebotomine flies; one (Icoaraci) has been recovered from both sandflies and mosquitoes in nature. Three others have been isolated from wild caught mosquitoes but never from sandflies; 2 of these, Arumowot and Itaporanga, have been shown to multiply in Aedes albopictus and Culex fatigans following intrathoracic inoculation, raising the possibility that the PHL group consists of mosquito-borne as well as sandfly-borne agents (Robert B. Tesh, personal communication 1973). Three others have been isolated only from naturally infected vertebrate hosts and not from arthropods.

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 strains of sandfly fever have been shown to cause large outbreaks. Three of 13 are considered to be proved arboviruses, 2 are probably arboviruses, while in regard to the remaining members, there are inadequate 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 members of the Bunyamwera supergroup.

Tables 15 and 16. Tick-borne Groups other than Group B Viruses: Table 15 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 showed morphology characteristic of the Bunyaviruses when examined by electron microscopy. With one exception (Silverwater), these agents have been recovered 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. In the latter form (CHF), the virus has been implicated in hundreds of cases of disease in the USSR. Little is known of the serologically related Hazara virus from West Pakistan.

The 2 members of the Ganjam group have been recovered repeatedly from ticks and occasionally from culicine mosquitoes; the Dugbe virus of Africa has frequently been isolated from the blood of cattle and from a rodent. Both have been associated sporadically with febrile illnesses in man.

Members of the remaining 3 groups (KSO, THO, and UUK) have not, with the single exception of Thogoto virus, been reported from cases of human disease. The Kaisodi group consists of 3 members, of which 2 have been isolated in Asia from ticks collected in forest undergrowth or from forest rodents, and one in North America from hares and their ticks. The Thogoto group contains only one registered virus, recovered in Africa and Europe, and found primarily in viremic cattle or ticks from cattle. Of the 5 members of the UUK group, 3 have been found in Europe and 2 in Asia. Most were isolated from ticks taken in the vicinity of bird nesting places, while Uukuniemi virus has also been recovered from birds, a rodent, and ticks feeding on domestic cattle.

Table 16 also lists members of 5 antigenic groups which are tick-borne but not belonging to Group B. They differ morphologically, however, from those listed under the previous table. The largest of these serogroups, KEM, contains 11 members, several of which 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; only 2 have been recovered from vertebrates. Four of these viruses have been

found only in North America, 3 only in Europe, 1 in Africa, 1 in Asia, and 2 in more than one continent. None has been reported from South America or Australasia. Association of infected ticks with bird nesting places, especially seabirds, is frequently noted; but in some, the association is with forested areas or domestic animals.

The 4 minor antigenic groups listed in Table 16 have not been classified taxonomically, but are included in this tabulation because they are tick-borne or, at least, tick-associated. The 4 members of the DGK group consist of 3 viruses from Asia and one from Africa. None has been isolated from any vertebrate host, nor from arthropods other than ticks. As with the previous group, infected ticks were collected most frequently from the immediate vicinity of bird colonies (AH, KS, and PT), while other infected ticks were taken from camels (DGK).

The Hughes and Soldado viruses of the HUG group are serologically related by the CF test; they are reported only from the western hemisphere. Both have been isolated from naturally infected ticks collected in areas frequented by sea birds. The HUG virus has also been recovered from the blood of sea birds but not from other vertebrates. The 2 members of the QRF group have likewise been isolated from ticks collected in bird nesting areas, Johnston Atoll from nests of terns in the Central Pacific area as well as from Australasia, and Quaranfil from trees in an egret rookery and from pigeon houses in Africa.

The 2 members of the QYB group, Bandia and Qalyub, on the other hand, have been reported only in Africa and from ticks taken from rodent burrows or (BDA) from rodents themselves.

Only 2 of the 21 viruses listed in Table 2 have been associated with human disease. One has been classified as a proved arbovirus, 3 as probable arboviruses, the remaining 17 as possible arboviruses.

Tables 17, 18, and 19. Minor Antigenic Groups of Viruses: These tables include a number of antigenic groups of viruses consisting of one to 5 registered viruses per group, none of which has been shown to cause disease in man. 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 17 are listed 5 small 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 vertebrates or man. 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 recovered 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 lower animals or man. All 3 members of the TUR group have been isolated from mosquitoes and 2 also from birds (TUR and UMB), and one (TUR) from hares. Individual members of the group are found in different geographic areas, with M'Poko (Yaba-1) in Africa, Turlock in North and South America, and Umbre in Asia.

Table 18 lists members of 8 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 animals, namely AHS in horses, mules and donkeys; BLU in both wild and domestic ruminants; and EHD in deer. The first two are widespread in geographic occurrence, with bloodsucking gnats (Culicoides) involved in their transmission. EHD has been reported only from North America and no arthropod has yet been incriminated.

The CGL group consists of 2 members, one of which has been isolated repeatedly from phlebotomine flies and once from man (Changuinola), while the other (Irituia) 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 variously with mosquito or biting gnat vectors and are reported from the eastern hemisphere. Little information is available concerning their role in nature.

In Table 19 are listed 3 minor antigenic groups the members of which show a morphology characteristic of the rhabdoviruses, followed by 4 small groups which have not yet been classified taxonomically.

The largest and probably the best known of these groups is the Vesicular Stomatitis group comprising 5 rhabdoviruses, at least 4 of which have been shown to cause infection in man. The Chandipura virus occurring in Asia (India) and Africa (Nigeria) has been reported once from a pool of phlebotomine flies and has been isolated also from 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 frequently from livestock and on several occasions from man. The Indiana strain has been isolated many times from phlebotomine flies and once from mosquitoes, while the New Jersey type has not yet been shown to be associated with arthropod vectors. The Piry virus is antigenically related most closely to Chandipura; it has been isolated from man and an opossum but not from arthropods. The Cocal virus is serologically related to VSI and has been recovered once each from a horse, a rodent, and mosquitoes, with 2 isolations reported from mites.

Little is known of the other rhabdoviruses listed in Table 19, although the Mossuril virus has been recovered repeatedly from mosquitoes and birds in Africa.

Among the unclassified viruses, Boteke and Nyando viruses have been isolated from mosquitoes and the latter also from man. The 2 members of the Matariya group represent bird isolates from Egypt; while the 2 viruses of the Timbo group have been recovered only from reptiles in Brazil.

Table 20 contains a listing of those viruses placed in the Tacaribe antigenic group. It has been recognized in the past several 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 this group are associated predominantly with rodent hosts and there is little or no evidence that they are transmitted by arthropod vectors in nature.

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

In Tables 21, 22, and 23 are listed all the remaining registered viruses which, with one notable exception, have not yet been found to be related antigenically to any other known virus and, thus, are presently ungrouped. Table 21 includes ungrouped viruses which, if arthropod-borne, are probably transmitted by mosquitoes under natural conditions. These mosquito-associated viruses are further sub-grouped according to their taxonomic status so far as is known. It will be noted that among the rhabdoviruses, Flanders and Hart Park are listed as ungrouped viruses although they have been shown to be very closely related to each other serologically. It is still not entirely clear whether these viruses form a group or complex of viruses or whether they should be regarded as variants of a single virus. Two of the listed viruses are considered by the SEAS to be probably not or definitely not arboviruses, although they are here tabulated as mosquito-associated. The Nodamura virus from Japan was recovered from a pool of wild-caught mosquitoes and has been shown to multiply in arthropods and to be transmitted experimentally by mosquitoes, but it is considered quite unlikely that this would be its usual mode of transmission in nature. The Cotia virus 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.

In the same manner, ungrouped viruses which appear to be associated predominantly with arthropod vectors other than mosquitoes are tabulated in Table 22, and again are subdivided by taxonomic status. Adequate epidemiologic and laboratory evidence has accumulated in several instances to permit representatives of several different genera to be classified as arthropod-borne viruses.

Finally, in Table 23 are listed the remaining registered viruses which have been isolated only from man or lower vertebrates, and no association with arthropod vectors can be adduced.

Tables 24 through 28 provide a further condensation of data which may be extracted from the Tables 4 through 23. They serve merely to draw attention in a single table to certain aspects of the information contained in multiple tabulations.

Table 24 summarizes distribution of viruses in different antigenic groups by continents as determined by actual isolations of the viruses. It can be seen that only a few viruses, 14 or 4.5%, 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. Almost 83% of all registered viruses have been found only on a single continent, while 95.5% of all viruses have been reported from 1 or 2 continents only. The largest total number of viruses have been isolated in Africa followed closely by South America, and then North America, Asia, Australasia, and Europe, in that order.

Table 25 lists the numbers of viruses by antigenic group which have been isolated from various classes of arthropods. 173 (55%) of all registered viruses have been recovered from mosquitoes, 64 (about 20%) from ticks, and 48 (15%) from all other classes. 57 viruses have never been isolated from any arthropod host including 13 members of the large Group B, and 18 of the ungrouped category. By far the largest number of viruses which have been isolated from any arthropod, 233 of 257, have been reported from one class only.

Table 26 shows that a few viruses can 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 (133 of 198 or 67%). The largest number of viruses have been isolated from man and rodents, followed by birds and other hosts.

In Table 27 are listed the number of viruses in each serogroup which have been found 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, 28% of all registered viruses have been implicated in human infections.

The SEAS ratings of registered viruses summarized in Table 28 show that data are considered adequate in 140 registrations (44.6%) to indicate that the viruses are arthropod-borne or probably arthropod-borne. In 23 additional instances, viruses have been rated with some degree of confidence as not arboviruses, or probably not arboviruses. However, it will be noted that in 151 registrations, or 48%, data are missing which would permit classification of the registered viruses other than as possible arboviruses.

FIGURE 1

YEAR OF ISOLATION OF 314 VIRUSES REGISTERED IN THE CATALOGUE, WITH CUMULATIVE PERCENTAGE OF ISOLATIONS BY YEAR

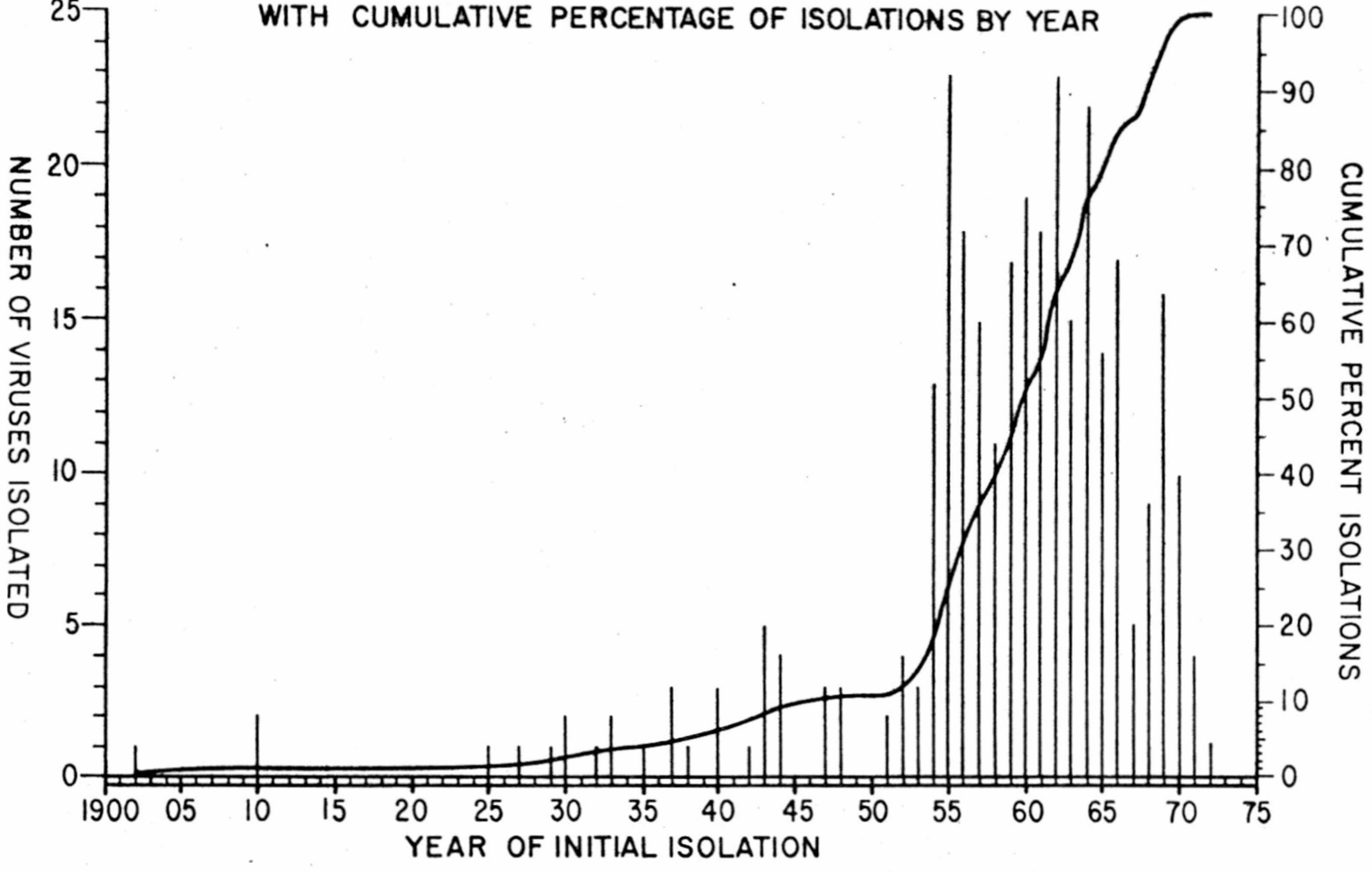


Table 1. Alphabetical Listing of 314 Viruses Registered as of 31 December 1973 with Recommended Abbreviations and Antigenic Grouping

Name	Abbr.	Ant. Group
Absettarov	ABS	B
Abu Hammad	AH	DGK
Acado	ACD	COR
Acara	ACA	CAP
African Horsesickness	AHS	AHS
African Swine Fever	ASF	
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
Bahig	BAH	TETE
Bakau	BAK	BAK
Baku	BAKU	KEM
Bandia	BDA	QYB
Bangui	BGI	
Banzi	BAN	B
Batai	BAT	BUN
Bauline	BAU	KEM
Bebaru	BEB	A
Belmont	BEL	
Bertioga	BER	GMA
Bhanja	BHA	
Bimiti	BIM	GMA
Bluetongue	BLU	BLU
Bocas	BOC	CAL
Boracea	BOR	ANB
Botambi	BOT	SBU
Boteke	BTK	BTK
Bouboui	BOU	B
Bujaru	BUJ	PHL
Bunyamwera	BUN	BUN
Burg el Arab	BEA	MTY
Bushbush	BSB	CAP
Bussuquara	BSQ	B
Buttonwillow	BUT	SIM
Bwamba	BWA	BWA
Cache Valley	CV	BUN
California Enc.	CE	CAL
Calovo	CVO	BUN
Candiru	CDU	PHL
Capim	CAP	CAP
Caraparu	CAR	C
Catu	CATU	GMA
Chaco	CHO	TIM
Chagres	CHG	PHL
Chandipura	CHP	VSV
Changuinola	CGL	CGL
Charleville	CHV	
Chenuda	CNU	KEM
Chikungunya	CHIK	A
Chobar Gorge	CG	
Cocal	COC	VSV
Colorado Tick Fever	CTF	
Congo	CON	CON
Corriparta	COR	COR
Cotia	COT	
Cowbone Ridge	CR	B
D'Aguilar	DAG	PAL
Dakar Bat	DB	B
Dengue-1	DEN-1	B
Dengue-2	DEN-2	B
Dengue-3	DEN-3	B
Dengue-4	DEN-4	B
Dera Ghazi Khan	DGK	DGK
Dhori	DHO	
Dugbe	DUG	GAN
East. Equine Enc.	EEE	A
Edge Hill	EH	B
Entebbe Bat	ENT	B
Ep. Hem. Dis.	EHD	EHD
Eubenangee	EUB	EUB
Everglades	EVE	A
Flanders	FLA	
Gamboa	GAM	SBU
Ganjam	GAN	GAN
Germiston	GER	BUN
Getah	GET	A
Gossas	GOS	
Grand Arbaud	GA	UUK
Great Island	GI	KEM

Name	Abbr.	Ant. Group
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
Hughes	HUG	HUG
Hypr	HYPR	B
Icoaraci	ICO	PHL
Ieri	IERI	
Ilesha	ILE	BUN
Ilheus	ILH	B
Ingwavuma	ING	SIM
Inkoo	INK	CAL
Irituia	IRI	CGL
Israel Turkey	IT	B
Issyk-Kul	IK	
Itaporanga	ITP	PHL
Itaqui	ITQ	C
Jamestown Canyon	JC	CAL
Japanaut	JAP	
Japanese B Enc.	JBE	B
Jerry Slough	JS	CAL
Johnston Atoll	JA	QRF
Joinjakaka	JOI	
Juan Diaz	JD	CAP
Junin	JUN	TCR
Jurona	JUR	SBU
Jutiapa	JUT	B
Kairi	KRI	BUN
Kaisodi	KSO	KSO
Kamese	KAM	MOS
Kammavanpettai	KMP	
Kannamangalam	KAN	
Kao Shuan	KS	DGK
Karimabad	KAR	PHL
Kasba	KAS	PAL
Kemerovo	KEM	KEM
Kern Canyon	KC	
Ketapang	KET	BAK
Keterah	KTR	
Keuraliba	KEU	
Keystone	KEY	CAL
Kokobera	KOK	B
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	
Langat	LGT	B
Lanjan	LJN	KSO
Lassa	LAS	TCR
Latino	LAT	TCR
Lebombo	LEB	
Le Dantec	LD	
Lipnovik	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
Manawa	MWA	UUK
Manzanilla	MAN	SIM
Mapputta	MAP	MAP
Maprik	MPK	MAP
Marco	MCO	
Marituba	MTB	C
Matariya	MTY	MTY
Matruh	MTR	TETE
Matucare	MAT	
Mayaro	MAY	A
Melao	MEL	CAL
Mermet	MER	SIM
Middelburg	MID	A
Minatitlan	MNT	SBU
Minnal	MIN	
Mirim	MIR	SBU
Mitchell River	MR	WAR
Modoc	MOD	B
Moju	MOJU	GMA
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

* Rabies related

Name	Abbr.	Ant. Group
Nairobi Sheep Dis.	NSD	
Nariva	NAR	
Navarro	NAV	
Ndumu	NDU	A
Negishi	NEG	B
Nepuyo	NEP	C
Ngaingan	NGA	
Nkolbisson	NKO	
Nodamura	NOD	
Northway	NOR	BUN
Ntaya	NTA	B
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
Ossa	OSSA	C
Pacora	PCA	
Pacui	PAC	PHL
Pahayokee	PAH	PAT
Palyam	PAL	PAL
Parana	PAR	TCR
Pathum Thani	PTH	DGK
Patois	PAT	PAT
Pichinde	PIC	TCR
Piry	PIRY	VSV
Pixuna	PIX	A
Pongola	PGA	BWA
Ponteves	PTV	UUK
Powassan	POW	B
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
Sakhalin	SAK	
Salehabad	SAL	PHL
Samford	SAM	SIM
San Angelo	SA	CAL
Sandfly F. (Naples)	SFN	PHL
Sandfly F. (Sicilian)	SFS	PHL
Sango	SAN	SIM
Sathuperi	SAT	SIM
Sawgrass	SAW	
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
Sokoluk	SOK	B
Soldado	SOL	HUG
Sororoca	SOR	BUN
Spondweni	SPO	B
St. Louis Enc.	SLE	B
Stratford	STR	B
Tacaiuma	TCM	ANA
Tacaribe	TCR	TCR
Tahyna	TAH	CAL
Tamiami	TAM	TCR
Tanga	TAN	
Tataguine	TAT	
Tembe	TME	
Tembusu	TMU	B
Tensaw	TEN	BUN
Tete	TETE	TETE
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

Name	Abbr.	Ant. Group
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 Nile	WN	B
West. Equine Enc.	WEE	A
Whataroa	WHA	A
Witwatersrand	WIT	
Wongal	WON	KOO
Wongorr	WGR	
Wyeomyia	WYO	BUN
Yaquina Head	YH	KEM
Yellow Fever	YF	B
Yogue	YOG	
Zaliv Terpeniya	ZT	UUK
Zegla	ZEG	PAT
Zika	ZIKA	B

Table 2. Antigenic Groups of 314 Viruses Registered in Catalogue

Antigenic Group	Abbreviation	No. Registered Viruses in Group		%
A	A		20	6.4
African Horsesickness	AHS		1	0.3
Anopheles A	ANA		3	1.0
Anopheles B	ANB		2	0.6
B	B		51	16.2
Bakau	BAK		2	0.6
Bluetongue	BLU		1	0.3
Boteke	BTK		1	0.3
Bunyamwera Supergroup			84	26.8
Bunyamwera	BUN	17		
Bwamba	BWA	2		
C	C	11		
California	CAL	11		
Capim	CAP	6		
Guama	GMA	6		
Koongol	KOO	2		
Patois	PAT	4		
Simbu	SIM	15		
Tete	TETE	4		
Unassigned	SBU	6		
Changuinola	CGL		2	0.6
Congo	CON		2	0.6
Corriparta	COR		2	0.6
Dera Ghazi Khan	DGK		4	1.3
Epizoot. Hem. Dis.	EHD		1	0.3
Eubenangee	EUB		1	0.3
Ganjam	GAN		2	0.6
Hughes	HUG		2	0.6
Kaisodi	KSO		3	1.0
Kemerovo	KEM		11	3.5
Kwatta	KWA		1	0.3
Mapputta	MAP		3	1.0
Matariya	MTY		2	0.6
Mossuril	MOS		2	0.6
Nyando	NDO		1	0.3
Palyam	PAL		4	1.3
Phlebotomus fever	PHL		13	4.1
Qalyub	QYB		2	0.6
Quaranfil	QRF		2	0.6
Tacaribe	TCR		9	2.9
Thogoto	THO		1	0.3
Timbo	TIM		2	0.6
Turlock	TUR		3	1.0
Uukuniemi	UUK		5	1.6
Vesicular stomatitis	VSV		5	1.6
Warrego	WAR		2	0.6
Ungrouped viruses	UNG		62	19.7
	TOTAL		314	

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
		South Africa	AHS
		Uganda	BWA, WN
	Asia	Japan	JE
		U.S.S.R.	RSSE
	North America	U.S.A.	EEE, SLE, WEE
	South 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 *
	North America	U.S.A.	CE, CTF, TVT
	South America	Brazil	ILH
		Colombia	ANA, ANB, WYO
1950-59	Africa	Egypt	CNU, QRF, QYB, SIN, WM
		Nigeria	ILE, LB
		South Africa	BAN, GER, ING, LEB, MID, MOS, NDU, NYM, PGA, SIM, SPO, TETE, USU, WIT, WSL
		Uganda	CHIK, CON, ENT, NDO, ONN
	Asia	India	ARK, BHA, GAN, KAS, KSO, KFD, MIN, PAL, SAT, VKT, UMB, WAN
		Israel	IT
		Japan	AKA, APOI, NOD, SAG, TSU
		Malaya	BAK, BAT, BEB, GET, KET, LGT, TMU
	Australasia	Australia	MVE
		Philippines	DEN-3 *, DEN-4 *
	Europe	Czechoslovakia	HYPR, TAH
		Finland	KUM
		U.S.S.R.	ABS
	North America	Canada	POW
		Panama	BOC, LJ, PCA
		U.S.A.	CV, EHD, HP, MML, MOD, RB, SA, TUR, VSNJ
	South 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.	BOT, BOU, BTK, MPO
		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
		Uganda	KAM, MEB, TAN
	Asia	India	CHP, DHO, KAN, KMP, SEM, THI, TPM, VEL
		Iran	KAR*, SAL *
		Japan	AINO
		Malaya	KTR, LJN
		Pakistan (West)	DGK, HAZ, MWA
		U.S.S.R.	OKH, SAK, TYU, ZT
	Australasia	Australia	ALF, ALM, BEL, CHV, COR, DAG, EH, EUB, JAP, JOI, KOK, KOO, KOW, KUN, MAP, MPK, MR, RR, SAM, SEP, STR, TRU, UPO, WAR, WON
		New Zealand	WHA
		Pacific Island	JA *
	Europe	Czechoslovakia	CVO, KEM, LIP, TRB
		Finland	INK, UUK
		France	GA, PTV
	North America	Canada	SIL
		Guatemala	JUT *
		Mexico	MNT, TLA *
		Panama	CHG, CGL, GAM, JD, LAT, LS, 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, PAH, SAW, SC, SHF, SR, TAM, TEN, UMA
	South 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
		Surinam	KWA
		Trinidad	COC, MOR, NAR, RES, SOL
1970-73	Africa	Cent. Afr. Rep.	BGI
		Egypt	AH, KS, PTH
	Asia	India	CG
		U.S.S.R.	IK, SOK
	Australasia	Australia	NGA, WAL, WGR
	Europe	U.S.S.R.	BAKU
	North America	U.S.A.	BAU, GI, NOR, YH

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

TABLE 4. GROUP A ARBOVIRUSES

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

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

TABLE 5. GROUP B ARBOVIRUSES, MOSQUITO-BORNE

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anophelline	Tick. Ixodid	Tick. Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	HUMAN DISEASE: Natural Infection	Lab. Infection	SEAS RATING *	TAXONOMIC STATUS
Alfuy	+										+										+			21	Flavivirus
Banzi	+							+							+				+			+		20	"
Bouboui	+	+						+											+					22	"
Bussuquara	+							+		+					+	+	+		+			+		20	"
Dengue-1	+							+											+	+	+	+	+	20	"
Dengue-2	+							+	+								+		+	+	+	+	+	20	"
Dengue-3	+							+									+		+	+	+	+	+	20	"
Dengue-4	+							+												+	+	+		20	"
Edge Hill	+	+																			+			21	"
Ilheus	+							+			+				+	+	+					+	+	20	"
Japanese Enc.	+	+						+			+	+		+						+		+	+	20	"
Kokobera	+																				+			21	"
Kunjin	+							+			+										+		+	20	"
Murray Valley Enc.	+							+													+	+		20	"
Ntaya	+																		+					21	"
Sepik	+																				+	+		22	"
St. Louis Enc.	+	+					+	+			+	+		+		+	+					+	+	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 4.

TABLE 6. GROUP B ARBOVIRUSES, TICK-BORNE

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

* See footnote Table 4.

TABLE 7. GROUP B VIRUSES, NO ARTHROPOD VECTOR DEMONSTRATED

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anopheline	Tick. Ixodid	Tick. Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	HUMAN DISEASE: Natural Infection	Lab. Infection	SEAS RATING *	TAXONOMIC STATUS
Apoi										+										+			+	22	Flavivirus
Cowbone Ridge										+						+								23	"
Dakar Bat												+							+					24	"
Entebbe Bat												+							+					24	"
Israel Turkey Meningo.											+									+				22	"
Jutiapa										+						+								22	"
Koutango										+									+					22	"
Modoc										+						+								24	"
Montana Myotis Leuko.												+				+								24	"
Negishi								+				+				+				+		+	+	22	"
Rio Bravo												+				+						+	+	24	"
Saboya										+									+					22	"
Sokuluk												+								+				22	"

*See footnote Table 4

TABLE 8. BUNYAMWERA SUPERGROUP: BUNYAMWERA GROUP VIRUSES

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	ARTHROPODS: Mosq. Anopheline	ARTHROPODS: Tick. Ixodid	ARTHROPODS: Tick. Argasid	ARTHROPODS: Phlebotomine	ARTHROPODS: Culicoides	ARTHROPODS: Other	VERTEBRATES: Man	VERTEBRATES: Other Primates	VERTEBRATES: Rodents	VERTEBRATES: Birds	VERTEBRATES: Bats	VERTEBRATES: Marsupials	VERTEBRATES: Other	VERTEBRATES: Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	HUMAN DISEASE: Natural Infection	HUMAN DISEASE: Lab. Infection	SEAS RATING *	TAXONOMIC STATUS
Anhembi	+									+							+			+				22	Bunyavirus
Batai	+	+						+							+			+		+				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 4

** May be strain of Batai

TABLE 9. BUNYAMWERA SUPERGROUP: BWAMBA GROUP AND GROUP C VIRUSES

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

*See footnote Table 4.

TABLE 10. BUNYAMWERA SUPERGROUP: CALIFORNIA AND CAPIM GROUP VIRUSES

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

*See footnote Table 4.

TABLE 11. BUNYAMWERA SUPERGROUP: GUAMA, KOONGOL, AND PATOIS GROUP VIRUSES

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	Mosq.		Tick.																					
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	North America	South America	Europe	Africa	Asia	Australasia	Natural Infection	Lab. Infection		
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 4.

TABLE 12. BUNYAMWERA SUPERGROUP: SIMBU GROUP VIRUSES

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

*See footnote Table 4.

TABLE 13. BUNYAMWERA SUPERGROUP: TETE GROUP AND UNASSIGNED VIRUSES

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Tick.																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	North America	South America	Europe	Africa	Asia	Australasia	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	"
Minatitlan															+	+								22	"
Mirim	+																+							20	

*See footnote Table 4.

TABLE 14. PHLEBOTOMUS FEVER GROUP VIRUSES

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING *	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Tick.		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	North America	South America	Europe	Africa	Asia	Australasia	Natural Infection	Lab. Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Anhanga														+			+							22	Bunyavirus-like
Arumowot	+									+				+			+		+					22	"
Bujaru										+				+			+							22	"
Candiru								+						+			+					+		22	"
Chagres	+							+									+					+		22	"
Icoaraci	+	+			+					+	+				+	+	+							21	"
Itaporanga	+										+		+		+		+							20	"
Karimabad					+															+				22	"
Pacui					+					+							+							21	"
Punta Toro					+			+								+						+		22	"
Salehabad					+															+				22	"
SF-Naples					+			+										+	+	+		+		20	"
SF-Sicilian					+			+										+	+	+		+		20	"

*See footnote Table 4.

TABLE 15. TICK-BORNE GROUPS OTHER THAN GROUP B VIRUSES

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

* See footnote Table 4

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

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anopheline	Tick. Ixodid	Tick. Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	HUMAN DISEASE: Natural Infection	Lab. Infection	SEAS RATING *	TAXONOMIC STATUS
KEMEROVO GROUP																									
Baku				+														+						22	Orbivirus
Bauline			+													+								22	"
Chenuda				+															+					22	"
Great Island			+													+								22	"
Kemerovo			+					+			+								+	+		+	+	21	"
Lipovnik			+															+						22	"
Okhotskiy			+																	+				22	"
Sixgun City				+												+		+						22	"
Tribec			+							+					+									21	"
Wad Medani			+													+			+	+				22	"
Yaquina Head			+													+								22	"
DERA GHAZI KHAN GROUP																									
Abu Hammad				+															+					22	Unclassified
Dera Ghazi Khan			+																	+				22	"
Kao Shuan				+																+				22	"
Pathum Thani				+																+				22	"
HUGHES GROUP																									
Hughes				+							+					+	+							21	Unclassified
Soldado				+													+							22	"
QALYUB GROUP																									
Bandia				+						+									+					22	Unclassified
Qalyub				+															+					22	"
QUARANFIL GROUP																									
Johnston Atoll				+																	+			22	Unclassified
Quaranfil				+				+			+								+			+		20	"

* See footnote Table 4

TABLE 17: MINOR ANTIGENIC GROUPS OF VIRUSES

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

* See footnote Table 4

TABLE 18: MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anopheline	Tick. Ixodid	Tick. Argasid	Phlebotomine	Culicoides	Other	ISOLATED FROM: VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	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	+?	+?																			+				22	Orbivirus
PALYAM GROUP																									
D'Aguilar						+															+				22	Orbivirus
Kasba	+																			+					22	"
Palyam	+																			+					22	"
Vellore	+																			+					22	"
WARREGO GROUP																									
Mitchell River						+															+				22	Orbivirus
Warrego						+															+				22	"

* See footnote Table 4

TABLE 19. MINOR ANTIGENIC GROUPS OF VIRUSES

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq. Culicine	Mosq. Anopheline	Tick. Ixodid	Tick. Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	HUMAN DISEASE: Natural Infection	Lab. Infection	SEAS RATING *	TAXONOMIC STATUS
KWATTA GROUP																									
Kwatta	+																+							22	Rhabdovirus
MOSSURIL GROUP																									
Kamese	+																		+					22	Rhabdovirus
Mossuril	+										+								+					22	"
VESICULAR STOMATITIS GR.																									
Chandipura					+			+						+					+	+		+		21	Rhabdovirus
Cocal	+						+			+				+	+		+							20	"
Piry								+					+				+						+	22	"
VSV-Indiana	+				+			+						+		+	+					+	+	20	"
VSV-New Jersey							+	+						+		+	+					+	+	22	"
BOTEKE GROUP																									
Boteke	+																		+					22	Unclassified
MATARIYA GROUP																									
Burg el Arab											+								+					22	"
Matariya											+								+					22	"
NYANDO GROUP																									
Nyando		+						+											+			+		21	"
TIMBO GROUP																									
Chaco														+			+							22	"
Timbo														+			+							22	"

*See footnote Table 4

TABLE 20. TACARIBE (LCM) GROUP VIRUSES

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

*See footnote Table 4

TABLE 21. UNGROUPED MOSQUITO-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq.: Culicine	Mosq.: Anopheline	Tick.: Ixodid	Tick.: Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	HUMAN DISEASE: Natural Infection	Lab. Infection	SEAS RATING *	TAXONOMIC STATUS
Rift Valley Fever	+							+						+					+			+	+	20	Bunyavirus-like
Tataguine	+	+						+											+			+		22	"
Witwatersrand	+									+					+				+					21	"
Japanaut	+											+									+			22	Orbivirus
Lebombo	+						+	+		+									+					21	"
Umatilla	+										+					+								20	"
Nodamura	+																			+				23	Picornavirus
Cotia	+				+			+							+		+					+		24	Poxvirus
Flanders	+										+					+								22	Rhabdovirus
Hart Park	+										+					+								21	"
Joinjakaka	+																				+			22	"
Arkonam	+	+																		+				22	Unclassified
Aruac	+																+							21	"
Belmont	+																				+			22	"
Ieri	+																+							22	"
Kowanyama		+																			+			22	"
La Joya	+															+								22	"
Minnal	+																			+				22	"
Nkolbisson	+																		+					22	"
Okola	+																		+					22	"
Pacora	+															+								22	"
Tanga		+																	+					22	"
Tembe		+															+							22	"
Triniti	+																+							21	"
Venkatapuram	+																			+				22	"
Wongorr	+																				+			22	"

*See footnote Table 4

TABLE 22. UNGROUPED TICK-, CULICOIDES-, OR PHLEBOTOMUS-ASSOCIATED VIRUSES

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq.: Culicine	Mosq.: Anopheline	Tick.: Ixodid	Tick.: Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	HUMAN DISEASE: Natural Infection	Lab. Infection	SEAS RATING *	TAXONOMIC STATUS
Bhanja			+							+				+				+	+	+				22	Bunyavirus-like
Lone Star			+											+		+			+					22	"
Nairobi Sheep Disease			+					+						+					+			+	+	20	"
African Swine Fever				+										+			+**	+	+					20	Iridovirus
Colorado Tick Fever			+	+				+		+						+						+	+	20	Orbivirus
Charleville				+	+									+							+			22	Unclassified
Chobar Gorge				+																+				22	"
Dhori			+																+	+				22	"
Issyk-Kul				+								+								+				22	"
Keterah				+								+								+				22	"
Matucare				+													+							22	"
Ngaingan						+															+			22	"
Nyamanini				+							+								+					21	"
Sakhalin			+																	+				22	"
Sawgrass			+													+								22	"
Upolu				+																	+			22	"
Wallal						+															+			22	"
Wanowrie	+		+					+											+	+				22	"

*See footnote Table 4

**Cuba

TABLE 23. UNGROUPED VIRUSES: NO ARTHROPOD VECTOR KNOWN

VIRUS	ISOLATED FROM: ARTHROPODS: Mosq.: Culicine	Mosq.: Anopheline	Tick.: Ixodid	Tick.: Argasid	Phlebotomine	Culicoides	Other	VERTEBRATES: Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	ISOLATED IN: North America	South America	Europe	Africa	Asia	Australasia	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	"
Gossas												+							+					23	"
Kammavanpettai											+									+				22	"
Kannamangalam											+								+	+				22	"
Keuraliba										+									+					22	"
Le Dantec								+											+					22	"
Marco														+			+							22	"
Sembalam											+									+				22	"
Simian Hemorrh. fever									+							+				?				24	"
Thottapalayam														+						+				22	"
Toure										+									+					22	"
Yogue												+							+					22	"

*See footnote Table 4

TABLE 24. CONTINENTAL DISTRIBUTION OF GROUPED AND UNGROUPED VIRUSES

Antigenic Group	Total in Group	North America	South America	Europe	Africa	Asia	Austral-asia	No. of Continents involved 1	2	3	4	5
Group A	20	5	8	1	6	6	5	13	5	1	0	1
African Horse-sickness	1	0	0	1	1	1	0	0	0	1	0	0
Anopheles A	3	0	3	0	0	0	0	3	0	0	0	0
Anopheles B	2	0	2	0	0	0	0	2	0	0	0	0
Group B	51	10	6	7	16	19	12	37	10	3	1	0
Bakau	2	0	0	0	0	2	0	2	0	0	0	0
Bluetongue	1	1	0	1	1	1	0	0	0	0	1	0
Boteke	1	0	0	0	1	0	0	1	0	0	0	0
Bunyamwera Supergroup: Bunyamwera	17	8	6	2	3	1	0	14	3	0	0	0
Bwamba	2	0	0	0	2	0	0	2	0	0	0	0
C	11	5	8	0	0	0	0	9	2	0	0	0
California	11	8	2	2	1	0	0	9	2	0	0	0
Capim	6	3	5	0	0	0	0	4	2	0	0	0
Guama	6	2	5	0	0	0	0	5	1	0	0	0
Koongol	2	0	0	0	0	0	2	2	0	0	0	0
Patois	4	4	0	0	0	0	0	4	0	0	0	0
Simbu	15	2	2	0	8	5	2	11	4	0	0	0
Tete	4	0	0	2	3	1	0	2	2	0	0	0
Unassigned	6	2	3	0	1	0	0	6	0	0	0	0
Changuinola	2	1	1	0	0	0	0	2	0	0	0	0
Congo	2	0	0	1	1	2	0	1	0	1	0	0
Corriparta	2	0	0	0	1	0	1	2	0	0	0	0
Dera Ghazi Khan	4	0	0	0	1	3	0	4	0	0	0	0
Epizootic hem. disease	1	1	0	0	0	0	0	1	0	0	0	0
Eubenangee	1	0	0	0	0	0	1	1	0	0	0	0
Ganjam	2	0	0	0	1	1	0	2	0	0	0	0
Hughes	2	1	2	0	0	0	0	1	1	0	0	0
Kaisodi	3	1	0	0	0	2	0	3	0	0	0	0
Kemorovo	11	5	0	3	3	3	0	9	1	1	0	0
Kwatta	1	0	1	0	0	0	0	1	0	0	0	0
Mapputta	3	0	0	0	0	0	3	3	0	0	0	0
Matariya	2	0	0	0	2	0	0	2	0	0	0	0
Mossuril	2	0	0	0	2	0	0	2	0	0	0	0
Nyando	1	0	0	0	1	0	0	1	0	0	0	0
Palyam	4	0	0	0	0	3	1	4	0	0	0	0
Phlebotomus fever	13	2	6	2	3	4	0	11	0	2	0	0
Qalyub	2	0	0	0	2	0	0	2	0	0	0	0
Quaranfil	2	0	0	0	1	0	1	2	0	0	0	0
Tacaribe	9	1	7	0	1	0	0	9	0	0	0	0
Thogoto	1	0	0	1	1	0	0	0	1	0	0	0
Timbo	2	0	2	0	0	0	0	2	0	0	0	0
Turlock	3	1	1	0	1	1	0	2	1	0	0	0
Uukuniemi	5	0	0	3	0	2	0	5	0	0	0	0
Vesicular stom.	5	2	4	0	1	1	0	2	3	0	0	0
Warrego	2	0	0	0	0	0	2	2	0	0	0	0
Ungrouped	62	10	10	2	21	15	10	58	2	2	0	0
Totals	314	75	84	28	86	73	40	260	40	11	2	1

TABLE 25. NUMBER OF VIRUSES ISOLATED FROM WILD CAUGHT ARTHROPODS

Antigenic Group	Total in Group	Isolated from: Mosq.	Ticks	Phlebotomine Flies	Culicoides	Mites	Other	Number of Classes Involved: 1	2	3
Group A	20	20	0	0	1	4	2	16	3	1
African Horse-sickness	1	0	0	0	1	0	0	1	0	0
Anopheles A	3	3	0	0	0	0	0	3	0	0
Anopheles B	2	2	0	0	0	0	0	2	0	0
Group B	51	26	13	0	0	1	1	35	3	0
Bakau	2	2	1	0	0	0	0	1	1	0
Bluetongue	1	0	0	0	1	0	0	1	0	0
Boteke	1	1	0	0	0	0	0	1	0	0
Bunyamwera Supergroup: Bunyamwera	17	16	0	0	2	0	0	16	1	0
Bwamba	2	2	0	0	0	0	0	2	0	0
C	11	11	0	0	0	0	0	11	0	0
California	11	11	0	0	0	0	1	10	1	0
Capim	6	5	0	0	0	0	0	5	0	0
Guama	6	5	0	1	0	0	0	4	1	0
Koongol	2	2	0	0	0	0	0	2	0	0
Patois	4	3	0	0	0	0	0	3	0	0
Simbu	15	7	0	0	8	0	0	9	3	0
Tete	4	0	0	0	0	0	0	0	0	0
Unassigned	6	5	0	0	0	0	0	5	0	0
Changuinola	2	0	0	1	0	0	0	1	0	0
CHF-Congo	2	0	2	0	1	0	0	1	1	0
Corriparta	2	2	0	0	0	0	0	2	0	0
Dera Ghazi Khan	4	0	4	0	0	0	0	4	0	0
Epizootic hem. dis.	1	0	0	0	0	0	0	0	0	0
Eubenangee	1	1	0	0	0	0	0	1	0	0
Ganjam	2	2	2	0	1	0	0	0	1	1
Hughes	2	0	2	0	0	0	0	2	0	0
Kaisodi	3	0	3	0	0	0	0	3	0	0
Kemerovo	11	0	11	0	0	0	0	11	0	0
Kwatta	1	1	0	0	0	0	0	1	0	0
Mapputta	3	3	0	0	0	0	0	3	0	0
Matariya	2	0	0	0	0	0	0	0	0	0
Mossuril	2	2	0	0	0	0	0	2	0	0
Nyando	1	1	0	0	0	0	0	1	0	0
Palyam	4	3	0	0	1	0	0	4	0	0
Phlebotomus fever	13	4	0	7	0	0	0	9	1	0
Qalyub	2	0	2	0	0	0	0	2	0	0
Quaranfil	2	0	2	0	0	0	0	2	0	0
Tacaribe	9	1	1	0	0	3	0	3	1	0
Thogoto	1	0	1	0	0	0	0	1	0	0
Timbo	2	0	0	0	0	0	0	0	0	0
Turlock	3	3	0	0	0	0	0	3	0	0
Uukuniemi	5	0	5	0	0	0	0	5	0	0
Vesicular stom.	5	2	0	2	0	1	1	2	2	0
Warrego	2	0	0	0	2	0	0	2	0	0
Ungrouped	62	27	15	2	2	0	1	41	3	0
Totals	314	173	64	13	20	9	6	233	22	2

TABLE 26. 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
ANB		2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B		51	26	3	17	14	10	1	5	6	23	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		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bunyamwera Supergroup	BUN	17	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	11	2	0	2	0	1	0	0	0	5	0	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	6	0	0	0	1	0	0	0	0	1	0	0	0	0	0
CGL		2	1	0	1	0	0	0	0	0	2	0	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		4	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		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GAN		2	2	0	1	0	0	0	1	0	1	0	1	0	0	0
HUG		2	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		11	1	0	1	1	0	0	0	0	2	0	0	0	0	0
KWA		1	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		2	0	0	0	2	0	0	0	0	2	0	0	0	0	0
MOS		2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
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		13	5	0	4	2	0	1	0	2	8	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
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
UNG		62	9	1	7	8	8	0	4	5	31	5	0	0	0	0
Totals		314	84	9	72	50	25	17	29	30	133	39	11	10	3	2

TABLE 27. 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
Afr. horsesickness		1	0	0	0	
Anopheles A		3	0	0	0	
Anopheles B		2	0	0	0	
Group B		51	27	22	29	56.9
Bakau		2	0	0	0	
Bluetongue		1	0	0	0	
Boteke		1	0	0	0	
Bunyamwera Supergroup	Bunyamwera	17	4	2	5	29.4
	Bwamba	2	1	0	1	50
	C	11	9	2	9	81.8
	California	11	4	0	4	36.4
	Capim	6	0	0	0	
	Guama	6	2	0	2	33.3
	Koongol	2	0	0	0	
	Patois	4	0	0	0	
	Simbu	15	2	1	2	13.3
	Tete	4	0	0	0	
	Unassigned	6	0	0	0	
Changuinola		2	1	0	1	50
CHF-Congo		2	1	1	1	50
Corriparta		2	0	0	0	
Dera Ghazi Khan		4	0	0	0	
Epizoot. hem. dis.		1	0	0	0	
Eubenangee		1	0	0	0	
Ganjam		2	2	1	2	100
Hughes		2	0	0	0	
Kaisodi		3	0	0	0	
Kemerovo		11	1	1	1	9.1
Kwatta		1	0	0	0	
Mapputta		3	0	0	0	
Matariya		2	0	0	0	
Mossuril		2	0	0	0	
Nyando		1	1	0	1	100
Palyam		4	0	0	0	
Phlebot. fev.		13	5	0	5	38.5
Qalyub		2	0	0	0	
Quaranfil		2	1	0	1	50
Tacaribe		9	3	3	3	33.3
Thogoto		1	1	0	1	100
Timbo		2	0	0	0	
Turlock		3	0	0	0	
Uukuniemi		5	0	0	0	
Vesic. stom.		5	3	3	4	80
Warrego		2	0	0	0	
Ungrouped		62	6	3	6	9.7
Totals		314	84	46	89	28.3

TABLE 28. EVALUATION OF ARTHROPOD-BORNE STATUS OF 314 REGISTERED VIRUSES (SEAS)

Antigenic Group	Total in Group	Arbovirus	Probably an Arbovirus	Possible Arbovirus	Probably Not Arbovirus	Not an Arbovirus	Arbo or Probably Arbo		Not or Probably Not Arbo	
							No.	%	No.	%
A	20	14	3	3	0	0	17	85	0	
AHS	1	1	0	0	0	0	1	100	0	
ANA	3	0	2	1	0	0	2	66.7	0	
ANB	2	0	0	2	0	0	0		0	
B	51	27	6	12	1	5	33	64.7	6	11.8
BAK	2	0	1	1	0	0	1	50	0	
BLU	1	1	0	0	0	0	1	100	0	
BTK	1	0	0	1	0	0	0		0	
Bunyamwera Supergroup BUN	17	9	3	5	0	0	12	70.6	0	
Bunyamwera Supergroup BWA	2	1	1	0	0	0	2	100	0	
Bunyamwera Supergroup C	11	10	1	0	0	0	11	100	0	
Bunyamwera Supergroup CAL	11	6	2	3	0	0	8	72.7	0	
Bunyamwera Supergroup CAP	6	3	1	2	0	0	4	66.7	0	
Bunyamwera Supergroup GMA	6	4	0	2	0	0	4	66.7	0	
Bunyamwera Supergroup KOO	2	0	2	0	0	0	2	100	0	
Bunyamwera Supergroup PAT	4	1	1	2	0	0	2	50	0	
Bunyamwera Supergroup SIM	15	2	2	11	0	0	4	26.7	0	
Bunyamwera Supergroup TETE	4	0	0	4	0	0	0		0	
Bunyamwera Supergroup SBU	6	1	1	4	0	0	2	33.3	0	
CGL	2	0	1	1	0	0	1	50	0	
CON	2	1	0	1	0	0	1	50	0	
COR	2	0	0	2	0	0	0		0	
DGK	4	0	0	4	0	0	0		0	
EHD	1	0	1	0	0	0	1	100	0	
EUB	1	0	0	1	0	0	0		0	
GAN	2	0	0	2	0	0	0		0	
HUG	2	0	1	1	0	0	1	50	0	
KSO	3	0	1	2	0	0	1	33.3	0	
KEM	11	0	2	9	0	0	2	18.2	0	
KWA	1	0	0	1	0	0	0		0	
MAP	3	0	1	2	0	0	1	33.3	0	
MTY	2	0	0	2	0	0	0		0	
MOS	2	0	0	2	0	0	0		0	
NDO	1	0	1	0	0	0	1	100	0	
PAL	4	0	0	4	0	0	0		0	
PHL	13	3	2	8	0	0	5	38.5	0	
QYB	2	0	0	2	0	0	0		0	
QRF	2	1	0	1	0	0	1	50	0	
TCR	9	0	0	0	0	9	0		9	100
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	
VSV	5	2	1	2	0	0	3	60	0	
WAR	2	0	0	2	0	0	0		0	
UNG	62	5	7	42	5	3	12	19.4	8	12.9
Totals	314	94	46	151	6	17	140	44.6	23	7.3

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

During the past eight months the SEAS subcommittee has evaluated 34 newly registered viruses. Of these, one (Nola) is considered an arbovirus, four are considered "probable arboviruses", 27 "possible arboviruses" and two are "probably not arboviruses". One "old" virus, Koutango, was re-evaluated as a probable arbovirus on the basis of laboratory mosquito transmission experiments; the fact that there have been no isolates from feral arthropods prevented the subcommittee from giving it full arbovirus status. These evaluations are tabulated on the following tables, to be used in conjunction with the SEAS tabulations of other arboviruses presented in Info-Exchange No. 26 (March, 1974).

For the SEAS subcommittee,
Thomas H.G. Aitken, Chairman

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

SEAS - 1974 Virus Evaluations

Name of Virus	Abbreviation	Group Complex	Suspected Vector (T,M,C and P)*	Isol. from nat. infect. arth.			Biological arth. transmission demonstrated				Arth. inf. by inj.		Passage	Vertebrate viremia demonstrated			Rel.	Epi	Negative information				Rating					Original Source and Host Source
	a	b	c	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
				Once only	2-9 times	≥10 times	By arth. infected by feeding	"Isolated" sentinel animal infected	By wild naturally infected arth.	By arth. infected by injection	Isolation from salivary glands	Isolation from whole arthropod	Transtadial or transovarial	Experimentally infected animal or man	In "isolated" sentinel (col. 5)	In feral animal or man by other than lab infection	Antigenically related to proven arbovirus	Strong epidemiologic associations	Unusual physical, chemical or biologic characteristics	No replication in suspect arthropod	Virus found in vertebrate salivary glands, urine, or feces	Neg. transtadial passage of tick isolate	Arbovirus	Probable arbovirus	Possible arbovirus	Probably not arbovirus	Not arbovirus	
NEW EVALUATIONS Aguacate	AGU	PHL	P			X	(7♂	pools pos.)					X				X							X				Panama Phlebot.
Bangoran	BGN		M	X																					X			Cent. Afr. Rep. Mosquito
Barur (Rhabdo)	BAR		T		X																				X			India Rattus
Batken	BKN		M,T		X																				X			Kirghiz, USSR Tick
Bimbo	BBO																								X			Cent. Afr. Rep. Bird
Birao	BIR	BUN	M		X												X								X			Cent. Afr. Rep. Mosquito

*T, Tick; M, Mosquito; C, Culicoides; P, Phlebotomus

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

SEAS - 1974 Virus Evaluations

Name of Virus	a	b	c	Isol. from nat. infect. arth. 1	2	3	Biological arth. transmission demonstrated 4	5	6	7	Arth. inf. by inj. 8	9	Passage 10	Vertebrate viremia demonstrated 11	12	13	Rel. 14	Epi 15	Negative information 16	17	18	19	Rating 20	21	22	23	24	Original Source and Host Source
Cacao	CAC	PHL	P			X	(2♂	pools	pos.)				X				X							X				Panama Phlebot.
Caimito	CAI	PHL	P	X													X								X			Panama Phlebot.
Chilibre	CHI	PHL	P		X		(1♂	pool	pos.)			X	X				X							X				Panama Phlebot.
Frijoles	FRI	PHL	P	X													X								X			Panama Phlebot.
Garba	GAR	MTY														X									X			Cent. Afr. Rep. Bird
Gomoka	GOM		M		X											X									X			Cent. Afr. Rep. Mosquito
Gordil	GOR	PHL															X								X			Cent. Afr. Rep. Rodent
Huacho	HUA	KEM	T	X																					X			Peru Tick
Kadam	KAD	B	T		X					X		X		X			X							X				Uganda Tick
Karshi		B	T		X												X								X			Uzbekistan, USSR Tick
Kolongo	KOL																								X			Cent. Afr. Rep. Bird
Landjia	LJA																								X			Cent. Afr. Rep. Bird
Marburg												X		X					X		X					X		West Germany Man
Mono Lake	ML	KEM	T	X																					X			California, USA Tick
Nique	NIQ	PHL	P	X													X								X			Panama Phlebot.

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

SEAS - 1974 Virus Evaluations

Name of Virus	a	b	c	Isol. from nat. infect. arth.			Biological arth. transmission demonstrated				Arth. inf. by inj.		Passage	Vertebrate viremia demonstrated			Rel.	Epi	Negative information				Rating					Original Source and Host Source
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Nola	NOLA	SIM	M	X			X			X		X		X			X				X		X					Cent. Afr. Rep. Mosquito
Ouango	OUA															X								X				Cent. Afr. Rep. Bird
Pata	PATA	BLU	M	X															X						X			Cent. Afr. Rep. Mosquito
Phnom-Penh Bat	PPB	B					O							X			X				X					X		Cambodia Bat
Pretoria	PRE	DGK	T	X																					X			South Africa Tick
Punta Salinas	PS	HUG	T	X																					X			Peru Tick
Saint-Floris	SAF																								X			Cent. Afr. Rep. Rodent
Sandjimba	SJA																								X			Cent. Afr. Rep. Bird
Tettnang			T	X	?																				X			West Germany Tick
Yata	YATA		M	X																					X			Cent. Afr. Rep. Mosquito
Zinga	ZGA		M		X											X									X			Cent. Afr. Rep. Mosquito
Zingilamo	ZGO	BTK														X									X			Cent. Afr. Rep. Bird
Zirqa	ZIR	HUG	T	X	?																				X			Persian Gulf Tick
REEVALUATION Koutango	KOU	B	M											X		X	X							X				Senegal Rodent

DEVELOPMENT AND GUIDELINES OF THE ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

The first issue of the Arthropod-borne Virus Information Exchange appeared in April, 1960. Its 39 pages bore brief reports from 18 laboratories and research groups representing six countries, and the names of 60 individuals were on the mailing list. The current issue (No. 27) contains 192 pages and is being sent to nearly 250 arbovirus investigators or administrators of arbovirus programs in over 50 countries. Distribution is made twice each year, in March and September.

The purpose of the Info-Exchange is succinctly stated on the bottom of its title page:

"This exchange is issued for the sole purpose of timely exchange of information among investigators of arthropod-borne viruses. It contains reports, summaries, observations and comments submitted voluntarily by qualified agencies and investigators. The appearance of any information, data, opinions or views in this exchange does not constitute formal publication. Any reference to or quotation of any part of the exchange must be authorized directly by the person or agency which supplied the text."

The Info-Exchange is distributed free of charge, with only such restrictions on distribution as are necessary to assure effective fulfillment of the mission, namely, to provide active workers with needed information 6 months to 2 years in advance of publication through usual channels. To be a recipient of the Info-Exchange, one must be directly or indirectly engaged in some phase of arboviral research, and should periodically contribute brief (1 to 3 pages) summaries of current findings (in English) for the benefit of others in the field. If tables are included, they should be black on white and of such dimensions as to permit their use without retyping. Chronic noncontributors are dropped from the mailing list, as too large a mailing list increases the costs of editing, typing, printing and mailing beyond the resources available, and tends to defeat the purpose of the Info-Exchange as an aid to the active worker. Neither do available resources generally permit the sending of multiple copies to a single research organization, unless the size and complexity of that arbovirus operation preclude effective circulation of a single copy among all of its interested workers.

As the solicited reports represent unpublished work and are not citable references, the Info-Exchange is not sent to reference libraries, nor is it provided to students unless they expect to have a lasting working interest in arboviruses. Such open availability of the Info-Exchange might discourage the serious investigator from submitting his preliminary and often confidential results.

Newcomers with suitable qualifications are welcome, for if the Info-Exchange is to remain effective, it must continue to serve all active investigators in the field of arbovirology. Individuals or research organizations who believe they are qualified should write to Dr. T.O. Berge, Chairman, Subcommittee on Arthropod-borne Virus Information Exchange, briefly stating their qualifications and their intent to cooperate.

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

Between 1st May and 27th July 1974, sera were collected from 780 small forest rodents in the Yukon Territory of Canada between latitudes 61 and 66°N. Neutralizing antibodies to the Marsh Lake 23 strain of California encephalitis (CE) virus (snowshoe hare subtype) have been demonstrated in 37 of 637 (6%) ground squirrels (Citellus undulatus), 1 of 18 tree squirrels (Tamiasciurus hudsonicus) and 4 of 6 snowshoe hares (Lepus americanus) tested to date.

California encephalitis virus (snowshoe hare subtype) has been isolated from a pool of 31 Aedes canadensis collected near Carmacks (62°N, 135°W) on 10 June.

Localization of replication of CE virus (Marsh Lake 23 strain) within the cytoplasm of acinar cells of salivary glands of A. aegypti was demonstrated after 2 to 27 days incubation at 70°F or 55°F following intrathoracic injection with 100 to 400 mouse LD_{50}, and after 4 to 22 days incubation at the same reduced temperatures for A. canadensis, A. communis and Culiseta inornata mosquitoes after intrathoracic injection with the same virus doses. Infectivity titers of CE virus in thoraces of replicate mosquitoes attained 2.0 to 4.0 log mouse LD_{50} at the time immunoperoxidase reactions were demonstrated. In order to render the peroxidase staining reaction specific for CE virus antigen, single-injection rabbit antiserum to Marsh Lake 23 virus was passed through Sephadex G150 columns before and after coupling to Sigma type VI horseradish peroxidase, orthotolidine was substituted for benzidine to reveal the enzyme within infected tissues, and sodium deoxycholate was used to eliminate non-specific nuclear staining. Salivary glands from uninfected mosquitoes were always processed simultaneously with infected glands as controls.

D.M. McLean

REPORT FROM THE ARBOVIRUS RESEARCH UNIT
SCHOOL OF PUBLIC HEALTH
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

This report reviews field and laboratory studies on arboviruses in California from 1 May 1973 through 30 April 1974.

In Kern County California, measurements were maintained of: vector populations; immunological conversions to Western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE) and Turlock (TUR) viruses; climatic factors; and clinical encephalitis cases. The mosquito control program was effective as the female Culex tarsalis light trap seasonal indices were 2 or less per trap night at most urban-suburban sites and less than 10 at most rural sites. Notable exceptions were in the vicinity of the several sewer farms where weekly indices surpassed 100 per trap night. Serial blood samples from 140 sentinel chickens in 9 flocks revealed no evidence of WEE infection, only 1 of the birds had been infected with SLE and TUR virus had infected 19 percent of birds. Serologic tests on bloods from 199 House Finches confirmed the low levels of viral activity detected in sentinel chickens. One clinical case of encephalitis was confirmed to be SLE and this was in a 17 year old girl, resident of Bakersfield, and with possible exposure in a rural environment. There were no confirmed cases of WEE in humans or horses. Climatic conditions during 1973 were typified by significantly above normal summer temperatures and a slight deficiency in rainfall for the year.

A fifth and final year of study on the ecological impact of a large scale irrigation development on a desert environment on the West Side of Kern County continued to reveal little evidence of viral activity. Culex tarsalis breeding continued to be minimal, largely related to the use of sprinkler rather than furrow irrigation. Infiltration by adult female C. tarsalis into the area was minimal. The average light trap nightly indices at the 6 sites ranged from less than 1 to 6 for the summer. Avian and mammalian populations seemed to have stabilized in the past several years. Serological tests on bloods from 77 sentinel chickens, 54 wild birds and 55 wild mammals yielded little or no evidence of WEE or SLE viral infection and a wide spread but low level of TUR viral infection. No antibodies were detected in serological tests on mammalian sera with 6 other arboviruses. The principal findings of this study have been that: sprinkler irrigation produced minimal C. tarsalis populations, minimal populations of C. tarsalis supported transmission of TUR virus but not of WEE or SLE viruses, and avian populations rapidly became established once tree harborage and food were available.

Studies of arboviruses in Butte and Glenn Counties in the Sacramento Valley were continued for a fifth year. Surveillance of mosquito populations and viral infection rates at 15 study sites revealed that the light trap indices at most sites remained relatively constant over the 5 years. The mean number of female C. tarsalis per trap night continued to be higher (seasonal averages above 5 at 10 of the 15 sites) than the averages seen in Kern County. Viral tests on 9,280 C. tarsalis revealed the following minimal infection rates (MIR) per 1,000: SLE 0.1, WEE 0.3 and TUR 1.7. For the third consecutive year the MIR for WEE in Aedes melanimon was equal to or slightly higher than in C. tarsalis.

TUR virus was isolated once from Culex peus. Viruses of the California encephalitis (CE) group, Jamestown Canyon (JC), were isolated from 5 pools of A. melanimon. This was the fourth consecutive summer this virus was detected. Serological tests on sera from 223 chickens maintained as 16 sentinel flocks confirmed the pattern of virus occurrence in C. tarsalis as there were only 1.8 and 0.4 percent conversions for WEE and SLE antibodies respectively and a 17.5 percent conversion for TUR antibodies.

There were no confirmed cases of WEE or SLE in humans or horses resident in the Butte or Glenn County areas in 1973.

An effort was made to detect transovarian transmission of CE group viruses in A. melanimon. A total of 10,545 A. melanimon adults were reared from larvae and pupae collected in the summer and these were tested for virus with negative findings. From the same field areas in the same time period 12,769 A. melanimon females were collected and yielded 5 CE and 3 WEE viral isolations. We have no evidence of transovarian transmission of CE or WEE virus in A. melanimon.

Intensive ecological studies on the Llano Seco Ranch in Butte County confirmed the pattern of virological activity observed for the bi-county area. Ring-necked Pheasants and House Finches commonly were infected with SLE and TUR viruses and Mourning Doves with SLE virus. The principal antibodies found in jackrabbits were to 3 mosquito-borne viruses WEE, SLE and CE and 3 Culicoides-borne viruses Buttonwillow (BW), Lokern (LOK) and Main Drain (MD). Studies of blood-meals from mosquitoes collected at Llano Seco confirmed that C. tarsalis fed on Mourning Doves, passeriforme birds, bovids and leporids. Culex peus fed almost exclusively on birds and A. freeborni almost exclusively on large mammals.

The studies in 1973 confirmed the endemicity of a wide range of arboviruses in the Sacramento Valley and the occurrence of a jackrabbit-A. melanimon cycle for WEE virus that has potential significance for rapid spread of infection to humans and horses.

A study was continued at the Gray Lodge study site in Butte County to determine if the relative abundance of vectors influenced the proportion that fed on avian versus mammalian hosts. The procedure was to expose paired jackrabbit-chicken or jackrabbit-pheasant hosts in a stable trap and then to correlate the rate of feeding on each host when different numbers of vectors entered the traps. The principal observations were that there was an increased proportion of feedings on mammalian hosts when the largest numbers of mosquitoes were present and that individual chickens varied widely in their tolerance to vector attack. Pheasants were less tolerant than chickens to vector attack. Aedes melanimon and Anopheles freeborni attracted to the above traps fed predominantly on rabbits regardless of vector abundance. Culex erythrothorax is aviophilic but in stable traps it fed on jackrabbits more readily than on pheasants.

We continued to support the diagnostic studies of the California State Department of Health and have tested paired sera from suspected arbovirus infections in humans and horses that had their onsets in 1972. We tested sera from 264 humans and 39 horses against 11 arboviral hemagglutinins. We diagnosed 2 cases (SLE) in humans and showed there was a very low prevalence of WEE (2 percent), SLE (8 percent) or CE (2 percent) antibodies in this sample. The

low prevalence of antibodies indicates there has been a very low rate of infections in this sample of persons in recent years. We diagnosed 2 cases of WEE in horses. The prevalence of SLE, CE, MD, LOK, BW and Venezuelan equine encephalitis (VEE) antibodies in horses was low. The low prevalence of WEE and VEE hemagglutination inhibiting (HI) antibodies indicated a rapid waning of immunity 1 year after the intensive VEE vaccination program in 1971.

A follow-up was begun on the large bank of horse sera collected from 13 counties of California before and after the statewide VEE vaccination program in horses in 1971. We tested the prevaccination blood samples from 926 horses in HI tests with 10 arboviral antigens. A high proportion (73 percent) of horses had HI antibodies to WEE. Antibodies to SLE virus had the highest frequency in horses from the Sacramento and Imperial Valleys. Modoc (MOD) antibodies had a relatively high prevalence in all areas except Los Angeles County. Antibodies to BW, CE, TUR and LOK viruses had a low prevalence. Tests are now in progress on sequential sera from these horses and should provide data on the incidence of infections over a 1 year period and the rate of loss of antibodies for the same viruses.

A bank of 23 unidentified viruses accumulated from the Sacramento Valley in the 1970-1972 period. Detailed study of these agents, principally with fluorescent antibody techniques, revealed that 14 probably were related to Hart Park (HP) virus, another group may be related to Umatilla virus and others have remained unidentified. The reagents prepared for this study allowed us to identify 14 isolates of HP virus from Butte County in 1973. Five other isolates from 1973 are added to the unidentified agents from the previous years.

We detected chronic TUR virus infection in 1 of 11 House Finches infected one or more years earlier with this virus. Isolation of TUR virus was from fragment cultures of the kidneys. We have now recovered TUR virus from 1 or more organ cultures of 3 of 53 House Finches infected more than 1 year earlier. We were unsuccessful in the isolation of virus from thousands of C. tarsalis that had fed on these birds or from blood samples. The viral strains isolated from the fragment organ cultures are more difficult to work with than are isolates from mosquitoes primarily because they do not reach high titers on passage in duck embryo cell cultures (DECC). Studies are being continued on latent WEE and TUR viral infections in House Finches and on latent WEE, BUT and CE infections in jackrabbits.

The Ring-necked Pheasant is a very common bird in the Sacramento Valley. Experimental pathogenesis studies revealed that this bird is refractory to infection when inoculated with 1,000 plaque forming units (PFU) of CE virus. All birds inoculated with 50 PFU of SLE virus or 1,000 PFU of TUR virus developed HI and/or serum dilution neutralizing (SDN) antibodies. No birds developed TUR viremia and only 3 of 9 birds developed a transitory low grade SLE viremia.

A search was extended for in vitro markers, other than plaque size, for large and small plaque variants of WEE virus that had varied markedly in their pathogenesis for kangaroo rats. Addition of various concentrations of DEAE dextran to overlay agar medium did not modify plaque size. One step growth curves in DECC revealed that our small plaque variant grew better at 42 C than at 32 or 37 C but did not differ from the growth of our large plaque variant thus the small plaque agent is not a low temperature mutant. The small plaque variant was inactivated faster at 56 C than the large plaque variant.

A project, now in its third year, has as its objective to identify genetic and nongenetic variables that affect the ability of C. tarsalis to become infected with and to transmit arboviruses. If genetic factors can be identified and isolated these characteristics will be linked with chromosomal translocations for insertion into field populations to control vector populations and to stop viral transmission. During 1973, 30 field populations of mosquitoes were evaluated for their susceptibility to WEE and SLE viruses. Culex tarsalis populations varied as much as 1,000 fold in their susceptibility to WEE virus. At one site susceptibility to WEE increased 100 fold from 26 June to 13 August. Culex peus and Culex pipiens were refractory to WEE viral infection.

Studies with A. melanimon, reared from pupae collected in the Sacramento Valley, demonstrated that when this species was exposed to WEE virus by intrathoracic inoculation, feeding on pledgets or feeding on viremic chicks, it was as susceptible as C. tarsalis. Comparison of the infectivity threshold of A. melanimon with the WEE viremia profile of jackrabbits indicated that for more than a 48 hour period viremia in this host would be sufficient to infect 50 percent or more of the vectors. Infected A. melanimon readily transmitted WEE virus after 7-24 days extrinsic incubation at 24 C.

Variations in the susceptibility of C. tarsalis to SLE viral infection were less than for WEE virus, but in almost all populations a high proportion of individuals were refractory to any dose of virus. Culex peus was as susceptible to SLE virus as C. tarsalis.

Variations in susceptibility of C. tarsalis to WEE, SLE or TUR viral infection did not correlate with the degree of autogeny or organophosphorus resistance.

The detection of variations in viral susceptibility led us to a study of the susceptibility of 7 colonized strains of C. tarsalis to infection with WEE, SLE or TUR virus by intrathoracic inoculation and/or pledget feeding. The purpose was to identify susceptible and resistant strains for further genetic studies. The susceptibility of all strains to WEE virus was quite similar except for the Ft. Collins strain which was resistant. Variations in susceptibility to the 3 viruses were insufficient to serve as a basis for genetic studies. A study is now in progress to select and colonize subpopulations that vary in susceptibility and ability to transmit WEE virus. These studies are being initiated with geographic strains that already differ in the directions desired.

Collaborative studies are in progress with Dr. A. Ralph Barr of the susceptibility of genetic strains of C. pipiens with well identified phenotypic markers to 4 geographically representative strains of SLE virus, 2 from California and 1 each from Illinois and Texas.

A colonized strain of Aedes dorsalis was found to be an excellent vector of WEE virus and is a more efficient transmitter than A. melanimon. Preliminary efforts to demonstrate transovarial infection were unsuccessful.

Studies of nongenetic variables revealed that vector age or the concentration of blood used in viral suspensions did not affect the susceptibility of C. tarsalis to WEE viral infection. However, concentrations of sucrose of 5 percent or more in WEE virus-blood mixtures decreased the proportion of mosquitoes that became infected as compared with a concentration of 2.5 percent. Temperature is an important environmental variable that affects extrinsic incubation of arboviruses. We found that WEE virus multiplied sufficiently to complete extrinsic incubation in C. tarsalis within 7 days at 18 and 24 C. In contrast, SLE required 21 days at 18 C and possibly 10 days incubation at 24 C before they could transmit. WEE virus reached mean titers of $10^{4.5}$ within 8 days at 18 C and 3 days at 24 C while SLE required 28 and 8 days at the same respective temperatures to reach peak titers.

Our studies on vector competence along with the findings of other researchers on insecticide resistance have emphasized the need for basic studies on the genetics of C. tarsalis. Such studies are now in their second year. Twelve possible phenotypic mutations are under study and 4 seem to be established including a marker on each of the 3 chromosomes. The normal karyotype has been described including a description of the behavior of the chromosomes in mitotic and meiotic cycles and in sequential cell division. Seven radiation-induced heterozygous chromosomal interchanges have been cytologically identified and at least 1 is established between chromosome II and III in the homozygous condition and is carried by both sexes. Efforts are being made to control the rate of embryonation through temperature control as a means of extending the time between generations of the colonies.

A C. tarsalis cell line has been adapted for virological studies and WEE, SLE, CE, JC, Cache Valley, TUR, BW, MD and LOK viruses multiplied in the cell line. POW, MOD and Rio Bravo viruses did not multiply in the cells. Five rhabdoviruses were studied and HP and Vesicular Stomatitis (VSV) Indiana mulplied but Kern Canyon, VSV New Jersey and VSV Cocal probably did not. SLE and TUR were grown at 24, 32 and 37 C in the C. tarsalis cell line. TUR virus grew equally well at all 3 temperatures but SLE virus did not multiply as well at 37 as at 24 and 32 C.

Three theses have been completed and covered such diverse subjects as: epidemiological and immunological studies on VEE (TC-83) vaccine, the relation between hypersensitivity to the bite of Culicoides variipennis and infection of leporids with BW virus, and studies of autogeny in C. tarsalis.

This report represents the summary of our Annual Progress Report. A limited number of copies of this report and prior Annual Reports are available on request.

(W. C. Reeves)

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

Surveillance for mosquito-borne encephalitis during the 1973 season again confirmed the persistence of western encephalitis (WEE) and St. Louis encephalitis (SLE) viruses in their natural cycles in many areas of the state. However, vector control and equine immunization programs kept human and equine cases of disease at the low levels characteristic of the past two decades. As usual, surveillance activities were carried out in collaboration with many other local, State and Federal agencies and personnel.

At least 1,037 persons suspected of having an arbovirus infection during the year were screened serologically by the State Viral and Rickettsial Disease Laboratory or by county health department laboratories (Table 1). No human cases of WEE were detected, and there was no evidence of Venezuelan equine encephalitis (VEE) in California, nor any resurgence of this disease in the central and northern areas of Mexico which were affected in previous years. However, five cases of SLE were confirmed, as shown in Table 2:

(1) A 62-year-old woman from Riverside County, onset July 19, 1973;

(2) A 17-year-old girl from Kern County, onset August 12, 1973;

(3) A 29-year-old woman from San Joaquin County, onset August 24, 1973, who visited in Butte County shortly before her illness, but whose mosquito exposure was most likely in her home environment;

and two cases in San Diego County, detected by the local public health laboratory, and confirmed by the State Viral and Rickettsial Disease Laboratory;

(4) A 12-year-old boy with onset September 5, 1973, and exposure most likely in a San Diego suburban area; and

(5) A 31-year-old man presumably exposed in Imperial County, onset October 10, 1973.

Neutralization, hemagglutination-inhibition, complement-fixation, and indirect fluorescent antibody methods were all used to confirm these diagnoses. The patients all recovered without sequelae. No fatality from WEE or SLE has been recorded in California since 1962.

Fifty-six clinically suspect equine cases were reported to the State Health Department, but only two cases of WEE could actually be confirmed by serologic tests: a one-year-old unvaccinated horse from Yolo County, with onset August 12, 1973; and a four-month-old unvaccinated colt from Shasta County, with onset September 4, 1973 (Table 3).

The mosquito testing program was carried out as usual by the State Vector Control Section, the Local Mosquito Abatement Districts, and the State Viral and Rickettsial Disease Laboratory. Of 4,838 mosquito pools tested in suckling mice, the yield was 281 virus isolates, the highest recovery rate in recent years (Tables 4-7). There were 97 isolates of WEE virus and 75 of SLE virus, mostly from Imperial County where mosquito collection was emphasized because of concern that VEE might move northward from Mexico. In addition, 74 strains of Turlock virus, 26 of Hart Park virus, three of California encephalitis virus, two of Main Drain virus, three of Bunyamwera group virus, and one as yet unidentified virus were isolated.

Despite the frequency of Turlock and Hart Park virus isolations from Culex tarsalis (the common and efficient vector for WEE and SLE viruses), little or no evidence of human or equine illness, or even of infection with these viruses has been obtained in California. And despite the recent occurrence of California encephalitis virus in mosquitoes after many years apparent absence from the state, no human cases of disease have been detected.

The only other arbovirus disease of significance during 1973 was Colorado tick fever. A total of 25 cases occurred, the largest number since record keeping began in 1954, bringing the total proven cases in California to 162.

During the 1974 surveillance season (as of September 1, 1974), no human or equine cases of arbovirus encephalitis have been laboratory confirmed, although numerous suspect cases are under study. Over 800 mosquito pools have been tested, yielding 17 strains of Turlock virus, four strains of WEE virus, four strains of Hart Park virus, and two strains of SLE virus, all from Culex tarsalis. The WEE and SLE isolates were only from Imperial and Riverside counties in southern California. There have been 13 cases of Colorado tick fever confirmed thus far in 1974.

(Richard W. Emmons and Edwin H. Lennette)

Table 1.-Humans tested serologically for mosquito-borne arboviruses by the Viral and Rickettsial Disease Laboratory, California State Department of Public Health and by county health department laboratories, by county of residence and month of illness onset, California, 1973.

COUNTY	TOTAL	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	UNR
California														
Alameda	18	-	-	1	1	2	2	5	2	1	1	-	-	3
Butte	15	-	1	1	-	3	-	1	3	4	1	-	-	1
Colusa	2	-	-	-	-	-	-	-	1	-	1	-	-	-
Contra Costa	33	-	-	-	1	4	8	4	5	-	7	1	-	3
Fresno*	268	16	-	-	-	26	28	56	64	18	30	12	16	2
Glenn	3	-	-	-	-	-	-	-	1	2	-	-	-	-
Imperial	3	-	-	-	-	-	1	-	-	1	-	-	-	1
Inyo	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Kern	48	-	-	1	-	3	7	8	6	3	6	2	-	12
Kings	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Lake	5	-	-	-	-	-	-	-	-	3	-	-	-	2
Lassen	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Los Angeles**	52	-	-	1	5	11	11	5	4	12	1	2	-	-
Marin	14	-	-	-	-	2	3	2	3	1	-	1	-	2
Mendocino	4	-	-	-	-	-	-	2	2	-	-	-	-	-
Merced	1	-	-	-	-	-	1	-	-	-	-	-	-	-
Modoc	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Monterey	6	-	-	1	-	2	-	-	-	1	-	-	-	2
Nevada	6	-	-	-	-	-	-	1	4	-	1	-	-	-
Placer	4	-	-	-	-	-	-	1	1	-	-	-	-	2
Riverside	13	-	-	-	-	-	5	5	1	1	-	-	-	1
Sacramento***	79	-	-	-	-	1	23	16	13	15	4	7	-	-
San Bernardino	17	-	-	-	-	2	3	4	3	2	1	1	-	1
San Diego****	75	-	-	-	2	13	12	10	13	13	6	4	-	2
San Francisco	92	-	-	1	1	12	11	19	12	12	8	6	-	10
San Joaquin	19	-	-	-	-	-	2	3	5	2	4	-	-	3
San Luis Obispo	3	-	-	-	-	-	1	-	-	1	-	1	-	-
San Mateo	47	-	1	-	-	4	4	5	11	7	5	8	-	2
Santa Barbara	21	-	-	-	-	3	2	5	5	-	1	-	-	5
Santa Clara	92	-	1	1	2	9	5	17	21	14	10	3	-	9
Santa Cruz	12	-	-	-	-	2	3	1	5	-	-	-	-	1
Shasta	6	-	-	-	-	-	-	2	1	3	-	-	-	-
Siskiyou	2	-	-	-	-	-	-	2	-	-	-	-	-	-
Solano	11	-	-	-	1	-	1	1	1	4	1	1	-	1
Sonoma	13	-	-	-	-	2	2	1	2	1	2	1	-	2
Stanislaus	12	-	-	-	-	-	2	6	-	3	-	-	-	1
Sutter	2	-	1	-	-	-	-	-	-	-	1	-	-	-
Tehama	8	-	-	-	-	-	1	3	-	2	2	-	-	-
Tulare	5	-	-	-	-	-	-	1	3	-	1	-	-	-
Tuolumne	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Ventura	5	-	-	-	-	-	-	1	-	2	1	1	-	-
Yolo	16	-	-	-	3	3	1	3	1	2	1	1	-	1
Total	1,037	16	4	7	16	104	139	193	193	132	96	52	16	69

* Tested by County Health Department Laboratory (includes 9 patients tested by State VRDL)
** Tested by County Health Department Laboratory (includes 12 patients tested by State VRDL)
*** Tested by County Health Department Laboratory (includes 10 patients tested by State VRDL)
**** Tested by County Health Department Laboratory (includes 4 patients tested by State VRDL)

Table 2.— Human cases of arbovirus encephalitis in California, 1973

Patient identification and exposure	Clinical Course	Serologic test	Antibody Titer			Remarks
			Acute	Convalescent		
1. L.O., 62, female Blythe, Riverside County; no specific exposure stated.	Onset July 19, 1973; fever, lethargy, semi-stupor; CSF-57 mononuclear cells; 58 mgm % protein; hospitalized; good recovery.	SLE-CF SLE-CF SLE-HAI SLE-IFA SLE-IFA SLE-NI SLE-NI	July/23/73 1:4 1:8 1:40 1:512 1:256 3.3 3.9	Aug/20/73 1:8 - 1:80 1:512 1:256 3.4 -	Sept/5/73 - 1:16 1:20 - 1:256 - 4.9	CF tests for WEE, VEE, polio 1,2 & 3 negative; mumps and herpes low stationary; HAI for 9 other arboviruses negative.
2. A.A., 17, female, Bakersfield, Kern County; probable mosquito exposure at Hart Park.	Onset August 12, 1973; fever, headache, mild nuchal rigidity; CSF-74 WBC; hospitalized, good recovery	SLE-CF SLE-HAI SLE-IFA SLE-NT	Aug/17/73 <1:4 1:160 1:64 1.9	(sent)Sept/1/73 1:8 1:80 1:128 3.0	Sept/11/73 1:8 1:160 1:128 4.5	CF tests for WEE, VEE, herpes, negative; mumps 1:16 stationary; HAI tests for WEE, Turlock, negative.
3. L.H., 29, female, Stockton, San Joaquin County; possible mosquito exposure while water skiing in Delta; August 12 and 16; also visited Chico August 18-19.	Onset August 24, 1973, severe headache, backache, malaise, fever, stiff neck, vomiting; rapid recovery.	SLE-CF SLE-CF SLE-HAI SLE-IFA SLE-NI	Sept/3/73 1:32 - 1:40 1:128 6.2	Sept/13/73 1:64 1:32 ≥1:160 1:256 6.3	Oct/1/73 - 1:16 ≥1:160 1:256 6.4	CF tests for WEE, VEE, herpes, negative, mumps 1:8 stationary; HAI tests for 9 other arboviruses negative.
4. K.B., 12, male, LaMesa, San Diego County; probable mosquito exposure at Santee, near his home; SLE virus isolated from C. pipiens and SLE antibody found in pheasants and chickens in area.	Onset September 5, 1973; high fever, headache, stiff neck, vomiting, confusion, loss of memory, hallucinations; CSF - 150 WBC (80% polys)→67 WBC (85% lymphs); hospitalized; good recovery.	SLE-CF SLE-HAI SLE-IFA SLE-NI	Sept/8/73 <1:8 1:160 1:64 2.2	Sept/25/73 1:32 ≥1:640 1:256 QNS		CF test for WEE negative; no antibody rise for mumps or herpes; HAI tests for WEE, Turlock, negative.
5. E.S., 31, male Spring Valley, San Diego County; probable mosquito exposure near Picacho, Colorado River, September 29-30; possible exposure on job as mechanic in Santee Lakes area.	Onset October 10, 1973; headache, high fever, dizzy, vomiting, delirium; hospitalized; good recovery.	SLE-CF SLE-HAI SLE-IFA SLE-NI	Oct/14/73 <1:8 1:20 1:32 -	Oct/29/73 1:16 ≥1:160 1:128 -	Nov/28/73 1:16 1:80 1:256 -	HAI tests for 8 other arboviruses negative (<1:10); Rio Bravo HAI 1:10 - 1:20 - 1:10

Table 3 - Suspected clinical cases of arbovirus encephalitis in equines, by county and month, for California 1973.

COUNTY	MONTH OF ONSET												Undetermined* or not tested	Totals
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		
Totals	1	1	2	7	9	3	5	18	6	3	1	1	6	63
Butte				1				1						2
Contra Costa								1					1	2
Glenn								1						1
Humboldt								1						1
Imperial	1			1			1	1						4
Kern		1						1	1				1	4
Lake				1				1						2
Los Angeles								2		1				3
Marin				1										1
Mariposa							1							1
Mendocino					2				1					3
Placer													1	1
Riverside					2	1	1							4
Sacramento					1									1
San Bernardino				1										1
San Diego				2				2					1	5
San Joaquin					1	1		1		1	1			5
Santa Barbara							1	1						2
Santa Clara			2				1					1		4
Shasta					2				2**					4
Sonoma								1						1
Tehama						1		1						2
Trinity										1				1
Tuolumne					1			1	1				1	4
Yolo								1**	1				1	3
Yuba								1						1

* Inadequate serum specimens available for testing. Complement fixation tests performed by the Viral and Rickettsial Disease Laboratory, California State Department of Health.

** Only two cases confirmed serologically for WEE: a 1-year old horse with onset in August from Yolo County with no history of vaccination; rising antibody titers of 1:8 to 1:32 taken two weeks apart; and a 4-month old colt from Shasta County with onset in September, no history of vaccination, the antibody titer rising from 1:64 to 1:256.

Table 4. Numbers of mosquitoes and other Diptera tested, by County and Month, California, 1973, by the Viral and Rickettsial Disease Laboratory.*

County	January	February	March	April	May	June	July	August	September	October	November	Total
Colusa					167 (6)	250 (7)	1,001 (21)	2,326 (47)		519 (11)		4,263 (92)
Fresno					39 (6)	83 (6)	156 (5)	71 (8)	107 (8)	32 (3)		488 (36)
Imperial	2,023 (96)	2,198 (91)	4,396 (149)	8,268 (229)	20,062 (444)	11,215 (304)	6,324 (167)	14,351 (408)	4,581 (168)			73,418 (2,056)
Kern						29 (7)	1,103 (31)	452 (15)	2,479 (53)	27 (1)		4,090 (107)
Kings					722 (19)	126 (9)	96 (7)	97 (6)	404 (12)			1,445 (53)
Lassen								500 (10)				500 (10)
Los Angeles								1,469 (34)		17 (9)		1,486 (43)
Madera					51 (7)	21 (5)	250 (7)	435 (12)	218 (7)			975 (38)
Merced						519 (15)	525 (16)	800 (22)	488 (17)			2,332 (70)
Modoc						600 (12)						600 (12)
Orange									1,122 (23)			1,122 (23)
Placer					117 (4)	459 (11)	441 (10)	63 (6)	33 (3)			1,113 (34)
Riverside							1,114 (35)	8,100 (219)	1,933 (64)	58 (5)		11,205 (323)
Sacramento					22 (1)	74 (2)	173 (4)		32 (2)			301 (9)
San Bernardino							38 (6)	1,453 (72)		544 (44)		2,035 (122)
San Diego	156 (15)	2,345 (91)	1,929 (155)	5,300 (167)	1,257 (43)	5,409 (154)	2,273 (56)	1,279 (37)	1,178 (70)	2,335 (136)	1,498 (69)	24,959 (993)
San Joaquin						25 (1)	802 (18)		32 (1)			859 (20)
Shasta						231 (6)	333 (8)	1,177 (27)	591 (14)			2,332 (55)
Siskiyou							578 (13)					578 (13)
Stanislaus						362 (18)	1,278 (32)	195 (7)	669 (19)			2,504 (76)
Sutter					105 (3)	426 (11)	1,125 (23)	1,838 (39)		129 (4)		3,623 (80)
Tehama						416 (9)	557 (16)	48 (3)				1,021 (28)
Tulare						13 (4)	29 (2)	53 (6)	135 (4)			230 (16)
Ventura							557 (15)					557 (15)
Yolo									19 (1)			19 (1)
Yuba							46 (1)					46 (1)
Yuma, Arizona	33 (6)	203 (12)	827 (23)	1,903 (42)	8,723 (184)	2,119 (46)	267 (9)	5,042 (115)	2,343 (55)			21,460 (492)
Mojave, Arizona								714 (20)				714 (20)
Total	2,212 (117)	4,746 (194)	7,152 (327)	15,471 (438)	31,265 (717)	22,377 (627)	19,066 (502)	40,463 (1,113)	16,364 (521)	3,661 (213)	1,498 (69)	164,275 (4,838)

* Total mosquitoes (pools) tested.

Table 5. Number of mosquitoes and other Diptera tested, by species and month, California 1973, by the Viral and Rickettsial Disease Laboratory.*

Species	January	February	March	April	May	June	July	August	September	October	November	Total
Culex												
tarsalis	1,249 (49)	1,449 (56)	907 (70)	5,860 (183)	14,299 (336)	17,266 (422)	14,239 (362)	15,062 (436)	10,430 (279)	1,179 (58)	15 (7)	81,955 (2,258)
erythrothorax	769 (25)	2,641 (72)	3,588 (107)	8,662 (187)	6,705 (147)	1,763 (88)	766 (17)	6,170 (143)	2,413 (64)	1,748 (53)	1,395 (41)	36,620 (944)
peus		3 (3)	29 (15)			474 (14)	832 (26)	216 (8)	182 (10)	125 (19)	1 (1)	1,862 (96)
pipiens	33 (5)	7 (4)	21 (4)	35 (1)	311 (8)	117 (10)	54 (7)	133 (11)	415 (28)	83 (19)	4 (1)	1,213 (98)
restuans								4 (1)				4 (1)
Aedes												
melanimon					50 (1)	93 (5)	50 (1)	48 (2)		150 (3)		391 (12)
vexans			26 (4)	559 (15)	9,769 (201)	1,873 (44)		4,085 (94)	80 (9)	61 (5)		16,453 (372)
dorsalis			28 (10)	61 (3)	20 (2)	349 (9)		197 (21)	18 (5)	16 (3)		689 (53)
sierrensis								1 (1)				1 (1)
increpitus			2 (1)									2 (1)
squamiger			10 (4)	50 (3)							2 (1)	62 (8)
taeniorhynchus							1 (1)	2 (2)	4 (1)			7 (4)
Anopheles												
freeborni	2 (1)	2 (1)	5 (2)	26 (2)		262 (11)	82 (4)	53 (7)	65 (5)	25 (10)		522 (43)
franciscanus		3 (1)	23 (8)		12 (1)	57 (4)		142 (19)	89 (14)	45 (9)	57 (9)	428 (65)
punctipennis								18 (1)				18 (1)
Culiseta												
incidens	9 (3)	20 (5)	42 (22)	40 (2)		36 (3)		5 (2)	7 (7)	7 (6)	5 (2)	171 (52)
inornata	150 (34)	221 (37)	388 (54)	178 (42)	94 (20)	21 (6)	3 (2)		60 (14)	212 (25)	17 (6)	1,344 (240)
particeps								9 (1)		10 (3)	2 (1)	21 (5)
Psorophora												
confinnis					5 (1)	66 (11)	3,039 (82)	14,296 (355)	2,401 (83)			19,807 (532)
signipennis								22 (9)				22 (9)
Culicoides												
variipennis		326 (11)	1,895 (17)						200 (2)			2,421 (30)
freeborni		53 (1)										53 (1)
mixed species		5 (2)	144 (6)									149 (8)
Simulium griseum		16 (1)	44 (3)									60 (4)
Total	2,212 (117)	4,746 (194)	7,152 (327)	15,471 (438)	31,265 (717)	22,377 (627)	19,066 (502)	40,463 (1,113)	16,364 (521)	3,661 (213)	1,498 (69)	164,275 (4,838)

* Total mosquitoes (pools) tested.

Table 6.— Viruses isolated from mosquitoes by the Viral and Rickettsial Disease Laboratory, California State Department of Health, by county and month of collection, 1973

County	April	May	June	July	August	September	October	Total
Colusa				Hart Park(1)	Turlock(1)		WEE(1)	Turlock(1) WEE(1) Hart Park(1)
Imperial	SLE(1) Turlock(1)	SLE(1) Turlock(12) Main Drain(1)	SLE(12) Turlock(15) WEE(36)	SLE(18) Turlock(4) WEE(32) Unident.(1)	SLE(7) (Turlock(1) Main Drain(1) WEE(4)	SLE(9) Turlock(2)		SLE(48) WEE(72) Turlock(35) Main Drain(2) Unidentified(1)
Kern					Turlock(1)	SLE(2) CEV(1)		SLE(2) CEV(1) Turlock(1)
Kings		Hart Park(2)	Hart Park(2)					Hart Park(4)
Lassen					SLE(1) Turlock(1)			SLE(1) Turlock(1)
Madera				Hart Park(1)	Hart Park(1) Turlock(1)			Turlock(1) Hart Park(2)
Merced				Hart Park(1) Turlock(1)				Turlock(1) Hart Park(1)
Placer				Turlock(1)				Turlock(1)
Riverside				SLE(2) WEE(4) Turlock(1)				SLE(2) WEE(4) Turlock(1)
San Bernardino					WEE(2) SLE(3)		CEV(1)	SLE(3) CEV(1) WEE(2)
San Diego			Turlock(3) Hart Park(1)	Turlock(4)	Turlock(4)	Turlock(1) SLE(4) Hart Park(3)	Turlock(1) SLE(3) Bunyamwera Group(1) Main Drain(1)	SLE(7) Hart Park(4) Turlock(13) Bunyamwera Group(1) Main Drain(1)
San Joaquin				Hart Park(3) Turlock(1)				Turlock(1) Hart Park(3)
Shasta				Hart Park(1)	Hart Park(1) Turlock(1)			Turlock(1) Hart Park(2)
Stanislaus			Hart Park(1)	Hart Park(2)		WEE(1)		Hart Park(3) WEE(1)
Sutter			Hart Park(1)	Hart Park(4) Turlock(2)	WEE(1) CEV(1) Turlock(1)			Turlock(3) WEE(1) Hart Park(5) CEV(1)
Tehama				Hart Park(1)				Hart Park(1)
Tulare					Turlock(1)	SLE(1)		SLE(1) Turlock(1)
Ventura				Turlock(1)				Turlock(1)
Mojave, Arizona					SLE(3) WEE(4)			SLE(3) WEE(4)
Yuma, Arizona		Turlock(4)	SLE(4) Turlock(7) WEE(10)	SLE(1) Turlock(1) WEE(2)	SLE(3)	Bunyamwera Group(1)		SLE(8) WEE(12) Turlock(12) Bunyamwera Group(1)
TOTALS	SLE(1) Turlock(1)	SLE(1) Turlock(16) Hart Park(2) Main Drain(1)	SLE(16) Turlock(25) WEE(46) Hart Park(5)	SLE(21) Turlock(16) WEE(38) Hart Park(14) Unident.(1)	SLE(17) Turlock(12) WEE(11) Hart Park(2) Main Drain(1) CEV(1)	SLE(16) Turlock(3) WEE(1) Hart Park(3) CEV(1) Bunyamwera Group(1)	SLE(3) Turlock(1) WEE(1) Main Drain(1) CEV(1) Bunyamwera Group(1)	SLE(75) Turlock(74) WEE(97) Hart Park(26) Main Drain(3) CEV(3) Bunyamwera Group(2) Unidentified(1)
	2	20	92	89	44	25	8	281 isolates

Table 7.- Viral isolates from mosquito pools, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1973

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V5-3133	Imperial	Seeley	4/4	Culex tarsalis	50	SLE
V5-3255	Imperial	Winterhaven	4/5	C. tarsalis	50	Turlock
V5-3408	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3420	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3446	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3455	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3466	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3478	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3500	Imperial	Westmoreland	5/1	C. tarsalis	50	Turlock
V5-3535	Imperial	Niland	5/2	C. tarsalis	50	Turlock
V5-3573	Imperial	Winterhaven	5/3	C. tarsalis	51	Turlock
V5-3694	Imperial	Winterhaven	5/3	C. tarsalis	50	Turlock
V5-3723	Imperial	Winterhaven	5/3	C. tarsalis	27	Turlock
V5-3755	Imperial	Winterhaven	5/3	C. tarsalis	54	Turlock
V5-3779	Imperial	Winterhaven	5/3	A. vexans	50	SLE&Main Drain
V5-3974	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V5-3989	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V5-4018	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V5-4021	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V4-1241	Kings	Lemoore	5/29	C. tarsalis	50	Hart Park
V4-1253	Kings	Lemoore	5/29	C. tarsalis	50	Hart Park
V5-4031	Imperial	Holtville	6/5	C. tarsalis	47	Turlock
V5-4040	Imperial	Calexico	6/5	C. tarsalis	50	SLE
V5-4082	Imperial	Calexico	6/5	C. tarsalis	50	WEE
V2-2008	Stanislaus	Newman	6/5	C. tarsalis	17	Hart Park
V5-4094	Imperial	Calipatria	6/6	C. tarsalis	50	Turlock
V5-4098	Imperial	Calipatria	6/6	C. tarsalis	50	Turlock
V5-4102	Imperial	Seeley	6/6	C. tarsalis	50	Turlock
V5-4103	Imperial	Seeley	6/6	C. tarsalis	50	Turlock
V5-4104	Imperial	Seeley	6/6	C. tarsalis	48	WEE
V5-4119	Imperial	Brawley	6/6	C. tarsalis	50	SLE
V5-4120	Imperial	Brawley	6/6	C. tarsalis	50	WEE
V5-4121	Imperial	Brawley	6/6	C. tarsalis	50	SLE
V5-4123	Imperial	Brawley	6/6	C. tarsalis	50	SLE
V5-4125	Imperial	Brawley	6/6	C. tarsalis	54	Turlock
V5-4140	Imperial	Niland	6/6	C. tarsalis	50	WEE
V5-4142	Imperial	Niland	6/6	C. erythrothorax	50	WEE
V5-4149	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4152	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4155	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4162	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4163	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4165	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4176	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4177	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4178	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4179	Imperial	Andrade	6/7	C. tarsalis	12	SLE
V5-4187	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4192	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4201	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4206	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4234	Imperial	Winterhaven	6/7	C. tarsalis	45	WEE
V5-4236	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4242	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4244	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4247	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4251	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4255	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4256	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	SLE & Turlock
V5-4257	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	Turlock
V5-4258	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4260	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	SLE & Turlock
V5-4261	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4262	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4263	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4264	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4265	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	Turlock
V5-4269	Imperial	Winterhaven	6/7	C. tarsalis	51	Turlock
V5-4273	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4274	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE

Table 7.-(continued)

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V5-4275	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4277	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4278	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4281	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4282	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4285	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4286	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4287	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4293	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4294	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4300	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4301	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4302	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4306	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V6-1092	Imperial	Winterhaven	6/8	C. tarsalis	17	WEE
V6-1093	Imperial	Winterhaven	6/8	C. tarsalis	50	SLE
V6-1094	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1095	Imperial	Winterhaven	6/8	C. tarsalis	50	SLE
V6-1096	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1097	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1107	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1112	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	Turlock
V6-1114	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	Turlock
V6-1115	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1119	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	Turlock
V6-1120	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	SLE
V6-1123	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1131	Imperial	Winterhaven	6/8	C. tarsalis	50	Turlock
V6-1132	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1133	Imperial	Winterhaven	6/8	C. tarsalis	43	Turlock
V6-1148	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1149	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1152	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	SLE
V6-1154	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V4-1275	Kings	Lemoore	6/13	C. tarsalis	11	Hart Park
V4-1277	Kings	Lemoore	6/13	C. tarsalis	40	Hart Park
V2-1515	Sutter	Pleasant Grove	6/14	C. tarsalis	50	Hart Park
V5-6151	San Diego	Escondido	6/28	C. tarsalis	27	Turlock
V5-6121	San Diego	Chula Vista	6/29	C. tarsalis	50	Turlock
V5-6123	San Diego	Chula Vista	6/29	C. tarsalis	51	Turlock
V5-6128	San Diego	San Ysidro	6/29	C. tarsalis	35	Hart Park
V5-4417	Imperial	Calexico	7/3	C. tarsalis	9	SLE
V5-4421	Imperial	Calexico	7/3	C. tarsalis	7	SLE
V5-4427	Imperial	Holtville	7/3	C. tarsalis	50	WEE
V5-4428	Imperial	Holtville	7/3	C. tarsalis	50	WEE
V5-4430	Imperial	Holtville	7/3	C. tarsalis	43	SLE
V5-4432	Imperial	Seeley	7/3	C. tarsalis	50	SLE
V5-4435	Imperial	Holtville	7/3	C. tarsalis	19	SLE
V5-4440	Imperial	Holtville	7/3	P. confinnis	50	WEE
V5-4443	Imperial	Calexico	7/3	C. tarsalis	50	Turlock
V5-4444	Imperial	Calexico	7/3	C. tarsalis	34	WEE
V5-4446	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4449	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4450	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4451	Imperial	Calexico	7/3	C. tarsalis	50	SLE
V5-4452	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4456	Imperial	Calexico	7/3	C. tarsalis	36	WEE
V5-4458	Imperial	Brawley	7/4	C. tarsalis	61	SLE
V5-4468	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4469	Imperial	Seeley	7/4	C. tarsalis	50	SLE
V5-4475	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4476	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4477	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4480	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4481	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4482	Imperial	Westmoreland	7/4	C. tarsalis	50	WEE
V5-4484	Imperial	Westmoreland	7/4	C. tarsalis	56	WEE
V5-4489	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4494	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4495	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE

Table 7.-(continued)

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V5-4497	Imperial	Westmoreland	7/4	C. tarsalis	33	WEE
V5-4498	Imperial	Niland	7/4	C. tarsalis	50	SLE
V5-4500	Imperial	Seeley	7/4	C. tarsalis	46	SLE
V5-4504	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4505	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4506	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4509	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4510	Imperial	Seeley	7/4	C. tarsalis	50	Turlock
V5-4511	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4512	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4514	Imperial	Seeley	7/4	C. tarsalis	47	WEE
V4-1315	Merced	Merced	7/5	C. tarsalis	50	Hart Park
V5-4313	Imperial	Winterhaven	7/5	C. tarsalis	31	WEE
V5-4315	Imperial	Winterhaven	7/5	C. tarsalis	41	WEE
V5-4316	Imperial	Winterhaven	7/5	C. tarsalis	4	Turlock
V5-4324	Imperial	Winterhaven	7/5	C. tarsalis	48	WEE
V5-4326	Imperial	Winterhaven	7/5	C. tarsalis	15	WEE
V5-4329	Yuma, Arizona	Yuma	7/5	C. tarsalis	46	SLE
V5-4330	Imperial	Winterhaven	7/5	C. tarsalis	11	WEE
V5-4332	Yuma	Yuma	7/5	C. tarsalis	17	WEE
V5-4333	Yuma, Arizona	Yuma	7/5	C. tarsalis	50	WEE
V5-4335	Imperial	Winterhaven	7/5	C. tarsalis	50	WEE
V5-4339	Yuma, Arizona	Yuma	7/5	C. tarsalis	40	WEE
V5-4340	Yuma, Arizona	Yuma	7/5	C. tarsalis	24	Turlock
V2-1559	Sutter	Pleasant Grove	7/6	C. tarsalis	41	Hart Park
V2-1562	Placer	Lincoln	7/6	C. tarsalis	50	Turlock
V5-4341	Riverside	Blythe	7/6	C. tarsalis	7	SLE
V5-4351	Imperial	Palo Verde	7/6	C. tarsalis	19	Turlock
V5-4353	Riverside	Blythe	7/6	C. tarsalis	17	WEE
V5-4354	Riverside	Blythe	7/6	C. tarsalis	28	WEE
V5-4360	Riverside	Blythe	7/6	C. tarsalis	4	WEE
V5-4365	Riverside	Ripley	7/6	C. tarsalis	22	WEE
V5-4372	Riverside	Blythe	7/6	C. tarsalis	38	SLE
V5-4374	Imperial	Palo Verde	7/6	C. tarsalis	20	WEE
V5-4381	Imperial	Palo Verde	7/6	C. tarsalis	50	WEE
V5-4383	Imperial	Palo Verde	7/6	C. tarsalis	50	WEE
V5-4384	Imperial	Palo Verde	7/6	C. tarsalis	50	WEE
V5-4387	Imperial	Palo Verde	7/6	C. tarsalis	53	Unknown
V5-4390	Imperial	Palo Verde	7/6	C. tarsalis	31	WEE
V1-1034	Tehama	Gerber	7/10	C. tarsalis	50	Hart Park
V2-1574	Colusa	Princeton	7/12	C. tarsalis	21	Hart Park
V2-2038	San Joaquin	Escalon	7/12	C. tarsalis	50	Hart Park
V2-2039	San Joaquin	Escalon	7/12	C. tarsalis	50	Turlock
V2-2044	San Joaquin	Escalon	7/12	C. tarsalis	50	Hart Park
V2-2049	San Joaquin	Escalon	7/12	C. tarsalis	50	Hart Park
V1-1040	Shasta	Palo Cedro	7/13	C. tarsalis	32	Hart Park
V5-8504	Ventura	Thousand Oaks	7/14	C. peus	50	Turlock
V2-2058	Stanislaus	Newman	7/17	C. tarsalis	52	Hart Park
V2-2063	Stanislaus	Mountain View	7/17	C. tarsalis	44	Hart Park
V5-8001	Riverside	Riverside	7/19	C. peus	12	Turlock
V4-1332	Madera	Madera	7/24	C. tarsalis	41	Hart Park
V4-1346	Merced	Merced	7/25	C. tarsalis	19	Turlock
V2-1587	Sutter	Sutter City	7/26	C. tarsalis	50	Turlock
V2-1588	Sutter	Sutter City	7/26	C. tarsalis	50	Turlock & Hart Park
V2-1589	Sutter	Sutter City	7/26	C. tarsalis	50	Hart Park
V2-1590	Sutter	Sutter City	7/26	C. tarsalis	50	Hart Park
V5-6179	San Diego	San Ysidro	7/31	C. peus	11	Turlock
V5-6192	San Diego	San Ysidro	7/31	C. tarsalis	38	Turlock
V5-6208	San Diego	Santee	7/31	C. tarsalis	50	Turlock
V5-6209	San Diego	Santee	7/31	C. tarsalis	50	Turlock
V5-6236	San Diego	Rancho Santa Fe	8/1	C. tarsalis	31	Turlock
V5-6238	San Diego	San Diego	8/1	C. tarsalis	50	Turlock
V5-6240	San Diego	Rancho Santa Fe	8/1	C. tarsalis	55	Turlock
V5-6261	San Diego	Rancho Santa Fe	8/1	C. tarsalis	56	Turlock
V1-1066	Lassen	Wendel	8/2	C. tarsalis	50	SLE
V1-1074	Lassen	Wendel	8/2	C. tarsalis	50	Turlock
V5-4527	Imperial	Holtville	8/2	C. tarsalis	15	WEE
V5-4531	Imperial	Calexico	8/2	C. tarsalis	35	WEE
V5-4537	Imperial	Calexico	8/2	C. tarsalis	50	WEE
V5-4588	Imperial	Niland	8/3	C. tarsalis	47	SLE

Table 7.-(continued)

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V2-1684	Sutter	Sutter	8/3	A. melanimon	24	CEV
V2-1687	Sutter	Sutter	8/3	C. tarsalis	50	WEE
V5-4609	Imperial	Winterhaven	8/4	C. tarsalis	21	Main Drain
V5-4635	Imperial	Winterhaven	8/4	C. tarsalis	39	SLE
V5-4664	Yuma, Arizona	Yuma	8/4	C. tarsalis	50	SLE
V5-4682	Yuma, Arizona	Yuma	8/4	C. erythrothorax	50	SLE
V4-1380	Madera	Madera	8/7	C. tarsalis	42	Turlock & Hart Park
V4-1383	Tulare	Woodville	8/8	C. tarsalis	8	Turlock
V1-1081	Shasta	Palo Cedro	8/10	C. tarsalis	50	Turlock & Hart Park
V2-1642	Sutter	Meridian	8/12	C. tarsalis	50	Turlock
V2-1648	Colusa	Moon Bend	8/12	C. tarsalis	50	Turlock
V4-1409	Kern	Maricopa	8/15	C. tarsalis	36	Turlock
V5-9205	San Bernardino	Needles	8/17	C. tarsalis	47	WEE & SLE
V5-9212	Mojave	Bermuda	8/17	C. tarsalis	50	WEE
V5-9214	Mojave	Bermuda	8/17	C. tarsalis	50	WEE & SLE
V5-9215	Mojave	Bermuda	8/17	C. tarsalis	50	SLE
V5-9216	Mojave	Bermuda	8/17	C. tarsalis	50	WEE
V5-9217	Mojave	Bermuda	8/17	C. tarsalis	50	WEE
V5-9218	Mojave	Bermuda	8/17	C. tarsalis	53	SLE
V5-9228	San Bernardino	Needles	8/17	C. tarsalis	33	WEE
V5-9242	San Bernardino	Needles	8/17	C. tarsalis	50	SLE
V5-9243	San Bernardino	Needles	8/17	C. tarsalis	58	SLE
V5-5099	Imperial	Seeley	8/28	C. tarsalis	30	SLE
V5-5118	Imperial	Calexico	8/28	C. tarsalis	27	SLE
V5-5126	Imperial	Calexico	8/28	C. tarsalis	36	SLE
V5-5148	Imperial	Seeley	8/29	C. tarsalis	50	Turlock
V5-5181	Imperial	Niland	8/29	C. tarsalis	50	SLE
V5-4952	Imperial	Winterhaven	8/30	C. tarsalis	48	SLE
V5-5078	Yuma, Arizona	Yuma	8/30	C. erythrothorax	50	SLE
V5-5086	Imperial	Winterhaven	8/30	C. tarsalis	50	WEE
V5-6277	San Diego	Santee	9/5	C. pipiens	10	SLE
V5-6291	San Diego	Chula Vista	9/5	C. tarsalis	17	Hart Park
V5-6321	San Diego	Rancho Santa Fe	9/6	C. tarsalis	49	Hart Park
V5-6332	San Diego	Rancho Santa Fe	9/6	C. tarsalis	50	SLE
V5-6333	San Diego	Rancho Santa Fe	9/6	C. tarsalis	50	SLE
V5-6334	San Diego	Rancho Santa Fe	9/6	C. tarsalis	28	SLE & Hart Park
V5-6338	San Diego	Rancho Santa Fe	9/6	C. tarsalis	31	Turlock
V4-1481	Kern	Bakersfield	9/12	C. tarsalis	51	SLE
V4-1494	Kern	Maricopa	9/12	C. tarsalis	50	SLE
V4-1499	Kern	Maricopa	9/12	C. tarsalis	50	CEV
V2-2083	Stanislaus	Newman	9/13	C. tarsalis	45	WEE
V4-1520	Tulare	Woodville	9/18	C. tarsalis	50	SLE
V5-5297	Imperial	Calexico	9/25	C. tarsalis	57	SLE
V5-5300	Imperial	Calexico	9/25	P. confinnis	39	SLE
V5-5317	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5320	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5325	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5330	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5332	Imperial	Calexico	9/25	C. tarsalis	50	Turlock & SLE
V5-5333	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5336	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5347	Imperial	Seeley	9/26	C. tarsalis	50	Turlock
V5-5455	Yuma, Arizona	Yuma	9/27	C. inornata	3	Bunyamwera group
V2-1704	Colusa	Maxwell	10/3	C. tarsalis	27	WEE
V5-6378	San Diego	Rancho Santa Fe	10/4	C. tarsalis	38	Turlock
V5-6406	San Diego	Rancho Santa Fe	10/4	C. tarsalis	50	SLE
V5-6430	San Diego	Santee	10/19	A. franciscanus	4	Main Drain
V5-6460	San Diego	Lakeside	10/19	C. erythrothorax	21	SLE
V5-6465	San Diego	Santee	10/19	C. tarsalis	18	SLE
V5-6480	San Diego	Santee	10/19	C. inornata	25	Bunyamwera group
V5-9253	San Bernardino	Parker Dam	10/25	C. inornata	38	CEV

Comparisons of Western Encephalitis (WE) Virus Strains

Dissimilarities between WE virus strains collected in the field have led us to search for methods by which we might separate these strains into subtypes. From our own collection and through the kindness of other investigators, we have accumulated more than 100 strains of WE virus differing in source and location. From these we have selected 10 representative strains (Table 1).

In order to amplify the quantity of virus, one passage of each virus was made in newborn (<24 hours) chicks. These were bled by decapitation 18 hours after inoculation. These virus stocks were so heavily contaminated with microorganisms that one further passage of each was made in primary duck embryo (DE) cells. By this method, "clean" virus pools were obtained. The supernatant fluid from these infected cultures is, at present, being used as seed virus.

All 10 seed viruses were tested for hemagglutinin (HA) activity with goose erthrocytes. With the exceptions of 71V-1658 and R-1257, eight strains had HA activity at pH optima typical of group A arboviruses (pH 6.0-6.4). The patterns of activity, however, differed in that W-17791, 73V-2540, 73V-1570, and MW8-5AD appeared to plateau from pH 6.0 to 6.5 and slowly decreased in titer with increasing pH, while 73V-1492 and V-8853 had pH optima at 6.0 or 6.1 and decreased rapidly in HA titer at higher pH's. Strains M2-958 and TBT-235 seemed to fit neither category, plateauing to pH 6.4 but rapidly decreasing in titer at higher pH values.

Our inability to detect HA activity with 71V-1658 and R-1257 suggested that we were working with low titering material. Since two (73V-1570 and MW8-5AD) of the other eight strains produced plaques in DE cells but did not kill suckling mice (SM) when first isolated, all 10 seed viruses were titrated in DE and Vero cells and in mice, hamsters, guinea pigs, and chicks.

Results thus far indicate that all seed pools except 71V-1658 titer (per ml) from $10^{7.7}$ to $10^{9.0}$ PFU in DE, $10^{8.3}$ to $10^{9.4}$ $SMicLD_{50}$, $10^{8.0}$ to $10^{8.8}$ $SMipLD_{50}$, $10^{6.2}$ to $\geq 10^{7.6}$ 3-4 week old mouse (WM) $icLD_{50}$ and $>10^{10}$ newborn chick $scLD_{50}$. Strain 71V-1658 titered $10^{3.5}$ to $10^{6.2}$ per ml in these five systems, possibly accounting for our inability to detect HA activity. Retrospectively, this could not, however, have been the case with R-1257 which had high titers in all systems.

Therefore, an additional passage of all 10 seed viruses was made in Vero cells. Both 71V-1658 and R-1257 had high titer HA after such passage, both having pH optimum and titration patterns similar to 73V-1492 and V-8853. We have repeated this and duplicated our observation. This is not the first time that we have noted changes in the characteristics of WE strains passed from one cell type to another. Similar observations have led us to pursue more involved studies with 73V-1570.

In addition, M2-958 and 7BT-235 strains having similar HA characteristics, did not kill WM inoculated ip, while all the others titered $10^{5.2}$ to $10^{6.9}$ LD_{50} per ml, again with the exception of 71V-1658 which titers $10^{3.5}$.

Thus far, none of the strains kill guinea pigs inoculated ip.

We are in the process of a number of other comparisons with these 10 strains of WEE virus including serum dilution plaque reduction neutralization tests for cross testing using infection immune chicken sera.

(C. H. Calisher)

TABLE 1. Strains of WE virus used for preliminary comparisons.

Designation	Original host	Location	Date coll.	Source	Passage Level
W-17791	*Alectoris chukar*	Maryland	9-30-65	Blood	SM_1
M2-958	*Culex tarsalis*	Mexico	6-24-72	(Pool)	Original
TBT-235	*Gopherus berlandieri*	Texas	10-27-71	Blood	Original
73V-2540	*Culiseta melanura*	Massachusetts	8-7/8-73	(Pool)	Original
71V-1658	*Equus caballus*	Oregon	8-13-71	Brain	Original
73V-1492	*Passer domesticus*	Texas	8-2-73	Blood	Original
73V-1570	*Passer domesticus*	Colorado	8-8-73	Blood	Original
R-1257	*Equus caballus*	Brazil	3-5-60	Brain	SM_8
V-8853	*C. tarsalis*	California	8-6-71	(Pool)	Original
MW8-5AD	*Aedes triseriatus*	Maryland	8-2/4-68	(Pool)	Original

Evaluation of Thiotepa as a Chemosterilant for *Culex tarsalis*

As a preliminary step in studies on biological vector control through release of sterile male mosquitoes, experiments were undertaken to determine the effectiveness of thiotepa as a sterilant for *Culex tarsalis*. Pupae from the Fort Collins colony were immersed for 4 hours in solutions of 0.7 percent or 0.9 percent thiotepa, and emerging males were tested both for sterility and sexual competitiveness. In three tests using 0.7 percent thiotepa, the average hatch rate of 45 egg rafts from female mosquitoes sired by treated males was 5 percent, compared to 93 percent for 41 rafts from untreated controls. In competition trials between equal numbers of treated and untreated colony males, 29 of 62 matings (47 percent) were by treated males. In two trials using 0.9 percent thiotepa, the corresponding values were 2 percent (N=49), 80 percent (N=46), and 48 percent (N=44). In initial trials with wild females, sterilized colony males accomplished 7 of the 10 recorded matings when competing against equal numbers of untreated wild males. These results show that *C. tarsalis*, like *Aedes aegypti* and *Culex pipiens quiquefasciatus*, can remain competitive after chemosterilization. They also establish that males of a long-term colony strain can still mate with females of a natural population.

(William A. Rush)

Ecology of Western Encephalitis (WE) Virus in Morgan County, Colorado, USA

Studies of arbovirus activity in eastern Colorado were initiated in 1972, and the results obtained during 1972 and 1973 were summarized in our previous report (Arthropod-Borne Virus Information Exchange, No. 26, page 118). No typical WE virus strain was recovered from mosquitoes in 1973, but we did report that "WE-like" virus strains were isolated from 25 nestling house sparrows and also from a pool of *Oeciacus vicarius*, hemipteran swallow-nest bugs. Since all of the WE-like virus isolations were from a single location (Bijou Bridge), the 1974 studies were concentrated at that and one other site in Morgan County. Since the nestling house sparrows from which virus was obtained in 1974 were inhabiting abandoned cliff swallow nests, this year's project included study of nestling house sparrows (*Passer domesticus*), nestling cliff swallows (*Petrochelidon pyrrhonota*), nestling barn swallows (*Hirundo rustica*), *O. vicarius*, and mosquitoes.

We had hypothesized that returning migrant swallows, or overwintering *O. vicarius*, might initiate the "WE-like" virus cycle in the spring time and that the nestling swallows and sparrows may subsequently serve as amplifying hosts in the virus cycle. This year's data indicate that the *O. vicarius* may initiate the virus cycle in the spring by transmission among nestling sparrows; in the 2 previous years, no collections were made prior to the last week of June.

At the study site (Bijou Bridge, near Fort Morgan, Colorado), house sparrows were observed to begin nest building on March 8, 1974. Eggs but no nestlings were found on May 8. On May 15-16, cliff swallows were observed in the study area, and began nest building at the Bijou Bridge site. At this time, nestling house sparrows were collected and yielded WE-like virus strains, as did O. vicarius bugs taken from nests with virus-positive nestlings. Thirty virus isolations have been obtained from nestling house sparrows nestling through July 1974.

Cliff swallow nestlings were first obtained on June 12, and WE-like viruses were recovered from them and bugs from their nests. Five swallow isolates were made through July 8-9. Swallows were no longer nesting on July 30. As noted in our previous report, swallows and sparrows were nesting together at Bijou Bridge, and O. vicarius bugs were found in all nests sampled. A total of 25 virus strains were isolated from O. vicarius through July.

The first C. tarsalis mosquitoes were obtained in CDC light traps on May 8, but were not abundant. On May 15-16 (when the first isolations of WE-like strains were recovered from nestling sparrows and bugs), only four C. tarsalis entered light traps.

The first isolate from C. tarsalis was collected on August 7, 1974; unlike strains from nestling birds and O. vicarius, the isolate produced large plaques in DECC on day 1 and then appeared on day 2 in Vero (see below). Five virus strains other than group A arboviruses from C. tarsalis, and one from Aedes vexans, were isolated from mosquitoes collected between June 26 to July 31.

The WE-like virus strains obtained this year and last are not lethal for 2- to 4-day-old suckling mice inoculated i.c. and demonstrate lower infectivity in DECC than in Vero cell cultures. Their plaque size ranges from 2 to 8 mm in Vero cell cultures and from 2 to 4 mm in DECC. In contrast, typical WE virus strains obtained from Culex tarsalis or equines produce greater numbers and larger size plaques in DECC than in Vero. The 1973 strains were shown to be antigenically similar to WE virus on the basis of CF tests. The 1974 isolates from O. vicarius appeared to be a homogenous virus population (on the basis of plaque characteristics); whereas, the nestling bird isolates appear to contain virion subpopulations with different plaque morphology. Some of the nestling bird isolates obtained after June 1974 formed greater numbers of plaques in DECC than in Vero and were more similar to typical WE virus.

Laboratory studies to identify and further characterize the WE-like virus strains are in progress, and field studies will continue in an effort to follow the virus cycle through the coming seasons.

(Richard O. Hayes, John S. Lazuick, Gordon C. Smith, and W. Daniel Sudia)

Studies on Human Cases of Colorado Tick Fever

Despite the common occurrence of Colorado tick fever (CTF), much is unknown regarding the specific epidemiology of the infection, its natural history, and the frequency with which clinical complications occur. Reports in the literature, largely based on retrospective case analysis submitted to state laboratories, produce a conflicting picture of the severity of the disease, although it is generally accepted that the mortality from the classical illness is extremely low. Laboratory investigators have noted that some patients continue to circulate erythrocyte-associated virus or viral antigen for several weeks after the acute illness, but since large numbers of patients have not been followed on a prospective basis, neither the frequency nor clinical and epidemiological significance of this finding has been defined. Studies were thus initiated in 1973.

METHODS

1. 1973: Letters were sent to physicians and hospitals throughout Colorado requesting that blood specimens be sent to the VBDD laboratory along with completed history forms on any patients suspected of having CTF or having fever of unknown origin, unexplained acute CNS syndromes or hematologic abnormalities.

2. 1974: Twenty-five physicians from areas determined to be hyperendemic in 1973 agreed to submit histories and blood specimens on patients they suspected of having CTF and to obtain follow-up histories and blood specimens at 30-day intervals following acute illness as long as virus was detectable.

RESULTS

Between March and October 1973, over 300 diagnostic blood specimens were processed by suckling mouse inoculation and by direct fluorescent antibody staining of red blood cells. One hundred forty-three were positive for CTF virus by at least one test. From May through July 1974, an additional 80 cases have been confirmed in our laboratory. Results presented are based on a review of those positive cases from 1973 on whom adequate histories were obtained and the first 80 cases submitted for study in 1974.

1. Tick exposure and leukopenia <4000 were significantly more frequent in positive than in negative cases ($p < .01$). However, the history of tick bite in patients who reported exposure did not significantly increase the risk of infection (Tables 2 and 3).

2. There was no significant difference in symptom frequency between positive and negative cases. In fact, in addition to the classic presentation of fever, myalgia, and headache, the gastrointestinal symptoms of cramping abdominal pain and/or vomiting were seen in up to 20% of patients and about 16% recalled that persistent nausea was prominant in the convalescent period. Pharyngitis was described in over 20% of acute illnesses (Table 4).

3. Although aseptic meningitis syndrome was frequently seen, acute meningoencephalitis was reported in only one patient, and no cases with bleeding complications were observed. Skin rash of any kind was rare, and petechial rash suggestive of Rocky Mountain spotted fever was reported in two cases.

   Two patients developed unusual clinical pathology about two weeks following their acute illness; CTF virus was isolated from the blood of both at the time the complication occurred, and each remained virus positive for at least two additional weeks. One of these, a 24-year-old male, developed unilateral epididymo-orchitis requiring hospitalization. The second, a 25-year-old male who had persistent weakness and malaise, was found to have a unilateral pulmonary infiltrate suggestive of atypical pneumonia. Whether either of these associated complications were etiologically related to their CTF infection has not been determined. Appropriate testing is to be done to evaluate other known causes of these syndromes. However, the patients' persistent viremia and failure to make a clinical recovery prior to onset of the complicating illness is highly suggestive of an etiological relationship to CTF.

4. About 20% of positive cases were hospitalized, and 55% of 73 patients on whom convalescent histories were obtained required three weeks or longer to recover. Fever and weakness were the most prominant symptoms one month following acute illness, but prolonged headache and myalgia were also reported in more than two-thirds of these cases (Tables 5 and 6).

5. In 1973, 86% of CTF confirmations were accomplished by virus isolation in suckling mice. Only 23% were positive by both direct FA and suckling mouse isolation (Table 7).

6. In 1973, 38 of 85 follow-up blood specimens obtained were positive for virus and in 23 of these instances, virus isolation was made in suckling mice. One blood specimen was positive for virus by FA staining 20 weeks following the acute illness. Fifty-six percent of specimens obtained four weeks following acute illness continued to have blood-borne virus infective for suckling mice (Table 8).

7. In 1973, one case, detected in a vacationer to the Rocky Mountain National Park, is noteworthy in that she conceived approximately 10 days after onset of her acute illness. Our experience, with two-week follow-up studies on 30 cases, revealed 87% were suckling mouse positive for CTF virus at this point in their convalescence. Based on this limited observation, persistent viremia was probable at the time this woman conceived. After a normal gestation, a healthy child was delivered; no virus could be demonstrated in the maternal or offspring red cells. A more definitive

observation was made in 1972 when a CTF infection was confirmed in the sixth week of pregnancy of a vacationer to Montana. She also delivered a normal child with no evidence of viremia in the mother or infant at parturition.

(H. C. Goodpasture, J. Poland, D. B. Francy, and C. H. Calisher)

Table 2

1973-74 Colorado Tick Fever Study

Epidemiology of 129 Cases of Acute CTF
Compared to That of 75 Controls*

Risk Factor	Positive	Attack Rate	Negative	Attack Rate	Total
Tick Exposure	118	.91	36	.48	154
Tick Bite	69	.58	17	.47	86
Altitude >7000	55	.43	27	.36	82
Adult ≥14	76	.61	49	.65	125
Male	50	.66	31	.63	81
Female	26	.34	18	.37	44
Child <14	48	.39	26	.37	76

*Controls were negative for CTF virus identification.

Table 3
1973-74 Colorado Tick Fever Study

White Blood Count During Acute Illness
CTF Patients vs. Controls

WBC	Positive	Negative	Total
<4000	49	16	65
>4000	39	35	74
Total	88	51	139

$p < .01$

Table 4
1973-74 Colorado Tick Fever Study

Acute Illness Symptoms of 129 CTF Patients
Compared with Those of 75 Controls

Symptoms	Positive	Percent	Negative	Percent	Total
Fever	123	95	72	96	195
Headache	97	75	67	89	164
Myalgia	97	75	55	73	152
Abd. Pain	23	18	21	28	44
Lethargy	71	55	46	61	117
Stiff Neck	19	15	19	25	38
Vomiting	13	10	12	16	25
Diarrhea	2	02	5	07	7
Rash	4	03	10	13	14
Pharyngitis	29	22	25	33	54
Petechiae	2	02	3	04	5
Bleeding	0	00	2	03	2

Table 5

1973-74 Colorado Tick Fever Study

Convalescent History of 73 CTF Patients and 7 Controls

Symptom	Positive	Percent	Negative	Percent	Total
Fever	68	93	7	100	75
Headache	47	64	5	71	52
Myalgia	50	68	4	57	54
Weakness	57	78	3	43	60
Nausea	12	16	3	43	15
Anorexia	6	08	0	00	6
Drowsiness	3	04	0	00	3
Rash	1	01	0	00	1

Table 6

1973-74 Colorado Tick Fever Study

Duration of Symptoms

Time	Positive	Negative
1 week	25	6
2 weeks	8	0
3 weeks	26	1
>3 weeks	14	0
Total	73	7

Table 7
1973 Colorado Tick Fever Study
Acute Blood Specimens Positive for CTF as Determined by Direct FA on Red Blood Cells and Virus Isolation in Suckling Mice

FA - SM	Specimens	Percent
Neg - Pos	75	52
Pos - Pos	33	23
Pos - Neg	20	14
Hem*- Pos	15	11
	143	100

*Hemolyzed

Table 8
1973 Colorado Tick Fever Study
CTF Virus Identification in 85 Convalescent Blood Specimens by FA and SM Test

	Positive/Total Tested	
Duration of Followup	FA	SM
2 weeks	4/10	7/11
4 "	12/22	15/27
6 "	1/10	1/10
8 "	2/8	0/9
10 "	1/3	0/3
12 "	0/0	0/2
14 "	1/3	0/3
16 "	0/1	0/2
18 "	0/2	0/2
20 "	1/11	0/14
Total	22/70	23/83

Report from the University of Wisconsin
Departments of Preventive Medicine
Entomology and Veterinary Science

Studies of the natural distribution of La Crosse virus:

During 1973 studies were concentrated in five small forested areas in rural Genoa and suburban La Crosse, associated with cases of California encephalitis.

Pre-season LAC virus antibody rates were slightly higher in sera collected from chipmunks 277/450 (62%) than squirrels 61/112 (54%). In prospective studies, antibodies neutralizing LAC virus first appeared in previously negative chipmunks in late June. Overall rates increased monthly from 49% during July to 83% during September. Antibodies were found in each of the areas, varying from 38% in one to 73% in another.

Aedes triseriatus collected in comparative aspirations and oviposition trips were first found during late June. Adult A. triseriatus averaged 7.9 per aspiration collection; with 5.8 in July, 6.3 in August, and 9.5 in September.

In fluorescent antibody studies research assistant Barry Beaty has observed fluorescence of LAC virus in infected BHK-21 tissue culture chamber slides, in smears of infected mouse brains and Aedes triseriatus.

Specific fluorescence has been detected in smears of transovarially infected 4th instar, pupal and adult stages of offspring of laboratory A. triseriatus mosquitoes. Adults were found to give the most obvious positives, and the severed abdomens were easily smeared. The remaining insect parts were inoculated into suckling mice for virus isolation.

This technique was utilized to investigate the prevalence of oviposition sites serving as overwintering foci in 4 study areas of known enzootic virus activity. Virus was detected in 13 collections from 8 different tree-holes before adult emergence in June. At least one overwintering isolate has been obtained from each of the 4 study areas. Studies of virus distribution are being continued throughout the mosquito season.

Studies on relative vector potential of A. triseriatus and A. hendersoni and their hybrids continue.

Studies on variants of California Group viruses:

Work continues on documenting the occurrence of variants of LAC and Jamestown Canyon (JC) viruses. Both large and small plaque variants were found in prototype LAC and JC viruses. Passage of LAC in chipmunks selected for the large plaque type and passage in grey squirrels selected for small plaques. Deer passage resulted in large plaque JC virus viremia. Attempts have been made to infect rhesus monkeys with the prototype, large and small plaque type LAC. One animal each was inoculated with the prototype virus, chipmunk uncloned

large plaque, cloned large plaque and cloned small plaque type viruses. Only the animal receiving the small plaque type virus developed antibody. No viremia was detected.

The relative neurovirulence of LAC-l and LAC-s viruses was determined. The mean death time for standardized doses of each plaque type were computed. The small plaque type appeared to kill mice more rapidly than did the large plaque type, suggesting that it is more neurovirulent. Further test are in progress.

Field studies in Colombia

Current studies are terminating. Typing of viruses isolated from mosquitoes, a sand fly, and a tabanid pool is still underway. We and our colleagues of the Facultad Veterinarian, Universidad de Antioquia, have been making an economic evaluation of two outbreaks of VSV (one of New Jersey and one of Indiana serotypes) in a dairy herd. Milk production is reduced by 20-30% during the two weeks following the outbreak, and some cows go out of production following infection.

W. H. Thompson, G. R. DeFoliart, T. M. Yuill, D. M. Watts, B. Beaty, P. R. Grimstad, W. R. Hansen, B. D. Nassif and F. N. Zuluaga

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

A National Arbovirus Reference Centre has been established in the Department of Medical Microbiology at the University of Toronto operating in conjunction with the Laboratory Centre for Disease Control in Ottawa.

Activities of the Reference Centre during the first year of operation include the following:

1. Collection of over 80 arbovirus strains with a particular emphasis on Canadian arbovirus isolates;

2. Compilation of publications concerning arbovirus studies performed in Canada;

3. Preparation of inactivated antigens and mouse ascitic fluids to Canadian isolates of the following arboviruses: Eastern equine encephalitis, Western equine encephalitis, St. Louis encephalitis, Powassan, California encephalitis (snowshoe hare) and Colorado Tick Fever viruses;

4. Participation in a limited serological survey of human and bird sera in Ontario in order to monitor for possible arbovirus activity;

5. Performing diagnostic services when requested by hospitals or Provincial Health Laboratories;

6. Undertaking of studies to identify two probable arbovirus isolates from mosquitoes in Saskatchewan.

The Reference Centre hopes to encourage Canadian arbovirus investigations by making available arbovirus reagents for serological studies as well as by providing facilities for the training of personnel in arbovirus techniques. Dr. D.M. McLean of the Department of Medical Microbiology, University of British Columbia has kindly agreed to act as an honorary consultant to the Reference Centre.

(L. Spence and H. Artsob)

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

Only 2 human cases of arbovirus infection have been identified through August 22 this year. One was a probable California encephalitis case in a 4-year-old boy resident of Albany County, with onset on June 23 of headache, stiff neck, fever, and an abnormal CSF with 100 WBC, 70% polys. A blood specimen taken 9 days after onset had a HI titer of 1:10 (CF <4) to the Snowshoe Hare (SSH) strain of CAL virus. No virus was isolated from a CSF sample taken at the same time. A second blood sample taken 24 days after onset still had no CF titer to either SSH or LaX, gave an unsatisfactory HI test, but had a mouse neutralization index of 4.3 logs against the Snowshoe Hare virus. The patient recovered uneventfully.

The other case was of probable Powassan infection in an 8-year-old boy resident of Otsego County with onset on July 12 of headache, stiff neck, fever, rash and abnormal CSF. A blood specimen taken 19 days after onset had a CF titer of 1:8, HI titer of 1:2560 and neutralization index of approximately 3.5 logs. The patient has recovered. A rash has not been reported previously in POW cases; herpes and measles CF titers were not significant.

Over 90,000 arthropods had been processed for attempted virus isolation by the middle of August, and 15 strains of virus had been isolated (Table 1). This is the first time that a CAL virus has been isolated from Aedes vexans in New York State, although that mosquito is abundant here, and a frequent vector elsewhere. Over 15,000 of this species collected State-wide had been processed this year before the isolation. The 2 isolations of a CAL virus from Quebec came from a collection of mosquitoes made at the request of the Canadian public health authorities.

A second strain of the new virus NY 73-51694/5 has been recovered. This was first isolated from Suffolk Aedes cantator in August 1973 and now from Schenectady Aedes communis group mosquitoes in July. It is an ether-sensitive agent with a titer of around 5 logs in suckling mice, and does not react by CF with any of the NIH reference arbovirus grouping fluids, which cover a combined spectrum of 226 grouped and ungrouped viruses.

No virus isolations have been made from over 9,000 Aedes canadensis, over 5,000 Aedes triseriatus, 1,672 Simuliidae or 2,007 ticks processed so far this year.

(John P. Woodall and Margaret A. Grayson)

Table 1

Viruses Isolated from Mosquitoes from New York State and Quebec, Canada, January 1 through August 7, 1974

Virus	No. of Strains	County	Month Collected	Species	Infection Rate
CE complex	3	Essex	June	Ae. communis gr.	1:262
CE complex	1	Hamilton	June	Ae. communis gr.	1:540
CE complex	1	Livingston	June	Ae. aurifer	1:162
CE complex	1	Cattaraugus	July	Ae. stimulans	1:468
CE complex	2	Quebec	July	Ae. communis gr.	1:403
NY 73-51694/5	1	Schenectady	July	Ae. communis gr.	1:89
Flanders	1	Chemung	July	C. pipiens	1:400
Flanders	1	Suffolk	July	M. perturbans	1:28, 668
Flanders	1	Suffolk	July	Cu. melanura	1:864
CE complex	1	Suffolk	July	Ae. cantator	1:3, 641
CE complex	1	Essex	August	Ae. communis gr.	NA
CE complex	1	Schenectady	August	Ae. vexans	NA

Inhibition of Arbovirus Hemagglutinins by Commercial 7.5% Bovine Albumin

During the course of propagating a group C arbovirus in cell culture, the serum in the maintenance medium is routinely replaced by bovine albumin to a final concentration of 0.4%. This is done in order to determine the various viral activities, including hemagglutination. We have employed a commercial (GIBCO) stock solution of 7.5% bovine albumin in PBS for this purpose.

In a recent experiment the harvested culture fluids did not show any detectable hemagglutinin although the infectivity assays indicated that the usual amount of virus was present in the fluids. The virus was concentrated from the harvested fluids by polyethylene glycol precipitation and still hemagglutinin was not detectable in the concentrate although infectivity assays indicated that the virus was properly concentrated. An identical experiment done approximately 4-5 months previously in which a different lot of commercial bovine albumin, also prepared by GIBCO, was successful in showing the presence of viral hemagglutinin. This matter was discussed with a representative of the company and assurances were offered that there had been no change in the method of preparing the bovine albumin. A sample of a new lot which had recently come off production was sent to us for evaluation. Both recent samples were diluted in borate-saline buffer to 0.4% concentration and were employed as HA diluents for several standard HA preparations. Our routine HA diluent, prepared from Armour powdered bovine albumin, was employed as a control diluent. The HA results of the GIBCO preparation

as compared with our standard diluent are shown in the Table. The GIBCO products resulted in complete inhibition of all HA antigens while the control showed the typical titers of the antigens.

The two commercial albumin solutions, as well as our routine HA diluent, were extracted with acetone according to the method of Clarke and Casals. Acetone extraction almost completely removed the inhibitory activity from the commercial albumin solutions while the control solution showed no change. Limited infectivity studies with Oriboca virus indicated that the commercial albumin solutions did not adversely affect viral infectivity.

(N. Karabatsos and D.H. Clarke)

TABLE
The Inhibitory Action of GIBCO Bovine Albumin Solution on Arbovirus Hemagglutinins

Virus	Diluent and HA Titer		
	GIBCO Lot A931209	GIBCO Lot A940315	Armour Control
Itaqui Serum HA	<2	4-8	>128
Oriboca BHK-21 TCF Lot 2	<2	-	>128
Oriboca BHK-21 TCF Lot 1	<4	8-16	>256
Chandipura HA	<10	<10	>5120
VSV-Indiana HA	<10	<10	160

Isolation of chikungunya virus contaminating Singh's Aedes albopictus cell line.

Singh's Aedes albopictus cell line obtained originally in 1971 from the ATCC and passed in laboratories in Maryland, Washington D.C., and Indiana was found at Purdue University to be contaminated with structures morphologically compatible with an alphavirus. At YARU rapid isolation of a cytopathic virus was effected by combining freeze-thawing or sonication, concentration with aquacide II, rate zonal centrifugation and subsequent plating of fractions on Vero cells under agar overlay. The virus was avirulent for infant and adult mice and produced cytopathic effect in Vero and BHK-21 cells. Serologically, the virus was identified as chikungunya by CF and plaque reduction neutralization test.

The same virus had previously been isolated from Aedes albopictus cells received from the Boyce Thompson Institute. Users of Aedes albopictus cells should be aware of the possibility of this difficult-to-detect contaminant. There is circumstantial evidence that chikungunya virus becomes overt in these cells only under stress conditions.

(A. Cunningham, S. Buckley, and J. Casals)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
GRADUATE SCHOOL OF PUBLIC HEALTH
UNIVERSITY OF PITTSBURGH, PENNSYLVANIA

In our attempts to isolate temperature sensitive (ts) mutants of dengue viruses, prolonged serial low temperature passage and treatment with mutagenic agents have been attempted. The latter approach has proved fruitful. To date, 7 mutants have been successfully cloned by the direct immunofluorescent method. The wild type material, designated P_3T_{10}, is a dengue 2, TH36 isolate at its tenth passage in primary hamster kidney cell culture (HKCC) following 3 suckling mouse brain passages. P_4 is virus in its fourth mouse passage.

The P_3T_{10} material was then allowed to replicate in HKCC containing the mutagen 5-azacytidine at concentrations ranging from 25 to 100 µg/ml at 33.5°C. This temperature was selected as the permissive temperature for all subsequent work since it occurs in peripheral skin and the upper respiratory tract of man, and had been used in the successful development of influenza virus ts mutants. This drug reduced virus yield to a slight extent (5 to 10-fold).

The yield from cultures containing 25 µg/ml 5-azacytidine was cloned as described. Sixty-eight clones were isolated and subsequently tested for their ability to produce FFU in HKCC, PFU on LLC-MK_2 monolayers, and to kill suckling mice. Plaque formation in LLC-MK_2 cells at 33.5 and 37° did not adequately discern any temperature sensitivity or altered plaque morphology in those clones tested. However, some temperature sensitivity was detected by the FFU method and mouse titration. To be sure, most of the clones were similar to wild type virus in that they produced nearly equal FFU titers at 33.5, 37, and 40°C, and readily killed suckling mice. The remaining clones exhibited varying degrees of temperature sensitivity and fell into four categories. Some clones were ts by FFU titration, but retain most of the wild type ability to readily kill suckling mice at high dilution, similar to P_3T_{10} stocks. Others were moderately reduced in mouse virulence, but exhibit no ts characteristics by the FFU test. The third group of clones were considered ts by both tests. The majority of clones constitute the fourth group, which exhibited no ts properties, similar to SMP3. The temperature sensitivity of these clones was confirmed by repeated testing. Additional clones were isolated from the 100 µg/ml azacytidine-treated cultures. In all, 7 mutants were isolated from 138 clones.

A summary of their properties obtained by titration in HKCC and suckling mice is shown in Table 1. All ts mutants showed substantial reduction in FFU titer at 40°C compared with 33.5°. The mouse neurovirulence was not appreciably decreased

by ts mutations, although on initial isolation, ts-1 was a virulent for mice. However, by the second HKCC passage, it had lost this property. It was concluded from this observation, and analysis of other clones, that mouse neurovirulence and the ts trait in HKCC varied independently of each other. Survival time analysis of mice inoculated with ts mutants, wild type clones, or mouse brain passaged DEN-2 TH36 virus showed identical relationship between $\log_{10}$ dose (FFU) inoculated and harmonic mean survival time. This observation further strengthens the conclusion that the ts mutants need not have altered neurovirulence for suckling mice.

The ts clones were also ts by propogation at the nonpermissive temperature. Table 2 showed that the replication of these viruses was substantially inhibited at 40°C, while that of the parental P_3T_{11} and P_4 materials was considerably less inhibited. In addition, the degree of fluorescence was markedly diminished at 40°C in ts-2 and ts-3 infected cells, somewhat diminished in cultures of the other 5 mutants, and not reduced at all in cells infected with P_4 or P_3T_{11}.

The stability of the ts trait was examined by several methods. Each mutant was serially passaged at 33.5°C in HKCC. The various passage levels were then titrated at 33.5, 37, and 40°C. Table 3 shows representative data for ts-1, -2, and -3. The ts phenotype, assayed by titration at the three temperatures, appears reasonably stable. Similar data exist for the other four ts mutants (not shown). Stability of the passage levels was also determined by propogation at 33.5 and 40°C, followed by titration of the yields at 33.5°C. Table 4 shows that ts-1, -3 and -5 retain the ts trait upon passage in HKCC at 33.5°C. Studies with the other mutants are incomplete at present. However, the latter method of determining the stability of temperature sensitivity by yield reduction at 40°C appeared to be more satisfactory than simple titration at the three temperatures.

Additional characterization of the mutants has involved the construction of growth curves at 33.5 and 40°C. Mutant ts-3 was drastically reduced in its growth potential at 40°C. Mutant ts-1 appeared to replicate as well at 40°C as the P_3T_{11} parent, except that virus production was delayed about 24 hr. When the amount of FA-reactive antigen was assessed in the culture vessels following removal of fluids for titration, an interesting phenomenon was observed. Cells infected with either parental strains showed maximum fluorescence at about 50 hr at either temperatures. However, ts-1 infected cultures showed peak fluorescence at about 72 hr. Viral antigens were present in very small amounts in ts-3-infected HKCC incubated at 40°C. Additionally, the drop in parental virus titer late in the infection cycle at 40°C was temporally correlated with the degeneration of the HKCC monolayer. Degeneration was not observed with either mutant at 40°C.

Limited numbers of complimentation tests have been conducted. Although the results have not been confirmed, ts-2 and ts-3 appeared to compliment each other, implying that the site of their respective mutations involves different viral cistrons. The lack of detectible complimentation between other virus combinations could mean that they belong to the same complimentation group, or more likely, that they contain multiple or other non-complimentable mutations, which defy detection in complimentation tests.

Each of the 7 ts mutants was evaluated in duplicate rhesus monkeys for subcutaneous inoculation into the left forearm. Monkeys were previously determined to be free of group B arbovirus antibody. Monkeys were monitored for viremia and HI antibody production. Monkeys injected with ts-1 or ts-2 demonstrated antibody production in the absence of detectible viremia. The other mutants resembled the parental viruses in that both viremia and antibody were observed. On the basis of this data, it was concluded that chemical mutagen treatment of dengue viruses may have value as a method of preparing vaccine condidates, as well as defined mutants for genetic studies. However, since virus was not recovered by inoculation of mice or HKCC with monkey serum we cannot make any statement concerning the genetic stability of ts-1 or ts-2 in monkeys.

(A.S. Lubieniecki, present address Meloy Laboratories, Inc., 6715 Electronic Drive, Springfield, Virginia 22151)

Table 1
Suckling Mouse and FFU Titers of DEN-2 TH36 Parental Strains and ts-Mutants

Virus[a]	Log_{10} FFU/0.1 ml assayed at temperature (°C) 33.5	37	40	log_{10} $SMICLD_{50}$ /0.1 ml[b]
ts-1	4.3	3.5	2.7	4.4
2	4.0	2.7	<2.0	4.2
3	4.4	3.8	2.6	3.0
4	4.2	3.4	2.8	4.7
5	3.0	2.3	<1.0	2.4
6	4.2	3.5	2.8	4.6
7	1.1	0	0	<1.0
P_3T_{11}	4.8	4.4	3.8	5.5
P_4	6.3	6.0	5.5	6.4

[a] ts numbers refer to clones previously identified as 1f2, 6h2, 3i3, 3i6, 15i2, 20i4, and 33i1, respectively. All material were tested in their second HKCC passage at 33.5°C following initial cloning.

[b] Titer corrected from a volume of 0.01 ml by multiplication by 10 before converting to log_{10} units.

Table 2
Effect of Propogation at Permissive and Nonpermissive Temperatures on Production of Infectious Virus by Dengue-2 Mutant and Parental Strains

Virus	Log_{10} FFU/0.1 ml[a] produced by propogation at temperature: 33.5C	40 C
ts-1	3.8	1.4
2	2.2	<0
3	4.2	<1.0
4	5.3	2.2
5	1.6	<0
6	4.9	1.0
7	2.1	0.7
P_3T_{11}	3.7	2.7
P_4	3.8	2.7

[a] Log_{10} fluorescent focus-forming units assayed at 33.5 C.

Table 3
Effect of Serial Passage at 33.5C on the ts Phenotype of ts-1, ts-2, and ts-3[a]

Virus	Passage Level	Log_{10} FFU/0.1 ml assayed at temperature: 33.5 C	37 C	40
ts-1	1	4.3	3.5	2.7
	2	4.2	3.7	2.6
	3	4.2	3.5	2.5
	4	3.8	3.6	2.3
	5	3.2	2.4	2.3
	6	4.7	4.5	>3.0
ts-2	1	4.0	2.7	<2.0
	2	2.0	1.9	<1.0
	3	3.8	3.4	2.4
	4	4.1	3.8	2.5
ts-3	1	4.4	3.8	2.6
	2	4.2	3.6	2.3
	3	3.4	3.2	1.5
	4	5.0	4.0	3.2

[a] 10^{-1} dilution of virus inoculated onto HK cells and incubated at 33.5 C for 4 days.

Table 4

Virus	Passage Level	Log_{10} FFU/0.1 ml[a] produced by propogation at temperature: 33.5C	40 C
ts-1	1	3.8	1.4
	2	3.4	1.7
	3	4.4	1.8
	4	4.4	<1.0
	5	4.5	1.2
	6	4.2	<1.0
ts-3	1	4.2	<1.0
	2	3.3	<1.0
	3	4.0	0
ts-5	1	1.6	0
	2	3.8	<1.0
	3	4.1	1.4

[a] Log_{10} fluorescent focus-forming units assayed at 33.5 C.

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

Two recent studies are summarized in this report, both of which concern Venezuelan equine encephalomyelitis. The report by Dr. Pedersen provides an additional means for characterizing virus strains and a report by Dr. Jahrling provides a new insight into the pathogenesis of the disease in hamsters.

ELECTROPHORETIC STUDIES OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUSES

Young and Johnson (Am. J. Epidemiol. 89:286-307, 1969) separated members of the Venezuelan equine encephalomyelitis (VEE) complex into four distinct subtypes (I-IV), with subtype I exhibiting five antigenic variants. Using discontinuous electrophoresis Schlesinger and Schlesinger (Virology 47:539-541, 1972) recently described the presence of three proteins in Sindbis, the prototype alphavirus, one in the nucleocapsid and two in the envelope. We detected variations in the envelope proteins of certain alphaviruses (Pedersen et. al. Virology 60: In press) and have extended these observations to members of the VEE virus complex.

VEE virus propagation in BHK-21 roller bottles and the concentration and purification procedure employed have been described (Pedersen, et. al. Infect. and Immun. 8:901-906, 1973). High pH, discontinuous SDS-disc electrophoresis was performed essentially as described by Maizel (Meth. in Virology V:180-244, 1971) using 7.5% (w/v) acrylamide resolving gel. Purified, concentrated virus preparations were reduced and alkylated, and electrophoresis was performed at 2 mA/gel for ca. 4 hr. Gels were stained with 0.25% Coomassie brilliant blue R 250. Molecular weight determinations were performed in parallel gels by the method of Shapiro, et. al. (Biochem. Biophys. Res. Comm. 28:815-820, 1967), using calibrated protein molecular weight markers.

Electrophoresis amply demonstrated similarity of the core protein and variation in the envelope proteins of members of the VEE complex. Five distinct profiles were observed among the VEE viruses tested. The TC-83 strain of VEE virus showed a clear separation of the two envelope proteins (pattern α) with molecular weights in the range of 55 to 57,000 daltons and 47 to 49,000 daltons. The Ica strain of VEE virus possessed two clearly defined proteins with molecular weights identical to those viruses showing the α pattern but with a minor intermediate band. This was designated pattern β. The 3880 strain of VEE virus showed two proteins which migrated through the gel in very close proximity (pattern γ). Molecular weights were found to be in the range of 52 to 54,000 daltons and 47 to 49,000 daltons. Mena II and other subtype Ie VEE viruses demonstrated only one apparent envelope protein band with a molecular weight

in the range of 47 to 49,000 daltons and was designated pattern δ. Only one virus, Piura, had an ε pattern with a major envelope protein in the range of 50 to 53,000 daltons and a minor protein in the range of 53 to 56,000 daltons. Table I lists VEE viruses studied in relation to the observed envelope patterns.

We found that the envelope proteins of the same VEE strain were identical in electrophoretic mobility regardless of the tissue culture host of origin, and that like strains from different laboratories showed the same electrophoretic profiles. Observed profiles were also stable if viruses were harvested at 3 hr intervals from 15 to 36 hr postinoculation in BHK-21 cells. The Trinidad donkey strain gave a pattern identical to that of its progeny, strain TC-83; and in addition, small and large plaque variant progency of Fe 3-7c VEE virus had the same electrophoretic profile as the parent, although they demonstrated variations in plaque size and virulence. Since electrophoretic profiles of VEE viruses appear to be stable, they may represent a valid criterion for characterization and classification. However, the use of envelope protein migration patterns for segregating VEE virus strains is valid only when superimposed on an existing, reproducible serological classification system. For example, although TC-83 and the subtype II VEE viruses have identical PAGE patterns there are distinct serological and epidemiological differences in the viruses, which are expressed upon infection of a suitable host and in their biological characteristics in nature.

If we compare the subtype I strains of VEE viruses, the electrophoretic patterns appear to separate the epizootic VEE isolates into four groups; α, β, γ and ε. Although the γ pattern is seen in both epizootic and sylvatic strains of subtype I viruses, there are certain generalizations that are evident from the epizootic pattern groupings.

1. Within subtype I only the Trinidad donkey strain and its TC-83 progeny show pattern α.

2. All isolates from the Central America-Mexico-Texas epizootic exhibit the β pattern. Also in this category are the Ica and Pergamino strains and the prototype VEE isolate, Kubes strain.

3. All isolates recovered during the great Venezuelan epizootic from 1962 to 1967 have the γ pattern.

4. The Piura strain is unique in having the ε pattern.

Thus, the similarities among isolates from the same epizootic suggest that the patterns are stable markers within this closely related group of viruses. Although many of these viruses are difficult to reproducibly separate serologically, the electrophoretic pattern groupings appear to identify genetic and epidemiological groups within serological subtypes. Our current attempts at modification of this procedure may increase the sensitivity of the technique so that subtle biological relationships between virus isolates may be explained at the level of envelope protein biochemistry.

(C.E. Pedersen, Jr.)

TABLE I. ELECTROPHORETIC PATTERNS OF VEE VIRUSES TESTED

ENVELOPE PATTERN	SUBTYPE[a]	STRAIN
α	1-A	Trinidad donkey
	"	TC-83
	II	Fe 3-7c
	"	Fe 4-71k
	"	Fe 5-47et
β	1-A	Kubes (Beck-Wycoff)
	1-B	69Z-1
	"	PTF-39
	"	GJ9-1B-J
	"	1ca
	"	Pergamino
	"	MF-8
	"	9859
γ	1-C	V-198
	"	PHO-107S
	"	P-320
	"	P-614-MC
	"	P-676
	"	V-178
	1-D	3880
	"	202330
	"	Gamboa 1107-5
	"	Canito F-322
	"	V-209A
	III	Paramaribo
δ	1-D	BTH 3468
	"	BTR-65-139708
	1-E	Mena II
	"	BT-2607
	"	2177-B
	"	63-R-20
	"	65-U-64
	"	68-U-201
	III	Mucambo BeAn 8
	"	52049
	"	900807
	"	900809
	"	900811
	IV	Pixuna BeAr 35645
	"	Pixuna BeAr 40403
	"	Pixuna BeH 38120
ε	1-B	Piura

a. Based on classification of Young and Johnson (1969).

EFFECT OF ANTIBIOTIC THERAPY ON VEE INFECTION OF HAMSTERS

Infection of adult hamsters with VEE viruses from antigenic subtype-I results in a rapidly progressive illness characterized by severe necrosis of lymphoid and hematopoietic tissues in spleen, bone marrow, lymph nodes, and intestine. We have consistently observed that a terminal event is the development a bacteremia due to gram negative intestinal flora, which probably results from the transmural necrosis of the ileum around Peyers patches. Recent data from several lines of investigation suggest that the bacterial sepsis and endotoxemia may be more than simply terminal events, and may be important factors in the development of lesions previously ascribed to the growth of VEE virus in "target" lymphoid and hematopoietic tissues.

Bacterial sepsis develops between the second and third day following VEE inoculation. Forty-four hours after inoculation of VEE-I, strain Trinidad donkey, 0 of 10 blood specimens were positive for aerobic enteric bacteria. However, by 56 hours, 3 of 10 were positive, and by 74 hours 9 of 10 bloods contained E. coli, P. vulgaris, or S. faecalis. Endotoxemia appears to precede overt sepsis, since 5 of 5 bloods obtained 36 hours after infection contained high levels of endotoxin as detected by the experimental Limulus lysate procedure.

Antibiotic treatment of VEE-infected hamsters dramatically affected the disease course. Hamsters were inoculated daily via the intraperitoneal (ip) route with 1 ml of a solution containing 2.5 mg. tetracycline and 2.5 mg. neomycin, for three days prior to sc inoculation of VEE-I. Inoculation of antibiotics was continued at daily intervals after virus inoculation. Although all of 20 hamsters treated with antibiotics eventually died, survival was prolonged from a mean of 3.2 days for untreated hamsters to 7.6 days; two treated hamsters lived for 10 and 11 days. We observed that treated hamsters died precipitously, and were not detectably sick even several hours before death. Histologically, the most severe lesions detected in the tissues of two hamsters dying on days 7 and 8 were massive areas of hemorrhage along the olfactory tracts and in the pyriform lobe of the brain. Lymphoid tissues in the spleen lymph nodes, and intestine were histologically normal, as was the bone marrow except for several areas of minimal necrosis and focal hemorrhage. Thus the development of hemorrhagic lesions in the brain, and the absence of significant lesions in the lymphoid and hematopoietic tissues of antibiotic-treated hamsters dying late after VEE-I infection, resembles the lesions described for VEE, subtype III, Mucambo virus infection in hamsters, and for the occasional hamsters that die following attenuated VEE strain TC-83 inoculation.

To test the hypothesis that destruction of hematopoietic tissues in VEE-infected hamsters results from the endotoxemia secondary to the intestinal lesion, we compared the changes in bone marrow following ip inoculation of 1 mg. of endotoxin prepared from E. coli, with the changes associated with VEE-I infection. Total cell numbers in bone marrow decreased to 30-40% of normal following inoculation of either endotoxin or VEE; in both cases the decrease was due in part to a release of mature granulocytes into the circulation, and to the destruction of cells of the myeloid series. In

addition, increased phagocytosis of RBC was apparent, and dramatic vacuolization of cells, particularly monocytes, occurred following either endotoxin or VEE inoculation. These changes in bone marrow were reflected by fluctuations in lysosomal enzyme levels in bone marrow and plasma. Alkaline phosphatase and B-glucuronidase levels were elevated to 10 times normal in plasma following endotoxin or VEE; these changes were reflected by decreased levels of these enzymes in bone marrow, but not in liver or kidney. Similar morphologic and enzymatic changes with bone marrow cells were demonstrable with endotoxin in cell culture; however VEE alone did not produce these changes in bone marrow cells in vitro, although the virus did replicate to a moderate extent. The similarities in the character of the bone marrow lesions produced by endotoxin and VEE in vivo suggest that endotoxin could be the effector of bone marrow necrosis following VEE inoculation. The finding that antibiotic treatment of VEE-infected hamsters essentially eliminates the bone marrow lesion adds support for this theory.

Sequential sacrifice studies using VEE-infected hamsters treated with antibiotics will be needed to sort out the direct effects of virus versus the secondary effects of endotoxin on the development of histopathologic lesions, leukopenia, and interferon induction.

(Peter. B. Jahrling)

REPORT FROM THE ARBOVIRUS DIAGNOSTIC LABORATORY, VIROLOGY DIVISION
CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

Dengue in the Trust Territory of the Pacific Islands

In mid April, 1974, seven paired sera specimens were received from Dr. John Steele, Majuro Hospital, Marshall Islands of the Trust Territory of the Pacific. During the first week of March when the rains were beginning and mosquitoes (including Aedes aegypti) were breeding, Dr. Steele began to see patients with a syndrome suggestive of dengue fever. The specimens submitted to CDC were from patients representative of the disease syndrome he was observing. He described the illnesses as an abrupt onset of severe fever accompanied by headache, periorbital pain, muscle aching, malaise and prostration. Nausea, vomiting, diarrhea, and a mild cough sometimes accompanied the illness. Usually the illness was biphasic, that is illness for 2 to 3 days, better for a couple of days, followed by a second phase with maculopapular eruptions of the limbs and painful erythema of the palms and soles. WBC were depressed with counts between 2000 and 3000 with a relative neutropenia. Selectively, three pairs of the submitted specimens were from prolonged and severe cases while the others were relatively mild.

Early recognition of the illnesses and proper collection of the specimens made possible a classical laboratory diagnosis. The acute serum specimens were taken early in the illness, 5 to 8 days after onset, and convalescent specimens were collected 13 to 17 days later. HAI tests using several group B arboviral antigens (dengue 1, dengue 2, dengue 3, YF, JBE and SLE) showed a fourfold or greater rise in titer in six of the seven suspect cases. Of the six positive responses, four were of a primary type while two were broad B reactors. Based on the clinical picture and the HAI results, dengue infections were indicated.

To confirm dengue as the causative agent and to determine the subtype, serum dilution plaque reduction neutralization tests were performed. The four subtypes of dengue plus Japanese B and yellow fever as group B controls were tested against the six paired HAI group B reactors using LLC-MK_2 cell culture. The neutralization tests of the four primary infections confirmed dengue as the infecting virus and further identified the subtype as dengue 1. Listed below are the convalescent neutralization titers of the four primary cases. The acute sera titers were generally <10; two acute samples were 1:20 for dengue 1 only.

	den-1	den-2	den-3	den-4	YF	JBE
PR 820	640	20	40	10	<10	<10
PR 826	320	<10	<10	10	<10	<10
PR 828	320	20	20	20	<10	<10
PR 830	320	10	20	10	<10	<10

The identification as type 1 has been confirmed by Dr. Leon Rosen, Pacific Research Station, NIH, Honolulu.

Complement-fixation tests were performed; however, the titers obtained were not diagnostically significant due to lateness in CF antibody appearance. This finding further emphasizes the importance of the serum dilution neutralization test for laboratory diagnosis of group B arboviral infections.

Later in May four convalescent serum specimens from suspect dengue cases were submitted by Ms. June Yip of the Trust Territory Health Services Department. Of these specimens, three were positive by neutralization tests with two showing a primary response pattern to dengue type 1.

Ms. June Yip stated that dengue had not been reported in the Trust Territory for the past thirty years. The involvement of dengue type 1 is of particular interest, as dengue outbreaks on other Pacific Islands since 1971 have been caused by dengue type 2 (personal communication, Dr. Leon Rosen).

It is not clear how and where dengue 1 first appeared in the Trust Territory outbreaks. The Marshall Islands are located in the central Pacific just above the equator and west of the international dateline. Daily air service by Air Micronesia has passengers en route between Honolulu and other districts of Micronesia and Guam. Also weekly air service connects Australia and islands of the South Pacific with the Marshall Islands.

Recent information from Dr. John Steele indicates suspect dengue cases peaked in April; however, illnesses were being reported from the outer atolls as late as June 1. The hemorrhagic syndrome was not observed.

Essentially this same report also appears in the current Dengue Newsletter for the Americas.

(Helen S. Lindsey, Robert E. Fontaine and John Steele)

REPORT FROM THE FLORIDA DIVISION OF HEALTH
BUREAU OF LABORATORIES, JACKSONVILLE, FLORIDA

As part of the general virus diagnostic services provided to the medical community in Florida, we tested 344 patients' sera against a battery of antigens associated with central nervous system diseases during the period from January through June, 1974. Three patients' sera had constant low level group B arbovirus HI antibodies when tested against both SLE and dengue antigens. Patients were prior residents of a dengue endemic area.

Our state-wide surveillance including mammals and sentinel fowl is reflected in the following table.

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

Species	Number of Sera	Reactors
Human	344	3 SLE and Dengue**
Horses	10	1 WEE[1]
Field Specimens (mammals) Sentinel Fowl Sera	383	5 VEE[2]
Total	737	9

*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

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

[1] Horse stabled in Western Florida when he became ill. HI - WEE titer $\geq$1:640.
[2] Field specimens, South Florida study areas, where VEE (Fe3-7c) is endemic.

(Nathan J. Schneider and Elsie E. Buff)

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

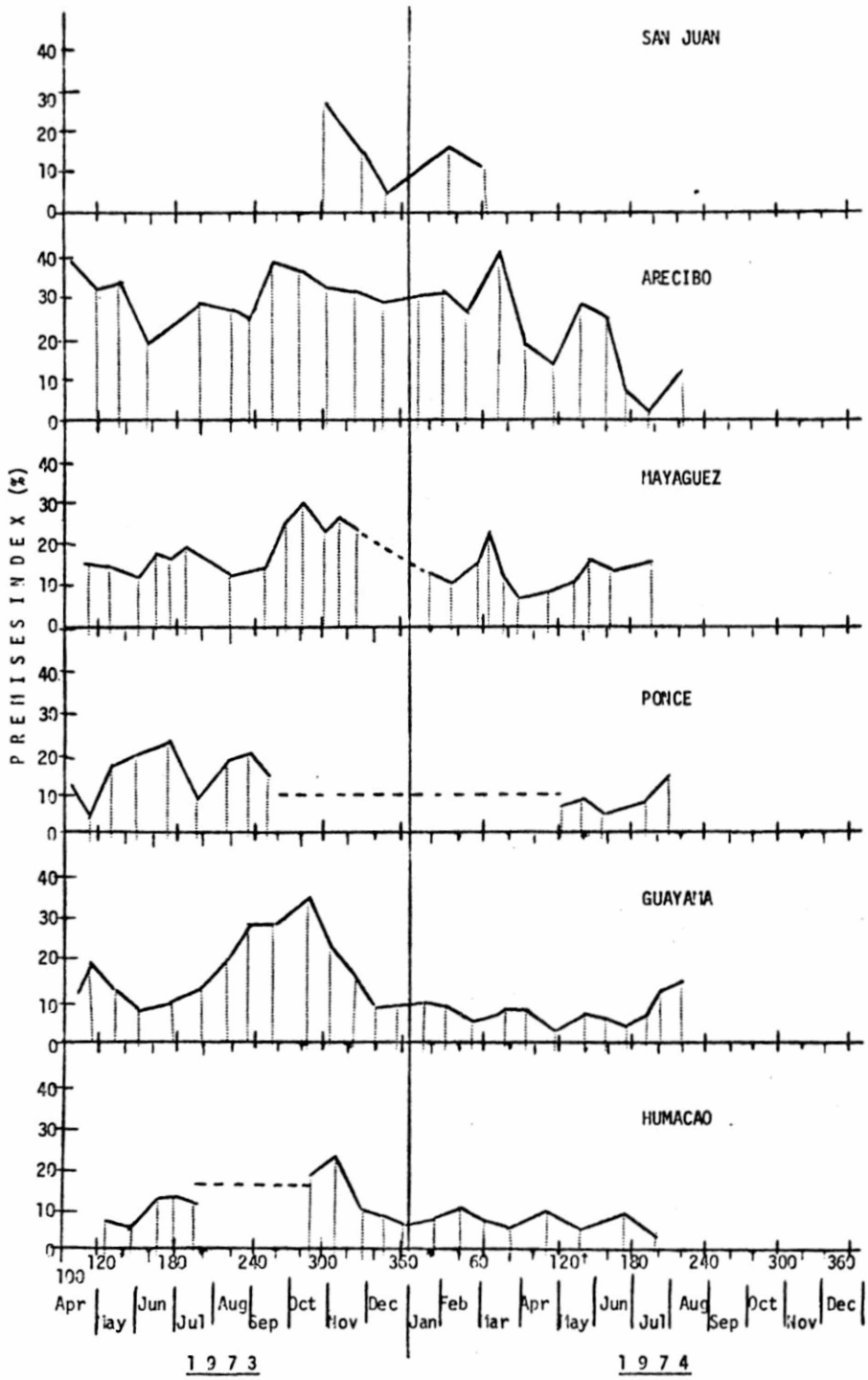
Dengue activity in Puerto Rico has been minimal during 1974. The last dengue infections, confirmed by HI and CF seroconversion, occurred in late January in the towns of Santa Isabel (2 cases), Villalba (1 case), and Guayanilla (1 case). Continuing surveillance has failed to document cases since that time.

A system to monitor population indices of Aedes aegypti in the six major urban zones of the Island (San Juan, Arecibo, Mayaguez, Ponce, Guayama, and Humacao) has now been in progress for approximately 16 months. In collaboration with the Puerto Rico Health Department, a systematic sample of 100 residences in each of these cities is inspected twice monthly for the presence of A. aegypti larvae, and all actual or potential breeding containers are enumerated. Preliminary results (Figure 1) indicate that the mosquito is found commonly in each of the six cities, that the premises index varies seasonally, and that some study areas, such as Arecibo, have consistently higher population indices than others. Work is in progress to determine the factors (rainfall, types of breeding containers, and other characteristics of the study areas) which correlate best with mosquito population indices.

Eleven agents isolated previously in suckling mice during an outbreak in Villalba were identified by CF as dengue-2. The isolates were from sera collected from febrile patients seen at the Villalba Health Center, and required 7 to 12 passages in suckling mice to permit identification by CF.

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

FIGURE 1
QUARTERLY REPORT OF AEDES AEGYPTI
SURVEILLANCE PROGRAM IN PUERTO RICO



Premises Index (%) = Number of cases positive/Total houses inspected x 100

8-30-74

REPORT FROM THE NATIONAL DIAGNOSTIC AND REFERENCE CENTER
INSTITUTE OF HEALTH AND TROPICAL DISEASES, MEXICO, D.F.

Retrospective study of a so-called outbreak of VEE among humans with possible resulting abnormalities in newborns in the state of San Luis Potosi, Mexico.

Since an epizootic outbreak of VEE occurred 3 years ago in the state of San Luis Potosi, the public health authorities (Dr. Alfredo Villalobos Rodas, Jefe de los Servicios Coordinados en el Estado and Dra. Maria J. de Jouguitud, Jefe del Departamento de Planeacion de los Servicios Coordinados del Estado de SLP) undertook a clinical study in various localities to look for neurological sequelae in people who had been ill at that time. They found numerous children about 3 years of age with birth defects such as blindness, deafness, muscular abnormalities and mental retardation.

In the retrospective examination of the mothers of the children, it was determined that all had presented with febrile illnesses which had been diagnosed "encephalitis" coincident with the epizootic and that, at that time, they were found to be in different stages of pregnancy.

Studies were carried out in 28 localities and in a total of 59 mother-child binomials. A serum specimen was obtained from each individual and sent to this laboratory for the determination of antibody content to VEE. The serum of 57 binomials was tested with the HA antigens of VEE, WEE, and SLE using 4 units of antigen and the microplate technic.

In 10 serums (mothers only) we found titers between 10 and 80 against SLE. All the sera were negative against WEE at 1:10, the lowest dilution tested. The results against VEE, as shown in table 1, were that 47.4% of the mothers had titers less than 10, and 3.5% of them had titers of 160 to 320. Only three children showed titers of 10; their mothers had titers of 20, 160 and 320 respectively. The remaining children were negative.

Of the 28 localities under study, the sera from 9 were completely negative for VEE antibody, and the sera which had titers of 160 and 320 all occurred in the same locality; in the remaining 18 localities, sera with titers of 10 to 40 were found.

The high percentage of negatives in a group highly suspected of having had VEE, the low percentage (5.2) of children with titers for VEE (and these were very low, only 1:10) and the presence of definite congenital defects in each of the children have caused us to look into other viral causes; for these reasons we are proceeding to investigate antibodies against rubella in 40 representative binomials from the 28 localities. The possibility exists that the state of San Luis Potosi had two outbreaks of viral disease at the same time, and that the VEE masked the diagnosis of rubella.

(Dra. Maria Luisa Zarate Aquino and Margarita Guerrero Sarinana, Q.I.)

Table 1. Ha antibody titers to VEE in 57 serums of mothers suspected of having had VEE during the epizootic in 1971.

Antibody titers	*No. of sera.*	*%*
- 10	*27*	*47.4*
10	*18*	*31.6*
20	*6*[a]	*10.6*
40	*4*	*7.*
160	*1*[a]	*1.7*
320	*1*[a]	*1.7*

[a] *Serum from the child of one mother had a titer of 10.*

REPORT FROM GORGAS MEMORIAL INSTITUTE-
MIDDLE AMERICA RESEARCH UNIT, BALBOA HEIGHTS, CANAL ZONE

I. Enzootic Wave of Jungle Yellow Fever in Panama

In spite of serologic evidence that yellow fever (YF) virus had crossed the Panama-Colombia border in a westerly direction during the 1970 rainy season, infecting monkey populations, no human cases of YF were recognized in Panama until February 1974 at the end of an unusually prolonged rainy season. At this time, a young man living in the forest on the southern slopes of the Bayano River basin was hospitalized in the capital with a provisional diagnosis of YF, a diagnosis which was confirmed serologically although virus could not be recovered. A second case, from the northern slopes of the Bayano River watershed, came into the hospital about a week later. This time YF virus was isolated from serial blood specimens and from the liver and lung at autopsy.

Retrospectively, a few additional fatalities brought to our attention in these same areas and one other area of the Bayano were considered as probably due to YF. Serologic survey of residents of the small settlements where the YF patients lived revealed at least another 9 probable infections (plaque N titer $\geq$ 1:512, and CF titer $\geq$ 1:64). Past vaccination appeared to result in lower YF antibody titers than recent natural infections, regardless of status of antibodies to other group B viruses.

The Ministry of Health of the Republic of Panama undertook a vaccination program in the sparsely populated forested areas of the Bayano basin as well as in the rest of the country. During the rest of the dry season, no further cases were seen.

Hospitalized cases appeared again, however, on 31 July and 6 August, in males from two small communities located near the Bayano River some 25 km to the west of the earlier cases. Outcome was fatal in both cases. YF virus was isolated from the liver of both, although the titer was quite low. Low-level plaque neutralizing antibody was demonstrable in the serum of both patients. Serologic studies of members of the same communities are in progress.

At the beginning of rainy season 1973, YF surveillance was carried out for two months at Aguas Claras Arriba, site of YF activity in previous enzootic waves. Four sentinel rhesus monkeys did not convert and 225 pools of daytime-biting mosquitoes (mostly Haemagogus and Sabethes) captured with human bait in the forest canopy yielded no viruses (in suckling mice, i.c., and Vero cultures).

With the appearance of YF cases in 1974, surveillance at Aguas Claras Arriba was again instituted in March. YF virus was detected in mosquitoes from early June until collections were discontinued there in mid-July. Twelve pools of Haemagogus lucifer and one of H. equinus yielded YF virus. A rhesus was returned to the lab on 15 July, died with a fulminant YF infection on 19 July. In late June, collections were begun at two new camps some 20 km west of Aguas Claras, i.e. Punta Mama and El Llano-Cartí Road Camp, several km apart in a north-south direction. Virus activity in mosquitoes first was detected in collections of 24 July and 30 July, respectively, at these two

sites, and there have been 5 Haemagogus isolates thus far. Surveillance is being continued at El Llano-Cartí Camp, but in mid-August collecting was terminated at Punta Mama as a new camp (Cerro Azul) is being set up 40 km west to start collections on approximately 1 September. The virus is predicted to reach this latter area in mid-September. So far, the route and velocity of the virus are following rather closely the pattern seen in previous YF enzootic waves.

Surveillance so far has been limited to anthropophilic mosquitoes of the canopy and rhesus sentinels. Sonication has been used in preparing arthropod pools since mid-1973, replacing trituration with mechanically driven TenBroeck homogenizers. Vero cell cultures appear to be as sensitive as or better than suckling mice (i.c.) for YF isolation from mosquitoes and human and animal tissues. Use of mice for mosquito pool isolation was therefore suspended in mid-July 1974, thus permitting more rapid testing in Vero cells only. Preliminary identification of virus isolates in the Vero-1 passage, using direct IFAT, has also expedited the detection of YF virus in field materials.

(P. H. Peralta, Pedro Galindo, K. M. Johnson)

II. Experimental Transmission of Yellow Fever in Arboreal Vertebrates

Sylvan yellow fever is currently epizootic in central Panama. Some basic questions in the epidemiology of the sylvan infection in this area remain unexplained, such as, the mechanism of dry-season virus survival and the differential role of arboreal vertebrates as virus hosts. Some preliminary data has been obtained in an attempt to answer these questions.

Six species of mammals were inoculated with the virus strain recovered from a fatal human infection occurring in 1974 and passaged once in a night monkey (Aotus). These included two- and three-fingered sloths (Choloepus and Bradypus), kinkajous (Potos), coatis (Nasua), woolly opossums (Caluromys) and prehensile-tailed porcupines (Coendou). Of these, only Bradypus were frequently infected.

The Bradypus developed measurable viremia by day 3 post-inoculation, the titers generally peaked at a level of 5.5 logs/ml and, in one instance, 8.5 logs or greater and persisted from 12-14 days post-inoculation. All viremic Bradypus had developed neutralizing antibody by 28 days post-inoculation. Two of six Bradypus receiving a high infecting dose died on days 6 and 8 post-infection. Temperatures, hemoglobins, and white blood cell counts were monitored daily during the first two weeks after infection and remained stable. If an intercurrent infection occurred, then the white count became elevated, but the infections responded quickly to treatment with penicillin.

By giving large doses of virus we were able to detect low-level viremia (1.5 to 2.0 logs/ml) in two Choloepus; however, with inoculum doses closer to the titer expected from a mosquito bite, no viremia was detected. Neutralizing antibody resulted in 5 of 8 Choloepus inoculated.

We are at present collecting serum samples from sloths on Barro Colorado Island, an area where yellow fever activity was last noted 25 years ago, and in the Bayano River basin where yellow fever is presently active, in an effort to determine if sloths are naturally infected by yellow fever virus. We are in the process of setting up a field station in an area where virus is now actually present to assess population density of sloths by radio-tracking. This study is a cooperative venture with Dr. G. Montgomery of the Smithsonian Institution.

(P. A. Webb and K. M. Johnson)

III. Reduced Fertility in Calomys Infected with Machupo Virus

During studies of Bolivian hemorrhagic fever in San Joaquin, Bolivia, Calomys callosus were incriminated as the possible vector of the disease. Since then experimental information has been gathered which could explain the successful perpetuation of Machupo virus in nature by C. callosus.

Calomys inoculated with Machupo virus during the first week of life developed a persistent viremia (type A infection) without detectable humoral antibody. Infected females mated with normal males produced less than 10% of the expected number of offspring, and infected males mated with normal females resulted in 50% of the expected live births.

Normal female rodents were mated with normal males and then inoculated with Machupo virus 3, 5, or 7 days later. When sacrificed at 17-19 days of gestation, all embryos of females infected at 5 or 7 days were viable and virus could not be detected. However, individual embryos from females infected on day 3 were dead, resorbing and virus-positive. Females exposed for 24 hours to infected males and sacrificed 20 days after showed that infection by venereal contact is highly effective; only 3 out of 26 females were not infected, embryos were viable and without virus. Resorbing embryos were found in infected females in proportion of 2:1 to those viable (Table 1).

Twenty-eight Calomys females with persistent infection were impregnanted and 16 days later nine of them were found in stages of gestation. Calomys have a gestation period of 21 days and after autopsy of 2 animals at 18-19 days of gestation, the rest were left to complete their term. Five females did not deliver and when autopsied at 23, 24, and 26 days, viable embryos with virus and dead or resorbing embryos were observed. Resorbing embryos in normal Calomys are rare, only one embryo in 17 pregnant females was observed.

(G. Justines and K. M. Johnson)

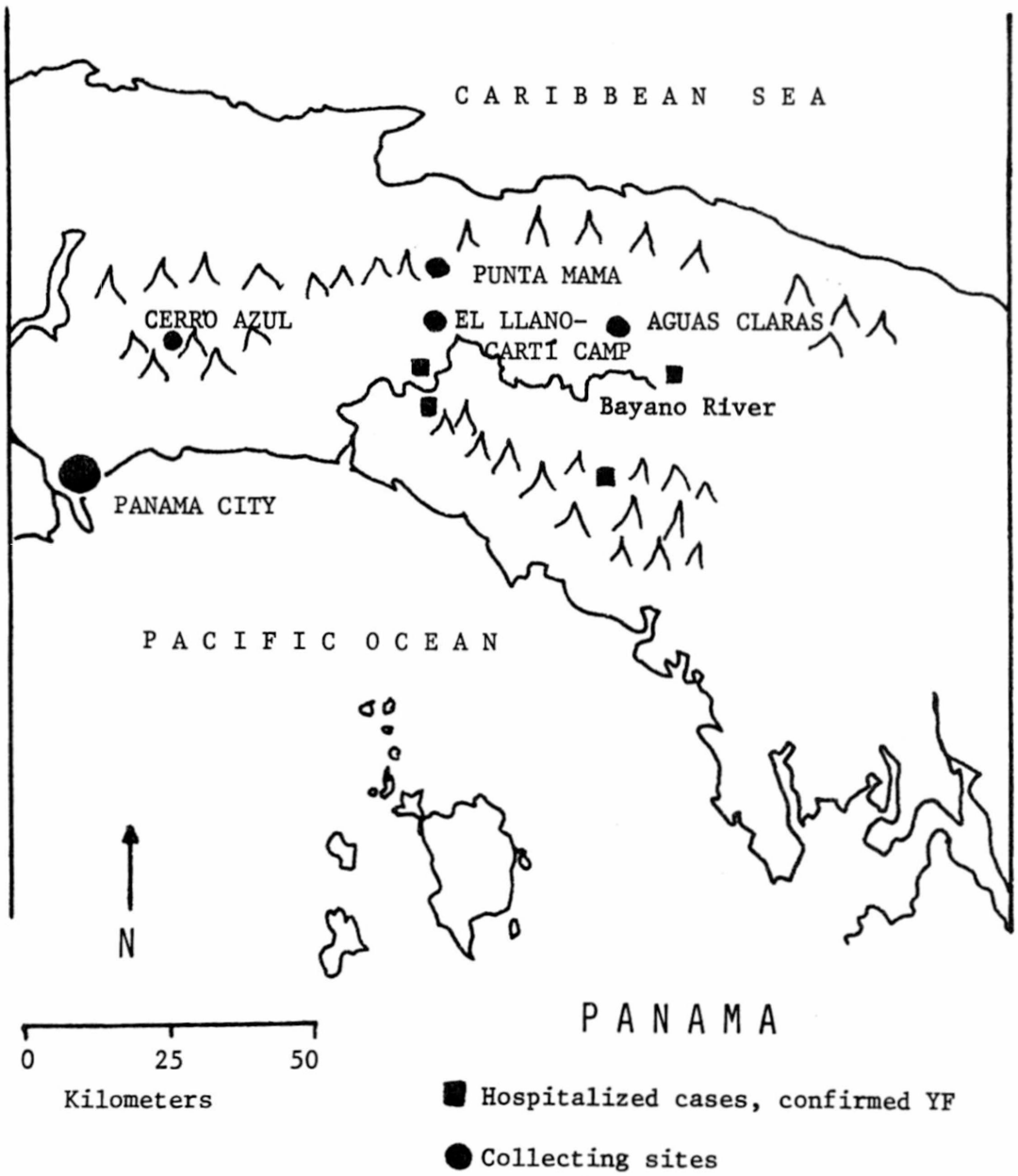

CARIBBEAN SEA
PUNTA MAMA
CERRO AZUL
EL LLANO-
CARTÍ CAMP
AGUAS CLARAS
Bayano River
PANAMA CITY
PACIFIC OCEAN
N
0
25
50
Kilometers
PANAMA
Hospitalized cases, confirmed YF
Collecting sites

TABLE 1. Normal *Calomys callosus* females impregnanted by Machupo type A male *Calomys*.

Calomys at 20 days gestation	Viable embryos			Dead embryos	Resorbing embyros			
	With virus	Without virus	Total		With virus	Without virus	Not tested for virus	Total
23 Viremic	29	16	45	2+	17	16	62	95
3 Nonviremic	0	18	18	0	0	0	0	0

+ Both with virus.

REPORT FROM THE SECTION OF ARTHROPOD-TRANSMITTED VIRUSES
INSTITUTE ADOLFO LUTZ, SAO PAULO, BRAZIL

The field station of Rio Guaratuba (23°45'S, 45°55'W), located in a primary forest at sea level, has been operated since 1968. The station is visited every 5 weeks to collect mosquitoes, wild mammals and birds and to expose sentinel mice and hamsters.

The viruses isolated by us at Rio Guaratuba field station during the first half of 1974 are shown in Table 1.

During the collecting trip of January, several sentinel suckling mice became sick in the last day of the trip. They were stored in liquid nitrogen, and on passage in the laboratory we isolated an agent which killed 2-day-old Swiss mice in 30 hours. The strain SPan 26550 was chosen as prototype and shown to cross with group C antiserum. In HI and CF tests it appeared to be different from Caraparu, Oriboca and Marituba, the 3 group C viruses used by the laboratory (Table 2).

The other virus isolated in that trip came from a pool of Culex sp. and was identified as a strain of Guaratuba virus, an agent related to Mirim virus.

From the 105 birds netted, 18 (17%) showed antibodies to An 26550 in HI tests, with titers ranging from 1/10 to 1/160. Only 2 bird sera showed cross-reactions with Caraparu and Oriboca antigens.

The 7 mammals captured were bats netted at night, and 2 of them had antibodies to An 26550. No other mammals were captured, probably due to the floods that occurred there in January, which is the rainy season in our area.

In March another collecting trip was made, and 2 viruses were isolated. They were identical to each other but different from our prototypes. Their identification is in progress.

From the 112 bird sera collected, 3 (2.6%) had antibodies to An 26550 in HI tests, with titers ranging from 1/10 to 1/40. Of 2 mammals captured in that trip, an armadillo (Dasipus novecinctus) had antibodies to An 26550.

In April, we performed another collection at Guaratuba field station and 6 viruses were isolated. Four of them were identified as EEE virus, 3 from sentinel mice and 1 from a ruby-crowned tanager (Tachyphonus coronatus). The other 2 viruses were identified as Guaratuba virus.

In HI tests with wild bird sera 7 had antibodies to EEE virus, and none reacted with the An 26550 antigen. The 6 mammals captured (4 rats and 2 bats) did not react with An 26550 or EEE antigens.

In May another trip was made. Two viruses were isolated from sentinel mice and their identification is in progress. No birds were trapped during that trip and the mammals captured did not react with EEE or An 26550 antigens.

Comments

We had not observed antibodies for group C viruses, using Caraparu, Marituba and Oriboca antigens, among the 4583 birds and 157 mammals collected in Rio Guaratuba since the beginning of the study in May 1968. Besides, no group C viruses were isolated either from 1320 sentinel mice or hamsters exposed or from the animals collected.

It appears to us that An 26550, the first group C virus isolated in our region, was apparently introduced between December 1973 and January 1974. We had searched in Rio Guaratuba on November 24-28, 1973, and none of the sera collected (76 birds and 11 mammals) showed antibodies to An 26550 and no strain of that virus was isolated.

It is also interesting to note that after April 1974, we did not find any further serological evidence of its presence in the region. The very low density of small mammals there, probably due to floods that occurred during January, suggests that An 26550 was unable to establish itself in that area. Only additional field studies in the region will confirm this hypothesis.

We have another field station nearby. Casa Grande (23°48'S, 45°55'W) is located at about 5 km from Rio Guaratuba but with an altitude of 800 meters. It seems that the mountain range acts as a barrier to the virus cycle, as we have not isolated the virus in Casa Grande or found any serological evidence of its presence as well. We collected there 291 wild birds, 34 rodents and 8 snakes during the first half of 1974 and no virus isolations or antibodies were observed.

EEE virus was reisolated in Rio Guaratuba field station after a 3-year quiescence. The last isolations were made in May 1971 from wild birds and sentinel mice.

EEE virus forest cycles continue to puzzle us. We have obtained isolations from sentinel mice but no virus was recovered from 66 mosquito pools processed. The forest vectors are still to be disclosed. No outbreaks in horses in our area were reported by raisers, in spite of EEE activity uncovered by our arbovirus surveillance.

(Oscar de Souza Lopes, L.A. Sacchetta, T.L.M. Coimbra)

TABLE 1

Virus isolated in Rio Guaratuba field station during the first half of 1974

Collecting Trips	Virus Isolations				
	Wild birds	Wild Mammals	Mosquito Pools	Sentinel Mice	Sentinel Hamsters
January 12 to 16	0/105*	0/7	1/43	22/55	1/4
March 9 to 13	0/112	0/2	0/17	1/22	1/4
April 30/3 to 3/4	1/106	-	0/6	5/43	1/6
May 4 to 8	-	0/4	-	2/40	0/6
TOTAL	1/323	0/13	1/66	30/160	3/23

* Number of isolation/Number of animal collected or exposed

TABLE 2

Comparison of SPAn 26550 with certain Group C Viruses

Antigen / Immune Ascitic Fluid		HI				CF			
		An 26550	Caraparu	Oriboca	Marituba	An 26550	Caraparu	Oriboca	Marituba
An 26550	3i	320	40	20	20	32/1024+ *	8/1024+	0/0	0/0
Caraparu	3i	80	160	20	20	8/1024+	16/1024+	-	-
Oriboca	3i	10	40	160	0	0	-	16/1024+	-
Marituba	3i	40	40	20	320	8/1024+	-	-	16/1024+

HI with 4 units of antigen

* Titer of sera/titer of antigen

\- Not done

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF OTAGO MEDICAL SCHOOL, DUNEDIN, NEW ZEALAND

EXPERIMENTAL TRANSMISSION OF JOHNSON ATOLL (J.A.) VIRUS BY *Ornithodoros capensis*

A strain of J.A. virus which was isolated from *O. capensis* collected in a gannet colony (Arthropod-borne Virus Information Exchange No. 25) was tested to determine whether it could be biologically transmitted by the tick.

O. capensis eggs were collected from a gull colony where no virus has been isolated from more than 500 ticks, and J.A. virus antibody was not detected in sera from 21 gulls. Larvae which hatched from the eggs were fed for four days on experimentally infected chicks which had a J.A. viraemia of about 100 plaque-forming units (pfu) per ml.

Larvae which were killed or died within five days of the start of feeding contained up to 1,000 pfu of virus, indicating that multiplication had occurred. Thirty-nine days after feeding, four ticks which had developed into second instar nymphs, were fed on day-old chicks but failed to infect them. However, 74 days after the infecting feed, two of four ticks transmitted J.A. virus. This was indicated by a low level of viraemia in the chicks five days after they had been bitten, and by the presence of J.A. virus neutralizing antibody in their serum 21 days afterwards.

(F.J. Austin)

Murray Valley encephalitis

Murray Valley encephalitis ("Australian X disease") occurred in epidemic form in southeast Australia in 1917, 1918, 1922, 1925, 1951, 1956 and 1971. The two most recent outbreaks were very small and most of our present understanding of the epidemic disease depends on 1951 findings. Distinguished work by Melbourne and Adelaide teams in that year gave clear indication of the basic epidemiology, but there were many aspects which could not be studied then because the investigators did not have time or because techniques were not available. For example the vector role of Culex annulirostris, confirmed by virus isolation in enzootic transmission by our earlier studies in northern Australia, had not (before 1974) had similar confirmation in an epidemic.

The occurrence of an epidemic of Murray Valley encephalitis in January-April this year was therefore important as an opportunity for research as well as for public health action. Accordingly the unit's work in 1974 was directed almost entirely to studies, in collaboration with southern laboratories, of this outbreak. Results to date may be reported under three headings.

Serological diagnosis. The Institute has received serum samples from many suspected cases from a wide area of Australia, either for primary diagnostic tests or to confirm findings by diagnostic laboratories in Brisbane, Melbourne and Adelaide. Forty-seven patients showed serological evidence of Murray Valley encephalitis--29 from Victoria, three from N.S.W., nine from Queensland, five from Northern Territory and one from Western Australia. It is understood that several additional cases were diagnosed in Adelaide. Geographical distribution was largely confined to the Murray-Darling basin (as in previous epidemics), but occurrence of cases at Alice Springs and at Duaringa and Springsure in Queensland extends the known epidemic area. Full details of all patients are not yet to hand but high incidence is evident in young children (five aged less than one year, and 18 aged less than 10 years) and people aged over 60 years (nine patients aged over 60). Twenty-five of 47 patients for which the information is available were male, a more even sex distribution than was recorded in earlier epidemics.

Serial serum samples were obtained from most of the 47 patients, and are being used to evaluate available serological tests. Results to date show that complement-fixing antibodies may appear up to several weeks after hemagglutination-inhibiting (HI) antibodies (and have not yet been detected in some cases) and that HI antibody response may in some cases be to only low titers. Many cases showed peak HI titers two to four weeks after onset with a pronounced fall in convalescence, a finding which may cast some doubts on the use of the HI technique in surveys of past infection. Our tests also confirmed that the antibody response is indeed to Murray Valley encephalitis virus and not to Alfuy, Kunjin or Japanese B encephalitis viruses. Other studies by diagnostic laboratories in Brisbane and Melbourne indicate the value in rapid diagnosis of the early appearance of HI antibody in IgM fractions.

Field studies. In earlier studies we maintained surveillance of arbovirus infection of sentinel chickens at Charleville in southwest Queensland, where we found no evidence of infection with Murray Valley encephalitis virus in 1964-1970, but widespread infection in 1971. In addition a case of Murray Valley encephalitis was confirmed at Charleville in 1971.

Accordingly field studies in 1974 were centered on Charleville. Mosquitoes were collected by several techniques in three periods February 4-12, March 24-29 and June 18-25, and domestic fowls known to be less than one year old were bled and banded in the first week of February, and bled again each three to four weeks. The results indicate heavy activity of several viruses in early 1974. Thus of 86 domestic fowls bled in the first week of February, 45 had HI antibody to Murray Valley encephalitis virus, and 42 to Sindbis virus, and several birds seronegative then converted to one or other virus by the end of February or by the end of March. Mosquitoes and chicken bloods (from chickens exposed in traps) collected in early February yielded 27 virus strains from 345 pools, identified as Murray Valley encephalitis (2, from Culex annulirostris and sentinel chicken), Kunjin (2, from C. annulirostris), Wongal (2, from C. annulirostris), Mapputta group, possibly Gan Gan virus (12, from C. annulirostris, Aedes normanensis, Ae. pseudonormanensis and Ae. eidsvoldensis), Sindbis (1, from C. Annulirostris) and BP8090 (1, from Ae. normanensis) viruses, or so far unidentified (7, from C. annulirostris and Anopheles amictus amictus). Nine strains isolated from mosquitoes collected in March (137 pools) were identified as Kunjin (7) or Sindbis (2), and two others remain untyped. All were isolated from C. annulirostris. The processing of this collection was made difficult by an unusually high level of bacterial contamination requiring extra steps of centrifugation or filtration, or addition of kanamycin to the diluent. Three isolations were made after filtration of the original suspensions.

Two strains isolated from sentinel chickens in the Murray Valley by Dr. D. Hore, Veterinary Research Institute, Melbourne, and three strains isolated from brains of fatal human cases by Dr. I. Gust, Fairfield Hospital, Melbourne, were tentatively identified as Murray Valley encephalitis virus. Over 7,000 of 10 species of mosquitoes, mainly Culex annulirostris, Aedes bancroftianus and Aedes imperfectus, were collected in the Murray Valley from February 5 to 14 by Professor David Lee, School of Public Health and Tropical Medicine, University of Sydney, and submitted for study. No viruses were isolated. A further collection of mosquitoes from Darwin was received from Mr. N. Rajapaksa, Department of Health, Darwin, and is under study.

In an attempt to map the extent of Murray Valley encephalitis virus infection in Queensland, the Department of Primary Industries has been asked to provide serum samples from domestic fowls from a wide area of Queensland. Sera reactive to group B viruses were found from Mt. Isa, Mitchell, Charleville and Inglewood, all west of the Dividing Range. Only one serum from east coastal Queensland, from Ayr, was reactive. This study is continuing.

Analysis of rainfall. Earlier workers noted the correlation of heavy spring rainfall in northern Australia with summer-autumn epidemics of Murray Valley encephalitis in southeast Australia, and suggested that the correlation might be used to predict epidemics, or at least to predict periods of risk. The rainfall pattern which preceded the 1974 epidemic showed some but not all

of the features they described: for example, rainfall in the Darling River watershed in November did not reach 200% of normal, a figure suggested as a basis for prediction. The unpredicted occurrence of Murray Valley encephalitis in Central Australia can also be associated with record spring-summer rainfall.

The above was extracted from our Annual Report for 1973-1974. Copies of the full report will be available later in the year to anyone interested.

(R.L. Doherty)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF WESTERN AUSTRALIA, PERTH, W.A., AUSTRALIA

Mosquito and arbovirus surveillance in the Ord River area is continuing. The Ord River dam (Lake Argyle) overflowed for the first time in January 1974. It will be some time before the effects of erosion, etc., around the edges can be evaluated as to their effect on mosquito breeding. The presence of permanent breeding sites at the diversion dam has allowed year-round high-density breeding of some species (see Table 1).

Virus isolations to date are summarized in Table 2. All isolations were made from pools collected at Kununurra. Some idea of the seasonal difference in virus prevalence can now be obtained. Although it must be emphasized that the isolations obtained from the most recently studied group are putative only, it is clear that the isolation rate in this season of the year is very high.

The presence of Murray Valley encephalitis in the region has warranted a more detailed study into the biology of this virus. Thus a study of the three local strains has been initiated.

(N.F. Stanley, H.E. Paterson, P.F. Liehne, C.G. Liehne, J.F. Williamson, M.P. Alpers, K.H. Chan)

TABLE 1

Species	Mar./May 1972	Nov.1972/ Jan.1973	Mar./May 1973	Nov./Dec. 1973	Mar./Apr. 1974
Aedes notoscriptus		10	16 (3)	33 (32)	85 (83)
Aedes britteni				1	1
Aedes tremulus	23	8	12 (2)	25 (24)	18 (16)
Aedes alternans		2		4 (4)	6
Aedes lineatopennis				5	1
Aedes normanensis		52	52(44)	71 (67)	176(167)
Aedes vigilax		52		23 (21)	2 (2)
Aedeomyia catasticta		352 (278)	587(587)	461(460)	8 (8)
Anopheles bancrofti				4	2
Anopheles annulipes complex	10 (5)	227 (146)		19 (13)	13 (3)
Anopheles amictus	28(19)	6	11 (6)		13 (5)
Anopheles novaguinensis	8				
Coquillettidia.spp.		2	14 (10)	93 (93)	3 (3)
Culex annulirostris	1438(1372)	3646(3351)	5085(5085)	2028(2027)	7635(7591)
Culex australicus	66 (56)				
Culex bitaeniorhynchus		3	3		
Culex fatigans	13	7 (3)	19 (10)	11 (5)	1997(1941)
Culex starkiae			1		35 (25)
Culex vicinus					5
Culex pullus					141(138)
Mansonia uniformis				13 (8)	
Tripteroides punctolateralis	9	4	13	3 (3)	4 (3)

The numbers given are the total numbers of adult mosquitoes caught during each trip, and the numbers in brackets refer to the numbers processed for arboviruses.

TABLE 2

Date of collection	No. of pools	No. positive	Mosquito species	Virus type
May-June 1972	38	7	Culex annulirostris	OR1 MVE[1]
				OR2 MVE[1]
				OR3 MVE[1]
				OR4 Kunjin[1]
				OR5 Wongal[1]
				OR6 Sindbis[1]
				OR7[2]
Nov. 1972-Jan. 1973	69	1[2]	Aedeomyia catasticta	OR115
Apr.-May 1973	113	42[2] (putative isolations)	Culex annulirostris	(37)
			Aedeomyia catasticta	(3)
			Aedes tremulus	(1)
			Aedes notoscriptus	(1)

[1]Neutralization tests still to be carried out.

[2]Number of putative isolations, not yet proven or typed.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF SINGAPORE, REPUBLIC OF SINGAPORE

1. 1973 Dengue Haemorrhagic Fever Outbreak

Singapore experienced the largest outbreak of dengue haemorrhagic fever in 1973. Hospitals and outpatient clinics reported 1,255 cases with 26 deaths. Most of the patients were under 20 years of age. The University Department of Paediatrics admitted 139 cases from January to August, and of these 34 went into shock and 7 died.

The 1973 outbreak and the incidence in the previous three years (1970-1972) are shown in the accompanying figure. The estimated number of hospitalized cases with serological confirmation (complement fixation) was 1,479. The predominant dengue viruses isolated from patients' acute blood samples were types 1 and 3, but final identification of all virus isolates has not been completed.

During the outbreak, swing fogging with a mixture of Reslin 10/10 and kerosene was carried out by the Vector Control and Research Branch of the Ministry of the Environment to control the vector Aedes aegypti. Swing fogging operations began in May at construction sites throughout the city and were later extended to residential homes in August. Control of larval breeding by oiling was also carried out concurrently.

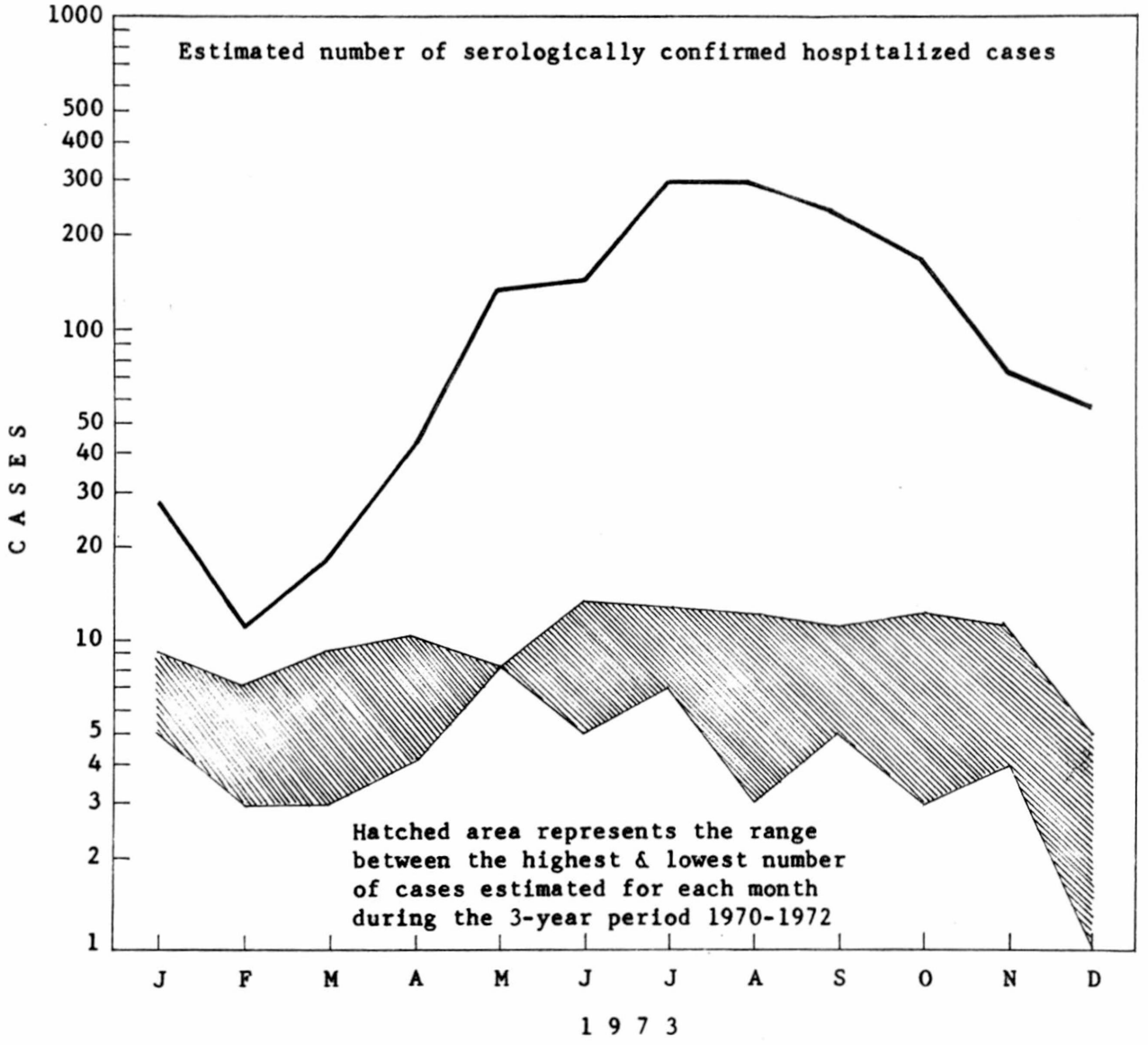
The expected outbreak in 1974 did not occur. From January up to the first week of August only 183 clinically diagnosed cases were reported.

2. Polyacrylamide Gel Analysis of Dengue Viruses

Electrophoresis of purified dengue type 2 viruses (New Guinea 'C' and local S-706/69 strains) prepared in suckling mouse brain in SDS-polyacrylamide gels revealed no distinctive differences in their polypeptide profiles. Six to nine nonstructural polypeptides and three structural polypeptides were obtained. It was also found that the number and type of polypeptides obtained were very much dependent on the amount of protein loaded on the gel. The purified virus preparations were screened for host protein contamination by immunochemical methods using anti-normal mouse brain sera; uninfected suckling mouse brains processed in the same manner served as controls.

(Chan Yow Cheong and Teoh Sek Hong)

DENGUE HAEMORRHAGIC FEVER - SINGAPORE 1973



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

Previous to our studies, eleven arboviruses had been isolated in Malaysia: bebaru, getah, and Sindbis in group A; dengue 1, Japanese encephalitis, Langat, and tembusu in group B; batai in the Bunyamwera group; bakau and ketapang in the bakau group; and Lanjan in the Kaisodi group. From the thousands of arthropod and vertebrate specimens collected and processed by us in Malaysia, we have added twelve viruses to this list, based on actual recovery and including dengue types 2, 3, and 4 (Table 1).

Of the known viruses new to Malaysia, Zika was previously recorded only from Africa, and Umbre from India. On the basis of serological surveys, Zika may have a cycle similar to dengue and yellow fever viruses. Zika antibodies can be demonstrated principally in man and monkeys and the virus was isolated from Aedes aegypti mosquitoes. Umbre, on the other hand, appears to circulate among birds and Culex mosquitoes (principally Culex annulus).

Seven of the viruses listed in table 1 are new to science: Batu Cave, Carey Island, and Jugra in group B; Seletar in the Kemerovo group; Puchong, which forms a new group with Sud Ar 1169 virus from the Sudan; and Keterah and Tanjong Rabok, which are presently ungrouped.

Batu Cave and Carey Island viruses were isolated from the salivary glands of bats. A serological survey to determine the incidence of antibody to these viruses in man and other vertebrates is planned.

Jugra virus was isolated from mosquitoes and the blood of a bat; Seletar from cattle ticks; and Keterah from bat ticks and the blood of a bat.

Tanjong Rabok virus was recovered from the blood of a monkey and antibody has been demonstrated in man.

Puchong virus, recovered from Mansonia uniformis mosquitoes, which are highly anthropophilic, may prove to be of special interest. Further work with this virus is planned.

Since the beginning of this project, 255 strains of dengue virus recovered in Malaysia from man, monkeys, and mosquitoes have been studied. Table 2 lists the strains by year and by dengue type. Types 2 and 4 appear to have been the most active in Malaysia in most years and type 3 the least active until 1973.

While only two types (1 and 2) have been recovered from monkeys, the first isolations from a vertebrate other than man, strong serological evidence in wild monkeys in Malaysia indicates that all four types are present in the forest. The sources of the dengue virus isolates are shown in table 3.

The principal interest of our unit in Malaysia is the ecology of dengue and we have been attempting to demonstrate that dengue (previously thought to have only an urban cycle involving man and domestic mosquitoes) has a jungle cycle involving wild primates and jungle mosquitoes.

The first phase of our studies began in 1962 for a period of several years. This was a general and widespread survey of urban areas, rural areas, and forest areas of several different types. The purpose was to determine the incidence and distribution of dengue in man, domestic animals, wild animals, and mosquitoes in all the major habitats.

Very briefly, the results of the general survey showed:

(1) that dengue disease was common in urban, town, and village areas of Malaysia, with cases occurring every month of the year, and that all four types of dengue were active in Malaysia;

(2) that dengue infection (but not necessarily illness) was common in rural areas as well as urban areas and also in deep forest among primitive aboriginal groups, as shown by serological surveys;

(3) that of the numerous species of mosquitoes and arthropods tested from many habitats, dengue virus could only be isolated from Aedes aegypti and A. albopictus mosquitoes in urban and rural areas;

(4) that monkeys of all species in all forest habitats had a high incidence and significant levels of dengue antibody, similar to that seen in man;

(5) that of the many vertebrate species examined, both wild and domestic, only monkeys appeared to be involved in a dengue cycle.

The second phase of these studies was devoted to the establishment and operation of several intensive study sites in isolated forest areas of mangrove swamp, freshwater peat swamp, and some relatively isolated primary lowland dipterocarp forest. Biweekly mosquito collections were made by several methods, but our principal effort was devoted to the use of sentinel monkeys permanently held in the canopy of these forests. These were bled twice per week for virus isolation attempts, and once per month for serological studies. A wild monkey capture-release-recapture program was also conducted and resulted in 314 captures (18% recaptures) in specific forest localities.

In brief, the second phase of the study resulted in additional evidence of specific dengue virus activity in isolated sentinel monkeys in mangrove and freshwater swamp forests, the detection of dengue viremia in a sentinel monkey, and excellent serological evidence of dengue activity in wild monkeys.

We then moved on to the third phase of our study, which is still in progress and is proving to be the most productive.

For this study, after a long search, we selected an extensive area of primary hill forest, which is as well isolated from normal human activity as can be found in Malaysia and still be accessible for routine study. It was necessary to provide our own access into the forest by constructing a trail of approximately 3+1/2 miles from the forest fringe.

We now felt that our efforts might be better rewarded if we could construct very large Magoon type traps capable of holding several monkeys (duplicating a small troop as it occurs in nature). We knew that several monkeys in a trap not only attracted more mosquitoes, but also attracted more mosquito species, and that there was an increase in the number of engorged mosquitoes.

We also hoped that the large size of the trap would induce mosquito species to enter that might not enter smaller traps.

We therefore proceeded with the construction of two very large traps, each weighing over 1000 lbs. One was placed in the high canopy and was baited with leaf monkeys; the second was placed at ground level and was baited with several macaques. In this way we hoped to more closely approximate natural conditions.

These traps have proved to be rewarding with the serological conversion for dengue of three leaf monkeys in the high canopy trap in December 1972; and the successful isolation of two strains of dengue type 2 and one strain of dengue type 1 from their blood samples. Additional leaf monkeys in the same trap also showed serological conversions in July 1973 and in February 1974.

It is of particular significance that, although the leaf monkeys in the high canopy trap (75' elevation) were infected with dengue of two different types, the macaques in the nearby ground trap were not infected during the same periods of time. This, then, appears to be convincing evidence of enzootic dengue activity in the high canopy of the forest. It also suggests that the forest vector involved in this case is more common in the canopy or that it prefers leaf monkeys to macaques. The latter seems less likely, since we proviously recorded several dengue conversions in wild macaques and since our serological surveys of wild monkeys revealed a high incidence of dengue antibody in all species of monkeys captured.

The mosquitoes collected in the trap during the periods that the dengue conversions occurred have failed to yield any virus so far. Studies are continuing in an effort to determine if the vector species are escaping from the trap. Nevertheless, studies of the biology of the canopy mosquitoes, we believe, will narrow the search for the vector. In combination with planned experimental transmission studies using suspect forest vectors, we hope to be able to determine which mosquitoes are important in the forest canopy. Details on the methodology and recent results and interpretations may be found in the UC ICMR Annual Progress Report for 1973.

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

TABLE I

Named Arboviruses and Probable Arboviruses Recovered

in Peninsular Malaysia to May 1974

Sero Group	Virus	Source							
		Mosquito	Tick	Man	Monkey	Horse	Pig	Bat	Bird
A	Bebaru	X							
	Getah	X							
	Sindbis	X							
B	Batu Cave*							X	
	Carey Island*							X	
	Dengue 1			X	X				
	Dengue 2*	X		X	X				
	Dengue 3*			X					
	Dengue 4*	X		X					
	Japanese encephalitis	X		X		X	X		
	Jugra*	X						X	
	Langat		X						
	Tembusu	X							
	Zika*	X							
Bunyamwera	Batai	X							
Bakau	Bakau	X			X				
	Ketapang	X							
Kaisodi	Lanjan		X						
Kemerovo	Seletar*		X						
Turlock	Umbre*	X							X
Ungrouped	Keterah*		X					X	
	Puchong*	X							
	Tanjong Rabok*				X				

*First isolations in Malaysia by this laboratory.

TABLE 2

Dengue Virus Isolations in Malaysia
(1962-1973)*

Year	DEN-1	DEN-2	DEN-3	DEN-4	Untyped	Total
1962-1964		16				16
1965	1					1
1966	2					2
1967	2	18	2	41	1	64
1968	2	16	1	21	3	43
1969		24		16		40
1970	1	21 (9)		4 (2)	1	27 (11)
1971	5 (4)	9 (4)		3 (2)		17 (10)
1972	11 (5)	8 (2)		2	1	22 (7)
1973	1 (1)	8 (7)	8 (8)	5 (2)	1	23 (18)
Total	25 (10)	120 (22)	11 (8)	92 (6)	7	255 (46)

*Numbers in parentheses show how many of the isolates were referred to us for identification by the Institute for Medical Research, Kuala Lumpur.

TABLE 3

Sources of Dengue Virus Isolates

in Malaysia (1962-1973)

	DEN-1	DEN-2	DEN-3	DEN-4	Untyped	Total
Human serum	24	113	11	89	6	243
Aedes aegypti		2		2	1	5
Aedes albopictus		2		1		3
Sentinel monkey blood	1	3				4
Total	25	120	11	92	7	255

REPORT FROM THE VIROLOGY DEPARTMENT, MEDICAL RESEARCH INSTITUTE
COLOMBO, SRI LANKA

1. Arbovirus Serology

1.1. From Clinical Cases

Table 1 gives the results of the arbovirus serology performed on blood from (mainly fever) patients received at the Medical Research Institute in 1972, 1973 and up to June 1974. The HI test is routinely used, followed by 2 ME treatment, CF and NT when required. The antigens are group A, chikungunya and Sindbis (since 1973); group B, dengue 1, dengue 2 and Japanese encephalitis.

Besides a sero-conversion or a fourfold or greater rise, an HI titer of 1:2560 for group B viruses and 1:1280 for group A viruses were taken as positives. NT and CF results have confirmed that there is continuing dengue 1 and 2 and Japanese encephalitis activity. But the increasing number of sero-negatives led to acute single sera also being examined from the beginning of 1973. It is seen that in 1972, 59.4% of paired and convalescent sera had no antibody.

In the case of chikungunya virus, activity seems to be even lower. Although there were no positives in 1974, 3 patients had titers of 640 suggesting a recent infection.

1.2. General Population Survey

This survey was conducted in 1973 in 3 different Medical Offices of Health areas on the outskirts of Colombo. Fifty sera (a fixed number per age group) were collected from each of eleven public health nurse (PHN) areas. Every tenth house was visited, making a total of 169 houses. Of 511 sera tested, arbovirus antibody was detected in 35.4%, with evidence of recent infection (HI titer of 640 or more) in 4.9%, including one case of chikungunya. The high level of sero-negatives (64.6%) reflecting the uninfected susceptibles, is surprising in the face of continuing viral activity in Colombo.

Determinations of Aedes index in Colombo by the Entomology Department of the M.R.I. show low values which may account for the above observation (as well as the low incidence of haemorrhagic fever).

2. Haemorrhagic Fever

In 1972 there were 19 cases of whom only 3 showed evidence of shock. One of these 19 was a dengue positive and one other had a low antibody titer. In 1973 there were 21 cases of whom 3 were in shock. There were 7 dengue positives while 6 others had antibody. Up to June in 1974, there have been 7 cases with one in shock. There was one dengue positive and one with antibody.

The reason for this low incidence is being investigated.

3. Japanese Encephalitis (JE)

Table No. 2 gives the number of patients from different parts of the country who had encephalitis and aseptic meningitis and from whom blood was received in 1972, 1973 and the first half of 1974. Paired sera were received in a minority of cases (19 out of 93 in 1974), and the number positive (a sero-conversion or fourfold or greater rise in titer) is small. These HI results were confirmed by neutralization test for JE. (Of those with antibody, 2 in 1973 and 2 in 1974 had HI titers of 2560 or greater so that they were probably positive.) One of the two positive cases was from Colombo and the other from Anuradhapura. There were no successful isolations in this period.

The impression among clinicians is that there has been an increase of encephalitis, often in patchy epidemics. The above results do not support the view that this is due to an increase of JE.

4. Sindbis Virus (this virus has not been detected in Sri Lanka before)

4.1. A field survey of a marshy area, Talawatugoda, on the outskirts of Colombo, gave the following results: Of sera from 40 domestic fowls 8 showed HI titers ranging from 1:4 to 1:320. Seven of these 8 showed neutralizing antibody to Sindbis virus. Sera from 23 humans in this area did not have Sindbis HI antibody. Virus could not be isolated from 10 pools of Mansonia crassipes mosquitoes.

4.2. From Clinical (mainly fever) Cases

863 sera in 1973 and 325 sera up to June 1974 were tested for Sindbis HI antibody. 15 showed titers ranging from 1:10 to 1:80 but there were no sero-conversions. They came from different parts of the country.

4.3. Jaundice Cases

Sindbis HI antibody was also detected in 16 jaundiced bloods, titers ranging from 1:10 to 1:640. In some cases the titers persisted in the convalescent bloods while in others it dropped sharply to less than 10. This phenomenon is being investigated further.

(U.T. Vitarana)

Table 1.

Arbovirus Serology (HI) results of blood from clinical cases received at the M.R.I., Colombo.

Year	1972	%	1973	%	1974 (up to June)	%
No. of specimens received.	1263		941		370	
No. Examined	475	100	863	100	325	100
No. Paired	244	54.4	165	19.1	49	15.0
No. Single	231	48.6	698	80.9	276	85.0
Group A No. Positive	-	-	6	0.7	Nil	-
No. with antibodies	43	0.9	79	9.2	17	5.2
No. Negative	432	91.0	778	90.1	308	94.8
Group B. No Positive	18	3.6	89	10.3	13	4.0
No. with Antibodies	183	37.0	336	38.9	97	29.8
No. Negative	292	59.4	438	50.8	215	66.2

Table 2.

Results of Japanese Encephalitis serology (HI) done on specimens (blood and CSF) received at the M.R.I., Colombo, from encephalitis and aseptic meningitis cases.

Year	No. of patients	No Paired	No. +ve JE	No. $\bar{c}$ Abs.	? +ve
1972	144	31	1	8	0
1973	120	45	1	18	2
1974 (up to June)	95	21	Nil	12	2

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

Virological studies on the first epidemic of Japanese encephalitis in India

An epidemic of encephalitis involving three districts in the state of West Bengal, (eastern part of India) broke out in June 1973. More than 700 clinical cases were reported. The epidemic subsided toward the end of 1973. Overall mortality was 40-50 percent. Previously, in 1958, an outbreak of Japanese encephalitis (JE) occurred in South India, when the virus was isolated from brain tissue of three patients. Subsequently, a few strains of JE virus had been isolated from mosquitoes, chiefly, Culex tritaeniorhyncus, all collected in South India.

Results of JE virus isolation in this epidemic are shown in Table 1.

Table 1. Number of specimens examined and number positive for virus.

Acute sera	CSF	Brain	Faeces	Throat swab	Mosquito pools
0* / 37	0 / 25	2 / 5	0 / 16	0 / 16	3 / 43

* Numerator - Number of isolations
Demoninator - Number of samples tested

The results of examination of mosquitoes collected in the affected houses and neighboring fields are shown in Table 2.

Table 2. Results of virus isolation from mosquitoes.

Positive			Negative	
Species	No. Pools	No. Mosquitoes	Species	No. Mosquitoes
C. vishnui	1	12	C. fatigans	256
A. hyrcanus	1	1	A. aegypti	6
A. barbirostris	1	1	A. vagus	49
			A. subpictus	115
			Armigeris obturbans	56
			C. tritaenior-hyncus	6
			C. vishnui	49
			A. annularis	25

Twenty-four out of 29 paired sera and 22 out of 41 single serum of clinical cases presented evidence of JE infection.

(S.K. Chakravarty, J.K. Sarkar, M.S. Chakravarty, M.K. Mukherjee, K.K. Mukherjee, B.C. Das. and A.K. Hati)

Dengue Infection at Children's Hospital of Bangkok

Patients suspected of having dengue virus infections were selected from the outpatient clinic and the infectious disease wards of Bangkok Children's Hospital. A standardized chart of pertinent signs, symptoms, and laboratory findings was instituted on each patient. Blood was obtained on the day of diagnosis and on day 3, 5, 15, 30 after the day of selection. Blood drawn on the first day was divided into plasma and serum portions. On subsequent collections blood was allowed to clot and serum was collected.

Plasma was used for isolation of viruses using a direct and delayed plaque technique. Isolates were identified by plaque reduction neutralization test using monkey antisera.

Sera were used for standard serology, hemagglutination inhibition (HI) tests were performed, using suckling mouse brain antigens prepared from dengue 1 (Hawaii), dengue 2 (New Guinea C), dengue 3 (H-87), dengue 4 (H-241), Japanese encephalitis (Nakayama) and Chikungunya (Ross). Sera were extracted with acetone and tested against 8 units of antigen. All sera were tested simultaneously. Aliquots of plasma and acute and convalescent sera were stored at -70°C for reisolation on certified cells and for specified dengue serologic tests against viral and non-viral antigens. At the conclusion of collection, clinical, isolation and serological data were used to identify individuals infected with dengue and to determine the type of antibody response and the severity of the illness. Patients were considered to have had dengue infection if a four-fold rise in antibody titer to at least two of the group B antigens was found between acute and convalescent serum or if convalescent antibody titers to at least two antigens equaled or exceeded 1:640. Criteria for the identification of primary or secondary dengue infected patients have been previously reported. Patients with HI antibody titers of $>$ 1:640 to at least two dengue antigens were considered to have secondary infections while those with convalescent antibody of $\leq$1:640 were considered to have primary infections. Grading of severity of DHF used criteria established by one of us (SN) and used in the past:

Grade I: Fever accompanied by non-specific constitutional symptoms; the only hemorrhagic manifestation is a positive tourniquet test.

Grade II: Fever and skin hemorrhage or other bleeding such as epistaxis or gingival hemorrhage.

Grade III: Circulatory failure manifested by rapid, weak pulse with narrowing of pulse pressure (20 mm Hg) or hypotension (systolic pressure 90 mm Hg).

Grade IV: Moribund patients with undetectable blood pressure or pulse.

Specimens were collected from 134 patients. These patients were seen and diagnosed as having illness compatable with dengue infection. Twenty-seven were collected in the outpatient clinic and 107 were obtained on hospital wards. Acute and at least one convalescent sera were obtained from 123 patients and 95 of these were diagnosed as dengue by viral isolation, by serological criteria or both. Of these 95, 91 were hospitalized for their illness. Twelve patients exhibited low level antibody responses characteristic of primary infection, and 8 of these were hospitalized. Sixty-two patients showed a four-fold rise in antibody titer and another 19 had high fixed titers. All of them were considered to have had secondary infections.

A breakdown of clinical and laboratory findings for these 95 patients is shown in Tables 1, 2 and 3. The findings were essentially similar to those of previous clinical studies. Comparison of patients showing primary and secondary responses demonstrates that DHF occurred in both groups. All but one patient with shock had secondary dengue infections. The exception was an 8 month old baby from whom only an acute plasma was obtained. Dengue 2 virus was isolated from this plasma at a time when no antibody was detectable; the mother unfortunately was not tested for antibody to dengue. Since no convalescent serum was collected, this patient could not be classified on the basis of antibody titer. He was considered to be a primary case because the acute plasma taken on the 5th day of illness had a titer of 1:20 to all group B antigens tested.

Between July and December of 1973, 22 strains of dengue were isolated from the 95 patients with evidence of dengue infection, representing an isolation rate of 23% (see Table 4 and 5). Nine strains were isolated from thirteen patients with serological evidence of primary disease; isolation was successful in 69% of primary cases. At the time plasma was obtained for isolation, only one individual had dengue antibody. In this case the titer for the homologous strain of virus was four-fold lower than that of the other dengue types suggesting antigen antibody complexes had been formed. The 82 secondary cases yielded 13 (16%) isolates. Dengue antibody was absent from the acute plasma in only three secondary cases, all of which had plasma obtained within the first two days of illness. The remaining ten had initial antibody titers ranging from 1:20 - 1:320 for the homologous virus (see Table 6). In the majority of isolations from secondary cases (11/13) the antibody to the homologous types was lower or equal to that of other types. Two had four-fold lower antibody titers to the infecting dengue type than to other dengue types tested, again possibly indicating complex formation.

Table 1. Summary of Clinical and Laboratory Findings in Thirteen (13) Primary Dengue Patients

Findings	UF* (5)**	Dengue Hemorrhagic Fever			
		Gr I (3)	Gr II (4)	Gr III (1)	Gr IV (0)
Fever	5/5 (100)***	3/3 (100)	4/4 (100)	1/1 -****	0/0 -
Hepatomegaly	0/5 (0)	1/3 (33)	2/4 (50)	1/1 -	0/0 -
Positive tourniquet test	0/5 (0)	3/3 (100)	4/4 (100)	1/1 -	0/0 -
Petechiae	0/5 (0)	0/3 (0)	4/4 (100)	1/1 -	0/0 -
Other signs of bleeding	1/5 (20)	0/3 (0)	0/4 (0)	0/1 -	0/0 -
Hemoconcentration	0/5 (0)	0/3 (0)	2/3 (67)	1/1 -	0/0 -
Platelet counts $\leq$ 50,000	0/5 (0)	0/3 (0)	2/4 (50)	1/1 -	0/0 -

* UF indicates undifferentiated fever
** Number of patients
*** Percentage of cases
**** 8 month old infant

Table 2. Summary Clinical and Laboratory Findings in 82 Secondary Patients

Findings	UF*	Dengue Hemorrhagic Fever			
	(2)**	Gr I (14)	Gr II (38)	Gr III (22)	Gr IV (6)
Fever	2/2 (100)***	14/14 (100)	38/38 (100)	22/22 (100)	6/6 (100)
Hepatomegaly	1/2 (50)	6/11 (56)	15/30 (50)	16/19 (84)	5/5 (100)
Positive tourniquet test	0/2 (0)	12/13 (92)	36/36 (100)	22/22 (100)	6/6 (100)
Petechiae	0/2 (0)	0/13 (0)	29/36 (81)	16/22 (73)	4/6 (67)
Other signs of bleeding	0/2 (0)	0/13 (0)	14/36 (39)	7/22 (32)	5/6 (83)
Hemoconcentration	0/2 (0)	7/11 (54)	18/36 (50)	18/19 (95)	6/6 (100)
Platelet counts $\leq$ 50,000	0/2 (0)	8/14 (57)	24/38 (63)	21/22 (95)	6/6 (100)

* UF indicates undifferentiated fever
** Number of patients
*** Percentage of cases

Table 3. Hemagglutination Inhibition Antibody Levels in Convalescent Sera from 94 Patients with Dengue

Grade (s) of Disease	Primary Infection (Titer $<$ 1:640)	Secondary Infection (Titer $\geq$ 1:640)
UF*	5	2
I & II	7	52
III	0	22
IV	0	6
TOTAL	12	82

* UF indicates undifferentiated fever

Table 4. Dengue Strains Isolated from Human Infections: Dengue Type and Convalescent Hemagglutination Inhibition Antibody Responses

Antibody Response	Number of Patients	Total Isolations	% Isolation	Strains Isolated		
				Type 1	Type 2	Type 3
Primary (<1:640)	13	9	69	3	3	3
Secondary (≥1:640)	82	13	16	1	11	1
TOTAL	95	22	23	4	14	4

Table 5. Dengue Strains Isolated from Human Infections: Dengue Type and Clinical Syndrome

Clinical Syndrome (No. of cases)	Number of Strains Isolated		
	Type 1	Type 2	Type 3
UF* (7)	2	2	2
G I (17)	1	0	0
G II (42)	1	6	2
G III (23)	0	5	0
G IV (6)	0	1	0
TOTAL	4	14	4

* UF indicates undifferentiated fever

Table 6. Distribution of Dengue Isolations by Hemagglutination Inhibition Titers to Dengue 2 or to the Homotypic Virus in the Acute Serum

Reciprocal Titer	No. of Patients	Dengue Isolates	
		No.	%
< 20	20	13	65
20	5	4	80
40	2	2	100
80	6	0	0
160	4	2	50
320	14	1	7
≥ 640	44	0	0
TOTAL	95	22	23

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

Dengue virus plaques on microplate cultures of BHK-21 cells

The microplate culture method was applied to dengue plaque formation and its neutralization test. Dengue virus type 1, Mochizuki strain, was mainly employed. BHK-21 cells, clone 13, were obtained from Dr. C.L. Wisseman, Jr., University of Maryland, through Dr. H. Aoki. Plaques were formed on the cells cultivated in plastic microculture plates (Falcon Microtest II, 124x82x14 mm, having 96 flat-bottom 7 mm wells) under overlay medium consisting of 1% methylcellulose and 2% heat-inactivated calf serum in Eagle's minimum essential medium. The plaques, approximately 1 mm in diameter, were clearly visible 5 days after the beginning of incubation at 37 C and were easily countable. Plaque reduction neutralization tests on the microplate cultures were carried out by use of the transfer-plate report by Catalano et al. (Appl. Microbiol., 18, 1094, 1969). The technique is comparatively rapid and easy to perform and can be applied to clinical diagnosis and epidemiological surveys of dengue and related virus infections.

Reference: Fujita, N., Tamura, M., and Hotta, S. Dengue virus plaque formation on microplate cultures and its application to virus neutralization (to be published).

(Susumu Hotta)

REPORT FROM THE NATIONAL INSTITUTE OF HEALTH, TOKYO, JAPAN AND THE VIRUS RESEARCH INSTITUTE, BANGKOK, THAILAND

1. Attempts to isolate virus from kidneys of bats trapped in Oita Prefecture, Japan.

In the past, Japanese encephalitis (JE) virus and several not yet identified viruses were isolated from bats caught in Japan (Sulkin et al., 1970; Miura, 1968). In December 1969, 80 bats were captured in a cave in Oita Prefecture. Attempts were made to isolate virus from their kidneys in tissue culture and by inoculation into suckling mice by the intracerebral route. No viruses were isolated but a bat kidney cell (BKC) line was established by serial passages.

2. Susceptibility of an established bat kidney cell line to some group A and B arboviruses.

The susceptibility of BKC to some group B arbovirus (JE, St. Louis encephalitis (SLE) and Apoi) and group A arboviruses (Sindbis and getah) was investigated as to whether or not cytopathic effect (CPE) could be seen (Table 1). Both JE and SLE viruses were found to produce CPE 2 to 3 days after inoculation, but Apoi and Sindbis viruses did not.

3. Persistent infection of JE virus in bat kidney cells.

It is noteworthy that some cells survived on the glass wall of the culture bottle after infection with JE virus. Such surviving cells were viable enough to grow when subpassaged. When the bottle culture was incubated at 37 C the virus could be recovered from the culture fluid in the absence of CPE. When the bottle was placed at 5 C for 118 days no virus yield was recognized but the virus appeared again in culture fluid when the bottle was incubated at 37 C. In other words, the BKC was persistently infected in the bottle at both temperatures.

References:

Sulkin, S.E., Allen, R., Miura, T., and Toyokawa, K. 1970. Studies of arthropod-borne virus infections in Chiroptera. 6. Isolation of Japanese B encephalitis virus from naturally infected bats. Am. J. Trop. Med. Hyg., 19, 77-87.

Miura, T. 1968. Study on a bat. Nihon Nettaiigaku Zasshi, 9, 7-9. (in Japanese)

(Ogata, T., Shimizu, A., and Kitaoka, M., National Institute of Health, Tokyo)

Table 1

Virus titration on bat kidney cells by tube method

Virus strain		PFU* on chick embryo cells	$TCID_{50}$** on bat kidney cells
JE	JaGAr-01	9.5	8.8
SLE		9.3	7.7

* PFU: plaque forming units on primary chick embryo cells (log 10/ml).

** $TCID_{50}$: 50% tissue culture infective doses (log 10/ml).

4. Mosquito-borne haemorrhagic fever in Thailand in 1972.

It is well known that dengue viruses of all types (1, 2, 3, 4, 5 and 6) and chikungunya virus are at least distributed concomitantly during the epidemic of mosquito-borne haemorrhagic fever (MHF) in Thailand. Table 2 indicates yearly reported cases of MHF in Thailand according to the Ministry of Public Health, Thai Government, during 1961 to 1972. The number of reported cases in 1972 was 23,782 with 685 deaths. This figure of 1972 is approximately twice that of 1971 and 3 to 5 times that in the other years. Such an increase in number may be due to the fact that the cases were reported not only from Bangkok but also from almost all provinces of Thailand, especially in the northeast region.

The age distribution ranged predominantly from 6 months to 15 years, with the greatest number in the 4 to 5 year age group, as given in Table 3. The season of case incidence of MHF in Thailand ranges from May to November, and is predominant in July and August. Table 4 indicates cases among suspected MHF cases, 1963-1972, which were confirmed at the Virus Research Institute, Yod-se, Bangkok, by HI and CF tests on paired sera using dengue virus antigens of all types and chikungunya virus antigen. Out of 3,723 cases tested 3,333 (89.5%) were dengue positive, 155 (4.2%) were chikungunya positive and 235 (6.3%) were double infections of dengue and chikungunya viruses.

In 1972, out of 1,219 samples submitted from Bangkok and the other provinces and tested by HI, 579 (47.5%) were dengue positive, 18 (1.5%) were chikungunya positive, 80 (6.6%) were double infections, 206 (16.9%) were inconclusive due to no fourfold rise of antibody, and 336 (23.6%) were not MHF. From the foregoing it can be seen that MHF in Thailand is still an important disease in children which needs to be controlled. About half of the reported cases are due to dengue virus infection, about one-tenth to chikungunya virus infection, and about one-fourth to neither dengue virus nor chikungunya virus.

(P. Tuchinda, S. Ahandarik, and S. Pisuthipornkul, Virus Research Institute, Bangkok; and M. Kitaoka, National Institute of Health, Tokyo)

Table 2
Number of reported cases of mosquito-borne hemorrhagic fever in Thailand during the period from 1961 to 1972

Year	No. of cases	No. of death	Morbidity rate per 100,000
1961	561	36	2.1
1962	5,947	308	21.4
1963	2,215	173	7.8
1964	7,663	385	26.3
1965	4,094	193	13.7
1966	5,816	137	19.1
1967	2,060	65	6.6
1968	6,430	71	20.2
1969	8,670	109	26.7
1970	2,767	47	8.3
1971	11,540	299	31.3
1972	23,782	685	64.5
Total	81,545	2,508	

(Case fatality rate, 3.1%)

Table 3
Age distribution of reported cases of mosquito-borne hemorrhagic fever in Thailand in 1969

Age group (year)	No. of cases	No. of death	Morbidity rate per 100,000 age population
under 1	916	19	71.6
1 - 2	1,127	11	
3 - 4	1,803	35	
5 - 6	1,655	26	62.9
7 - 9	1,537	11	
10 - 14	1,199	7	32.6
over 15	365	-	2.0
unknown	68	-	
Total	8,670	109	26.7

Table 4

Confirmed cases of mosquito-borne hemorrhagic fever in Thailand during the period from 1963 to 1972

Years	Dengue fever	Chikungunya	Double infection (dengue plus chikungunya)
1963	177	17	7
1964	320	14	13
1965	407	21	14
1966	461	10	8
1967	149	16	15
1968	233	18	7
1969	411	17	33
1970	287	16	32
1971	312	8	26
1972	576	18	80
Total	3,333 (89.5%)	155 (4.2%)	235 (6.3%)

5. Interferon induced in Vero cells infected by vaccinia viruses against JE virus growth

In the past outbreaks of encephalitis in horses which were transferred from JE nonendemic area such as the northern part of Hokkaido to endemic areas or were imported from a nonendemic country to Japan have served as warnings for outbreaks of human encephalitis to follow a few weeks later. In 1947 compulsory country-wide vaccination against smallpox was carried out to eradicate that disease in Japan. In the same year, a large outbreak of horse encephalitis occurred but the subsequent human case incidence of encephalitis was very low. The next year (1948) a large epidemic of encephalitis in both man and horse occurred. These facts suggested to the authors that the smallpox vaccination in man might have played an interfering role in respect to human JE. Therefore an experiment was undertaken to determine whether or not vaccinia virus induces interferon against JE virus in vitro.

Table 5 indicates the procedure of preparing interferon by using Vero cell culture infected with vaccinia virus. Table 6 indicates the method of

interferon assay. By similar procidure we also tried to demonstrate interferon in Vero cells infected with JE virus and Sindbis virus; interferon activity of original material and at dilutions of 1:2, 1:8 and 1:32 was presented as percentage of plaque reduction compared with control. As given in Table 7, the interferon induced by vaccinia virus markedly inhibited the growth of JE virus (92.4%) and also inhibited the growth of Sindbis virus (26.8%). Almost no interferon was induced in Vero cells infected with JE virus, but interferon was induced by Sindbis virus in amount adequate to inhibit the growth of Sindbis virus in Vero cells by 36.7%.

The interferon-like activity mentioned above is similar to that described in text books, but studies with actinomycin D as described by Toyoshima for possible presence of an antivirion, noninterferon agent have not yet been done. The relationship between smallpox vaccination and the case incidence of JE should therefore be further investigated.

(M. Kitaoka and H. Tsukeda)

Table 5 Preparation of interferon

Monolayers of vero cells (bottle)

↓ starting cell number: 2×10^5 cells/ml
incubated at $37^{\circ}C$ for 24 hrs

Washed twice the monolayered cells with Hanks-PBS

↓

Infected with induced viruses

↓ vaccinia, JE and s indbis viruses
absorbed at $37^{\circ}C$ for each 1 hr

removed the inoculated virus by pipetting

Added maintenance medium to each bottle

↓ incubated at $37^{\circ}C$ for 24 hrs

Harvested the fluids

↓ centrifuged at 1500 rpm for 10 min

Inactivated the residual virus by heating at $56^{\circ}C$
↓ for 2 hrs

Interferon samples were prepared (crude)

kept at $0^{\circ}C$ for 16 ~ 24 hrs for next
assay of interferon

Table 6 Assay of interferon

Monolayers of vero cells (dish)

↓ starting cell number: 1 x 10^6 cells/ml
incubated at 37°C for 24 hrs

Washed once the monolayered cells (stationary phase)

↓ with Hanks-PBS

Treated with the interferon samples in each dish

↓ incubated at 37°C for 3 hrs

removed the treated interferon samples
by pipetting

Infected with indicator viruses

↓ JE and sindbis viruses
absorbed at 37°C for 2 hrs

Added the 1st overlay medium to each dish

↓ incubated at 37°C for 48 hrs

Added the 2nd overlay medium to each dish

↓ incubated at 37°C for 48 hrs

Counted the plaque numbers

Table 7

Activity of interferon induced by vaccinia, JE and Sindbis viruses on the growth of JE and Sindbis viruses

Interferon	Indicator virus							
Dilution	JE virus 1		Sindbis virus 2		Sindbis virus 3		Sindbis virus 4	
	Titer PFU/ml	Inhibition rate %	Titer PFU/ml	Inhibition rate %	Titer PFU/ml	Inhibition rate %	Titer PFU/ml	Inhibition rate %
0	6.5×10^6	92.4	5.2×10^7	26.8	6.1×10^7	14.1	4.5×10^7	36.7
x 2	1.9×10^7	77.7	5.7×10^7	19.8	6.2×10^7	12.7	5.0×10^7	29.6
x 8	6.1×10^7	28.3	6.4×10^7	9.9	6.1×10^7	14.1	5.1×10^7	28.2
x32	7.3×10^7	14.2	6.6×10^7	7.1	6.1×10^7	14.1	5.7×10^7	19.8
Control	8.5×10^7		7.1×10^7		7.1×10^7		7.1×10^7	

1 and 2 using interferon induced by Vaccinia virus
3 using interferon induced by JE virus
4 using interferon induced by Sindbis virus

REPORT FROM DEPARTMENT OF HYGIENE AND EPIDEMIOLOGY
SAPPORO MEDICAL COLLEGE, SAPPORO, JAPAN

Serologic survey of arbovirus infections for man in Southeast Asia

In an attempt to determine serologically the geographic distribution of arboviruses in Southeast Asia, more than 2,000 serum samples were collected from man indigenous to Laos, Thailand and Indonesia. The places at which the collection was carried out were shown in Fig. 1. The following viruses were employed as antigens: Chikungunya(Ch), Getah, Sindbis and Ross River for group A arbovirus, and Japanese encephalitis(JE), Murray Valley encephalitis(MVE), type 2 dengue(D-2) and Negishi for the group B. Based on the preliminary examination, antibodies against the group A arboviruses were titrated using the hemagglutination-inhibition(HI) test, while those against the group B were by means of the neutralization(NT) test using a microplate. Of the serum samples collected at the 10 places, 20-35 samples were offered to the antibody examination for each age group with an interval of 10 years.

1. HI antibodies against the group A arboviruses.

Ch antibody was distributed widely in the study area with great geographic variations in the incidence(Fig.2). While in Laos and Thailand the antibody began to appear in early years of age, most people of the ages under 20 in Indonesia were devoid of the antibody. Only exception was Balikpapan at which 43% of people of that ages had the antibody. Seropositives to Getah and Sindbis viruses were detected more frequently in the regions east- and westmost of the study area than the region between both.

The geographic distribution of Ross River antibody was also bipolar in the pattern. The high incidence of

the antibody in Jayapura and the progressive decline of the incidence toward west in Wallacea were worthy to note. A steep rise in the number of seropositives to the virus with the increase in age was observed in Jayapura and Pontianak.

2. NT antibodies against the group B arboviruses.

Of the antibodies against the 4 group B arboviruses, that to D-2 was the highest in the titer. D-2 antibody extended with high incidence and uniformity over the entire area, except Pit River(Fig.3). The great majority of inhabitants have had the antibody by the age 30, regardless of their residence. Contrary to this, JE antibody was distributed almost exclusively in the regions westside of the Wallace's Line. MVE antibody extended also widely; and at the places eastside of Surabaya, the antibody predominated over the JE in the incidence, while at the westside of Balikpapan the relationship was reversed. Considering the results, the MVE antibody of the eastern region was in all probability due to the virus infection, whereas the same antibody of the western region was most likely that which was produced heterologously by JE virus infection. Nobody was found to be positive to Negishi, as far tested.

Our serologic survey made it clear that the two biogeographic boundaries, the Wallace's and Weber's Lines, affected largely the geographic distribution of some arboviruses in this area. Of interest is that the distribution of Ch antibody with high titers accords closely with the geographic distribution of monkeys known to be the main host of Ch virus. The lack of the antibody in young Indonesians and the recent outbreak of Ch epidemic in Balikpapan suggest that the epidemic potential of the disease is increasing in Indonesia.

Seropositives to JE virus were rare in the regions eastside of the Wallace's Line. This means that this line restricts strongly the habitation of host of the virus; however, considering serologically, the same line appears not to form a strong barrier to the habitation of the host of MVE virus. Serologic evidence was obtained suggesting that Ross River virus extended to West Irian and Wallacea, if the density of the virus was low in the latter region. Although the same antibody was detected in Pontianak and Vientiane, the geographic distribution of the antibody forms a deep gap at the region of Wallace's Line. Taking this into consideration, the Ross River antibody found in the western region is possibly due to infection of viruses related antigenically to Ross River, presumably those viruses different from Getah and Sindbis.

Wallacea is characteristic of its poor animal fauna, and this may explain the low incidence in man of Getah and Sindbis virus infection. If this explanation is true, hosts of the two viruses may be of the species different from those of Ch and MVE viruses. Pit River of West Irian is a village located at the place 2,600 meters high from sea level, and is free of malaria. The fact that nobody of inhabitants of the village had antibodies against either of the 7 mosquito-borne arboviruses is probably due to the absence of vector mosquitoes of the viruses there.

(Masatsugu KANAMITSU and Midori FUKUHARA)

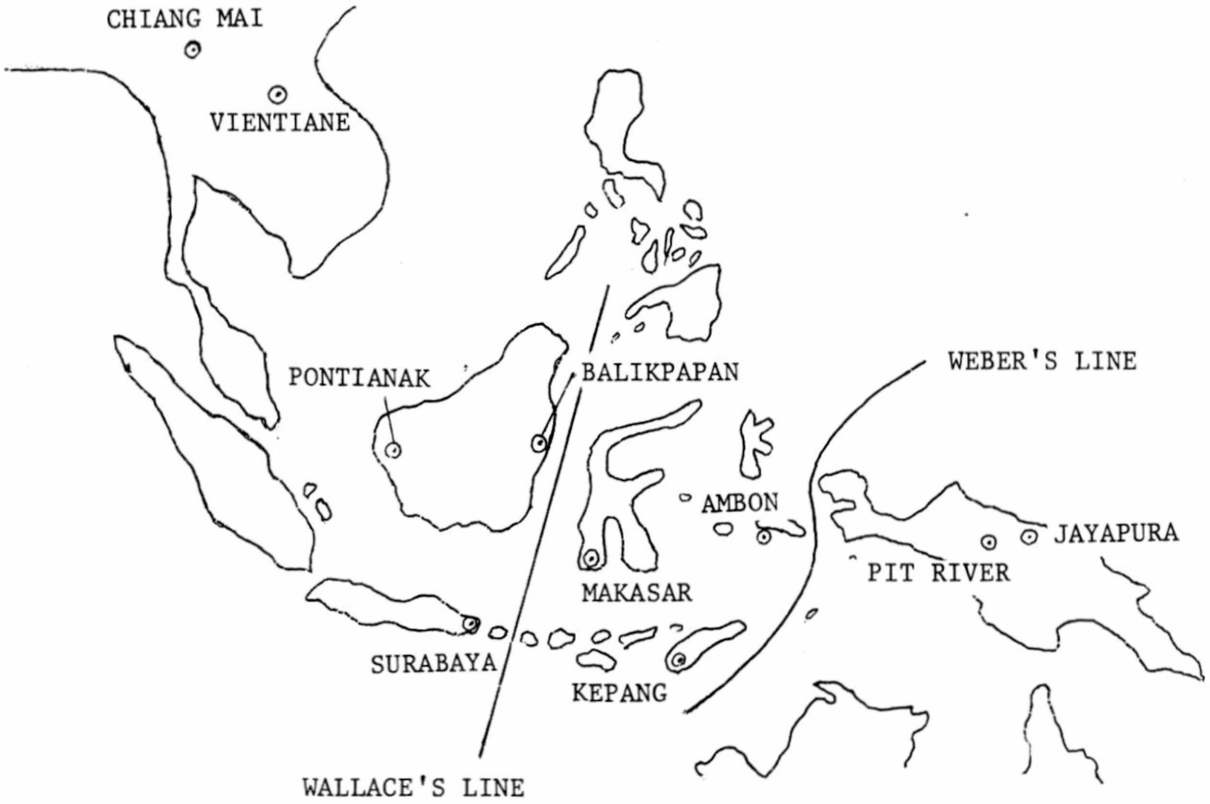


Fig. 1. Places of collection of serum samples.

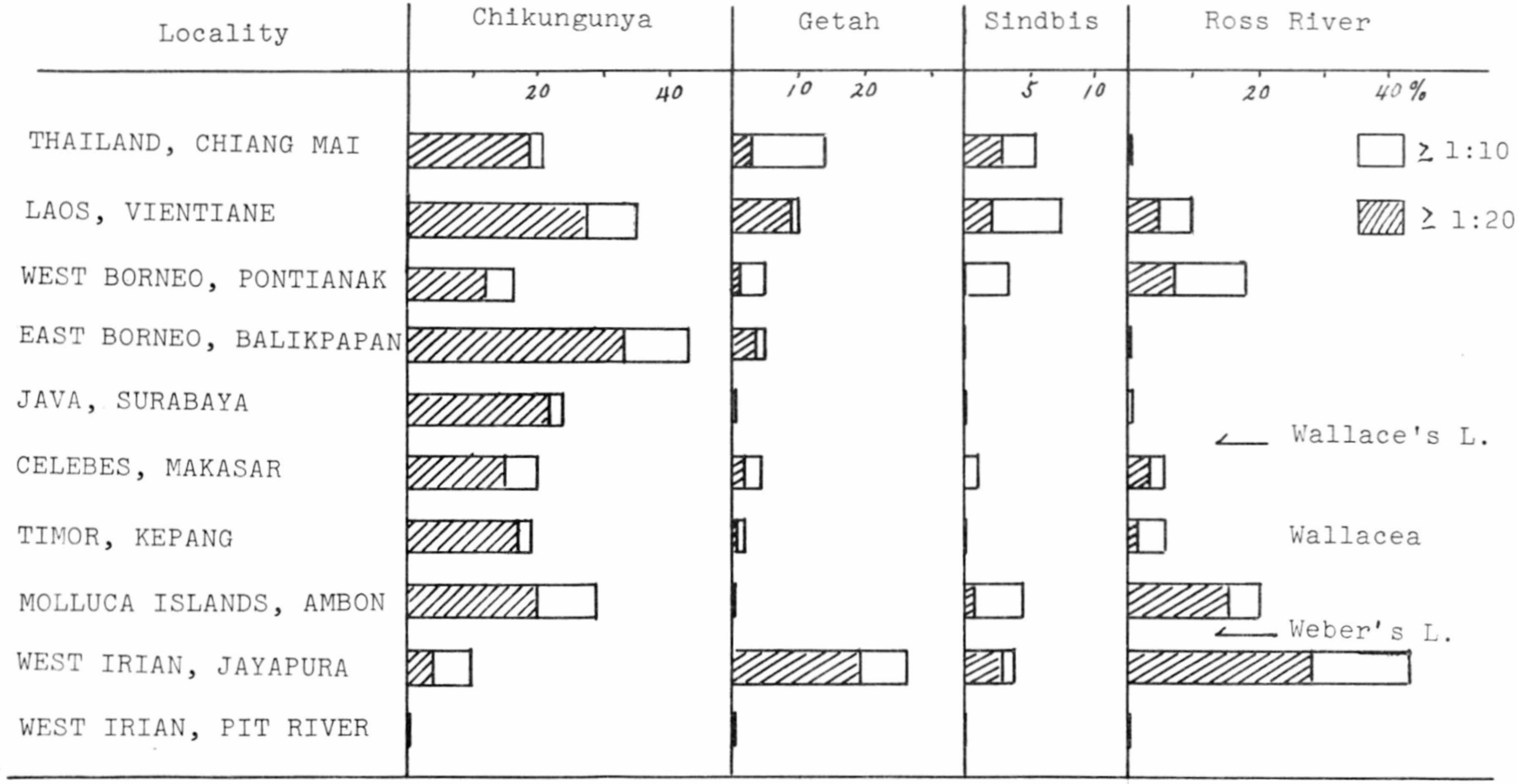


Fig. 2. Geographic distributions of HI antibodies against group A arboviruses.

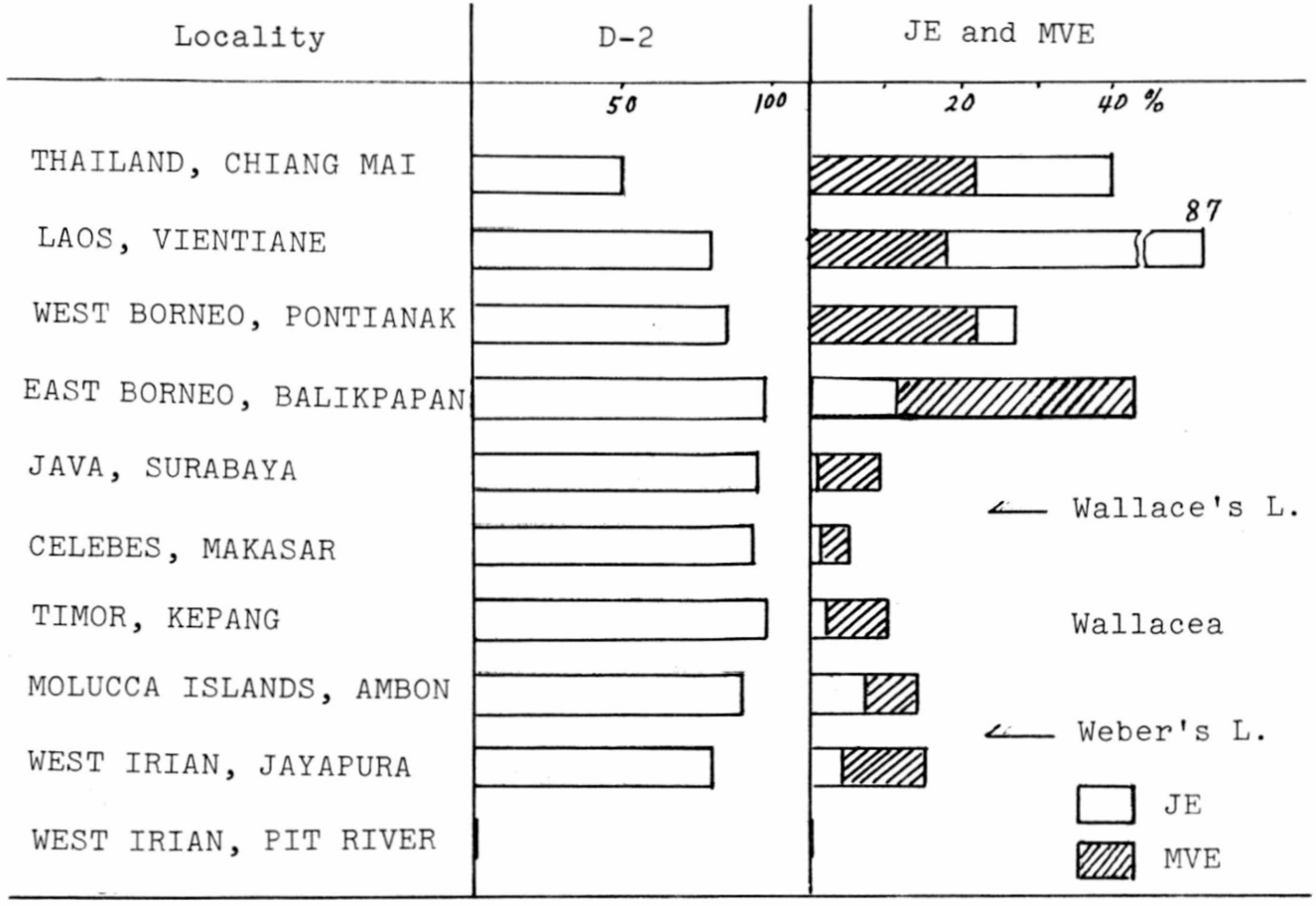


Fig. 3. Geographic distributions of NT antibodies(1:4 by the microneutralization test) against group B arboviruses.

REPORT FROM THE INSTITUTE FOR VIRAL DISEASES
KOREA UNIVERSITY MEDICAL COLLEGE
SEOUL, KOREA

Isolation of Japanese encephalitis virus from Aedes vexans nipponii

It has been widely known that Culex tritaeniorhynchus is the main vector of Japanese encephalitis, and it has also been reported that Japanese encephalitis virus was isolated from Culex pseudovishnui and Culex gelidus in a part of Southeast Asia.

In this study, 33,381 Aedes vexans nipponii were collected in the suburbs of Seoul in the summers of 1967 and 1968 in a search for Japanese encephalitis virus and other viruses. In 1967, 19,006 mosquitoes in 266 lots, and in 1968, 14,275 mosquitoes in 240 lots (total 500 lots) were tested for viruses in porcine kidney and chick embryo cells.

Four strains of Japanese encephalitis virus were isolated from Aedes vexans nipponii, 2 strains in July, 1967, and 2 strains in August, 1968.

(H.W. Lee)

Japanese Encephalitis Virus Isolation from A. vexans nipponii

Mosquito Species	Strain Name	Date and Place of Collection	Method of Isolation
A. vexans nipponii	M-67-AVN-219	7/18/67 Suburbs of Seoul	Porcine kidney and chick embryo cell culture system
A. vexans nipponii	M-67-AVN-222	7/21/67 Suburbs of Seoul	"
A. vexans nipponii	M-68-AVN-117	8/6/68 Suburbs of Seoul	"
A. vexans nipponii	M-68-AVN-131	8/13/68 Suburbs of Seoul	"

Retrospective study of the etiology of phlebotomus fever in Ashkhabad

After the 2nd World War, in 1945-50, outbreaks of phlebotomus fever were repeatedly recorded on the USSR territory. The disease was endemic in the following areas: Crimea, some regions of the Transcaucasus, Moldavia, republics of Central Asia. A number of strains were isolated from patients and an increase in antibodies to them was revealed in convalescents. However, the strains were not compared with Sicilian and Naples phlebotomus fever viruses and, in fact, remained unidentified. Thus until recently the fact that these viruses belong to the phlebotomus fever group was not proved.

The present study was directed to identification of the Central Asian strain isolated during an outbreak of phlebotomus fever in Ashkhabad in 1950. Since 1953 the virus was kept lyophilized in the All-Union collection of virus strains in the D.I. Ivanovsky Institute of Virology. The virus was reestablished only after two blind passages in 2-3-day old suckling mice. At the third passage level, the animals fell ill after a 7-day incubation period, then the latter shortened up to 3-4 days and thereafter remained at this same level.

When the virus titer in the brain reached 7 log LD_{50}/0.02 ml after a number of successive passages, a hemagglutinating antigen was prepared and the conditions for hemagglutination were studied. The titer of antigens of individual series was 1:32 to 1:128, sonication resulted in a 4-8 fold increase of the titer. The virus agglutinated goose erythrocytes at a pH range of 6.0-7.0, the optimum pH range being 6.0. Temperature did not exert any marked effect on HA but the results were somewhat worse at 4°C. The treatment of erythrocytes with trypsin considerably increased their sensitivity in the HA test. The virus agglutinated chicken erythrocytes equally as well as goose erythrocytes (Table 1). Human group O erythrocytes and rooster erythrocytes were also sufficiently sensitive. The treatment of erythrocytes with trypsin increased the receptory activity of all kinds of erythrocytes and made it possible to reveal agglutination with natively nonsusceptible erythrocytes (rabbit, hamster). Only sheep erythrocytes appeared to be completely refractory among those tested. Identification of the "Central Asian" strain with other viruses of the phlebotomus fever group was performed in HI, CF and ADP tests. Antigens of and immune ascitic fluids to the following 10 phlebotomus fever viruses were used: Anhanga, Bujaru, Iran-47, Candiru, Naples and Sicilian phlebotomus fever, Punta-Toro, Salehabad, Chagres, Karimabad. In the HI test, which is group specific for the complex of viruses under consideration, the "Central Asian" strain gives the strongest reaction with Naples phlebotomus fever virus (Table 2). In the CF test, which is more specific for the PHL group, the "Central Asian" strain reacted only with Naples phlebotomus fever, Iran-47 and Salehabad viruses which are antigenically closely related to the Naples serotype. By the ADP test, the close antigenic similarity between Central Asian and Naples viruses was confirmed (Table 3). The studies undertaken made it possible to allocate the Central Asian Strain to the Naples serotype of phlebotomus fever virus.

(S. Ya. Gaidamovich)

Table 1

THE HA TEST WITH ERYTHROCYTES DERIVED FROM VARIOUS SPECIES OF DONOR ANIMALS

Species of donor animals	Titer in HA test at pH 6.0 and 22°C with erythrocytes	
	Native	trypsin-treated
Goose	32	512
Chickens (one-day-old)	32	512
Rooster	16	256
Humangroup 0	16	128
Sheep	0	2
Rabbit	0	64
Guinea-pig	16	64
Hamster	4	128
Rat	32	128
Mouse	0	128

Designations: the HA titers are expressed in reciprocals of dilutions.

Table 2

ANTIGENIC RELATIONSHIPS BETWEEN CENTRAL ASIAN STRAIN AND OTHER VIRUSES OF THE PHLEBOTOMUS FEVER GROUP IN THE HI TEST

I A F	IAF titers with antigens: Central Asian	IAF titers with antigens: Homologous
Anhanga	0	320
Bujaru	0	160
Iran-47	160	160
Candiru	0	80
Karimabad	10	80
Naples phlebotomus fever	320	160
Punta-Toro	10	n.t.
Salehabad	20	80
Sicilian phlebotomus fever	0	160
Central Asian	1280	1280
Chagres	40	1280

Designations: IAF-titers are expressed in reciprocals of dilutian
n.t. - not tested

Table 3

ANTIGENIC RELATIONSHIPS BETWEEN CENTRAL ASIAN STRAIN AND NAPLES PHLEBOTOMUS FEVER VIRUS

IAFs to / Virus	Naples			Central Asian		
	HIT	CFT	ADPT	HIT	CFT	ADPT
Naples phlebotomus fever virus	160	320	32	160	160	16
Central Asian virus	320	160	16	1280	320	16

For designations see Table 2.

REPORT FROM THE DEPARTMENT OF THE ECOLOGY OF ARBOVIRUSES
INSTITUTE OF VIROLOGY, SLOVAK ACADEMY OF SCIENCES
BRATISLAVA, CZECHOSLOVAKIA
and
DEPARTMENT OF MICROBIOLOGY, MEDICAL SCHOOL
UNIVERSITY OF THESSALONIKA, GREECE

1. Isolation of Sindbis virus from the organs of a hamster in Eastern Slovakia

During field studies in East Slovakia in the spring of 1972, isolation experiments were carried out from the blood, brain and liver of 198 hamsters. One agent was isolated from the blood and liver of a hamster captured near the village of Trhoviste.

The virus was isolated in the first passage in suckling mice. The incubation period from the first to the 3rd passage was 6 to 7 days; in the 4th passage it was 4 days, and from the 5th passage, 3 days. The virus was pathogenic for suckling mice after intracerebral inoculation (6.5-8.0 log LD_{50}/0.01 ml) and after intraperitoneal inoculation (4.5 log LD_{50}/0.01 ml). In the first and second passage the virus was not pathogenic for three-week-old mice after intracerebral inoculation. After adaptation of virus (in the 4th and 5th passage), the virus became pathogenic for adult mice after intracerebral inoculation (2.5-4.5 log LD_{50}/0.03 ml). The virus was filterable (no loss of virus titer after passage through a Seitz filter), sensitive to ether (decrease of virus titer by 4.5 log/0.01 ml) and to sodium deoxycholate (decrease of virus titer by 3.9 log LD_{50}/0.01 ml).

Haemagglutination antigen was obtained in the 5th mouse passage, though not in the 4th passage; sucrose-acetone antigen made from baby mouse brain showed a haemagglutination titer of 1:5120 with goose cells at pH 6.2. A further study of antigenic interrelation of Michalovce strain and other arboviruses was performed by HI tests with Michalovce antigen and immune sera against the following viruses: western equine encephalomyelitis, eastern equine encephalomyelitis, Semliki, Sindbis, tick-borne encephalitis (TBE), West Nile, yellow fever, St. Louis encephalitis, Japanese encephalitis, and Calovo. Michalovce virus was found to be closely related to Sindbis virus in the HI test (Table 1). Additional serological comparison was conducted by complement-fixation test. The results shown in Table 2 indicate that Michalovce strain is indistinguishable from Sindbis virus. A difference between Michalovce strain and Sindbis virus was observed on a kinetic performance on the HI test.

To obtain more information about the pathogenicity of virus, experimental infection of hamsters with Sindbis virus was studied. Eighteen hamsters were inoculated ip with Sindbis virus (Michalovce strain) in its 4th suckling mice passage with $10^{6.5}$ LD_{50} of virus. The infected animals showed no signs of clinical illness. The virus was first detected in the blood 24 hours p.i. Soon after its appearance here, the virus was found in the brain 48 hours p.i. In the liver it was demonstrated on the 4th day p.i. All virus isolates were identified by the HI test. These results confirm the isolation of Sindbis virus from the blood and liver of a hamster captured on a field near Trhoviste village. After experimental infection of hamsters with Sindbis virus, HI antibody production was observed (Table 3).

The isolation of Sindbis virus from the blood and liver of a hamster along with the isolation of the same virus from the brain and liver of a warbler (Acrocephalus scirpaceus), confirmed the presence of this virus in Central Europe. Further studies on vectors of this virus may shed some light on the ecology of this virus in Central Europe. The question as to whether the virus is pathogenic for man in Slovakia remains open.

(M. Gresikova, M. Sekeyova and M. Batikova, presented at the IX Congress of Tropical Med. Malaria, Athens, October 14-21, 1973).

2. Haemagglutination-inhibiting antibodies to arboviruses in human populations in Greece.

In Greece an epidemic of dengue was observed in 1927-1928. Serological studies showed that the epidemic was caused by dengue 1 virus (Theiler et al. 1960, Pavlatos and Smith, 1964). Marinis in 1967, with sera collected 1961 to 1964, reported the same results and that a group B arbovirus was active in the south part of Greece. To elucidate the situation in the northern parts of Greece 300 human sera were collected in 1972, and were lyophilized and stored at -20°C until examined by HI test against antigens for the following viruses: Sindbis, tick-borne encephalitis (TBE), West Nile (WN), dengue types 1 and 2, and Tahyna. The sera were adsorbed by kaolin and goose erythrocytes before testing. The HI antigens were prepared by sucrose-acetone extraction of SM brain; 4 to 8 units of antigens were used in the tests.

Antibodies to Sindbis virus were found to be present in 0.7% of the sera and to Tahyna in 1% of the sera. There were a large number of sera with antibodies to West Nile virus (22.7%). Of the 300 sera, 9.6% and 10.6% reacted with dengue 1 and 2, respectively (Table 4). Titers to Sindbis and TBE were no higher than 1:20. Some of the TBE positives probably represented antigenic overlapping of group B antigen; WN titers generally were much higher than the TBE titers, up to 1:160 (Table 5).

In comparing the HI patterns obtained with the sera from humans born before 1928 and after 1928 it was found that 21.3% and/or 26.6% of those alive during the dengue epidemic had antibodies to dengue 1 and/or dengue 2, respectively, compared with 5.7% and/or 4.4% of those born later (Table 6). For West Nile virus it was found that the percentage of positive reactions was similar in both groups.

Table 7 gives the percentage of antibodies to various arboviruses in people from the different regions of Macedonia. The highest prevalence of antibodies to Sindbis virus was found in C. Macedonia. The highest prevalance of antibodies to group B antigens was found in E. Macedonia.

(J. Papapanagiotou, M. Batikova, M. Gresikova, M. Sekeyova: Haemagglutination-inhibiting antibodies to arboviruses in human population in Greece. Zbl. Bakt. Hyg., in press)

Table 1

Identification of Michalovce strain as Sindbis virus by haemagglutination - inhibition test.

Immune serum	HI titre with Michalovce antigen	HI titre with homologous antigens
WEE	40	320
EEE	40	5120
Semliki	20	2560
Sindbis	320	2560
TBE	0	5120
WN	0	2560
Y.F.	0	640
St. Louis	0	2560
Japanes encephalitis	0	320
Čalovo	0	320

Table 2

Identification of Michalovce strain as Sindbis virus by complement - fixation.

Antigen	Immune serum	
	Sindbis	Michalovce
Sindbis	128/512	16/64
Michalovce	64/256	8/64
Normal tissue	0	0

Table 3

Immune response of hamsters, experimentaly infected with Michalovce virus.

Hamster No	HI antibody titre			
	7	14	21	28 days
6	320	-		
7	80	-		
8	0	-		
9	40	20	-	
10	160	160	320	-
11	160	40	160	160
12	80	40	40	80
13	40	20	20	-
14	80	40	160	160
15	20	0	0	-
16	80	0	0	-
17	160	80	80	80
18	20	0	20	20

Table 4

HI antibodies to arboviruses in 300 human sera from Greece.

Group of arboviruses	Virus	Per cent of positive reactions
A	Sindbis	0,7
B	Tick - borne encephalitis	4,3
	West Nile	22,7
	Dengue type 1	9,6
	Dengue type 2	10,6
California	Ťahyňa	1,0

Table 5

HI antibody titres with arboviruses in human sera from Greece.

Age group /years/	No. of sera	HI antibody titres with antigens					
		Sindbis	Tick-borne encephalitis	West Nile	Dengue type 1	Dengue type 2	Tahyňa
0 - 4	282	0	0	20	0	0	0
	283	0	0	20	0	0	0
5 - 9	242	20	0	160	20	20	0
10 - 14	221	0	0	20	0	0	0
	226	0	0	20	20	0	0
	227	0	0	0	0	0	20
	228	0	0	20	0	0	0
	230	0	0	20	0	0	0
	233	0	0	20	0	0	0
	234	0	0	20	0	0	0
	235	0	0	20	0	0	0
	294	20	20	20	20	20	0
15 - 19	180	0	0	40	0	0	0
	181	0	0	20	20	0	0
	184	0	0	40	20	20	0
	190	0	0	40	0	0	0
	191	0	0	80	20	0	0
	192	0	0	20	0	0	0
	196	0	0	40	20	0	0
	199	0	0	80	0	0	0
20 - 24	201	0	0	20	0	0	0
	218	0	0	20	0	20	0

25 - 29	155	0
	158	0
	159	0
	161	0
	162	0
	163	0
	164	0
	165	0
	167	0
	169	0
30 - 34	127	0
	128	0
	134	0
	135	0
	140	0
	142	0
35 - 39	85	0
	87	0
	89	0
	91	0
	94	0
	96	0
	97	0
	99	0
	100	0
40 - 44	51	0
	54	0
	55	0
	61	0
	62	0
	68	0
	71	0

Table 5 (continued)

0	20	0	0	0
0	40	40	0	0
0	160	80	40	0
0	20	0	0	0
0	20	0	0	0
0	20	20	0	0
0	20	0	0	0
0	20	0	0	0
0	20	0	0	0
0	20	0	0	0
0	160	40	40	0
0	0	0	0	20
0	20	0	0	0
0	20	0	0	0
0	20	20	0	0
0	160	80	20	0
0	160	0	20	0
0	80	0	0	0
0	80	0	20	0
0	20	0	0	0
0	40	0	0	0
0	160	0	0	0
0	160	0	0	0
0	0	0	20	0
0	40	0	0	0
0	40	0	0	0
0	20	0	0	0
0	40	0	0	0
0	20	0	0	0
0	160	0	20	0
0	20	0	0	0
0	20	0	0	0

Table 5 (continued)

45 - 49	102	0	0	0	20	20	0
	111	0	0	40	20	20	0
	112	0	0	160	20	0	0
	120	0	0	20	0	0	20
	122	0	0	40	0	0	0
50 - 59	28	0	20	80	20	20	0
	27	0	0	0	0	20	0
	34	0	20	80	20	40	0
	35	0	20	80	40	40	0
	37	0	20	0	20	20	0
	40	0	0	80	40	20	0
	45	0	0	0	20	20	0
	47	0	20	20	0	20	0
Over 60	1	0	20	20	20	20	0
	3	0	20	160	20	80	0
	5	0	20	40	40	40	0
	7	0	20	160	40	40	0
	9	0	20	40	20	40	0
	11	0	20	20	20	20	0
	12	0	0	0	0	20	0
	14	0	20	40	20	40	0
	15	0	0	0	0	20	0
	16	0	0	20	0	20	0
	25	0	0	0	0	20	0

Table 6

Haemagglutination-inhibiting antibodies to arboviruses in human population born before and after 1928.

Antigen	Per cent of positive reactions	
	Born up to 1928	Born after 1928
Sindbis	0	0,9
Tick-borne encephalitis	16,0	0,4
West Nile	22,6	21,7
Dengue type 1	21,3	5,7
Dengue type 2	26,6	4,7
Ťahyňa	1,3	0,9

Table 7

Haemagglutination - inhibiting antibodies to arboviruses in different regions of Greece.

Region	No of serum samples	% of positive reactions with antigens					
		Sindbis	Tick-borne encephalitis	West Nile	Dengue type 1	Dengue type 2	Ťahyňa
C. Macedonia	135	1,4	2,2	19,2	6,6	6,6	0,7
E. Macedonia	66	0	12,1	26,3	18,1	19,7	3,0
W. Thracc	96	0	2,0	22,8	7,2	8,3	1,0

REPORT FROM THE ARBOVIRUS UNIT, VIRUS DEPARTMENT
INSTITUTO SUPERIORE DE SANITA, ROME, ITALY

Studies on tick-borne viruses

Surveillance and research activities have continued in some Italian regions during 1972. Ticks were collected in the spring either on vegetation or on vertebrates.

In Table 1 are summarized the number of ticks collected and processed for virus isolation according to species and stages. Seven virus strains were isolated from nymphs and females of Ixodes ricinus. One out of the five strains from females was isolated from samples collected in central Italy (Siena province). The other strains were isolated in northern Italy (Gorizia province). Four out of seven strains were successfully reisolated.

Antigens were extracted from all virus isolates and antiserum was prepared against the prototype (ISS.IR.560), which is the virus strain isolated in Siena province. Cross CF test demonstrated that the seven virus isolates were identical. The prototype strain, treated by sodium deoxycholate, showed a 3.9 logs LD_{50} infectivity reduction. Weanling mice were not susceptible to the intracerebral and intraperitoneal inoculation of the virus. Attempts to prepare a hemagglutinin were negative.

In Table 2 are reported the results of cross CF tests performed with some other tick-borne viruses. According to the CF test the strain ISS.IR.560 seems to be related to Tribec virus, but not to TBE, BHA and UUK viruses. The final identification was performed by Dr. M. Gresikova at the WHO Regional Reference Centre for Arboviruses, Bratislava, Czechoslovakia.

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

Table 1 - Number of ticks examined for virus and number of isolates from ticks collected in 1972 in northern and central Italy -

Species	Stage	Number of specimens	Number of isolations
Ixodes ricinus	Nymphs	2,713	2 *
	Males	247	0
	Females	339	5 *
Haemaphisalis concinna	Nymphs	8	0
	Males	124	0
	Females	9	0
Dermacentor marginatus	Nymphs	-	-
	Males	7	0
	Females	5	0
Rhipicephalus sanguineus	Nymphs	-	-
	Males	30	0
	Females	6	0
Rhipicephalus bursa	Nymphs	-	-
	Males	5	0
	Females	3	0
Hyalomma marginatum	Nymphs	8	0
	Males	322	0
	Females	113	0
Total		3,939	7 *

* Tribec virus

Table 2 - Results of CF test

Antigens	MIAF or immuneserum		
	ISS. IR. 560	TBE	Bhanja
ISS. IR. 560	128/64 +	< 8	< 8
Tribec	64/128 ++	n. d.	n. d.
TBE	< 8	32/32	n. d.
Bhanja	< 8	n. d.	64/64
Uukuniemi	< 8 ++	n. d.	n. d.

\+ serum titer/antigen titer

++ performed by Dr. M. Gresikova at the WHO Regional Reference Centre for Arboviruses, Bratislava, Czechoslovakia.

n. d. = not done

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUTE OF HYGIENE AND TROPICAL MEDICINE
LISBON, PORTUGAL

Serological survey for antibodies against arboviruses in Angola

In collaboration with the Institute for Public Health of Angola (IPSPA), Nova Lisboa, and the Institute for Scientific Research of Angola, (IICA), our laboratory has undertaken a large serological survey for antibodies against arboviruses in the human population of Angola.

Several serological surveys for antibodies against yellow fever virus have been done in Angola using the mouse neutralization test (1934, 1954, 1955, 1962) in order to define the possible areas where this virus was present. However, the first information about the geographical distribution of other arthropod-borne viruses of Angola was obtained only after the survey carried out by Kokernot and co-workers in 1960.

The epidemics of yellow fever and chikungunya in Luanda in 1971 (Bull. WHO, 49, 31-35 and 37-40, 1973) stimulated interest in reevaluating the geographical distribution of arbovirus immunity in Angola. More information was needed before beginning studies that would lead to the isolation of arboviruses and determination of their public health importance in Angola.

Angola has an area of about 1,246,700 Km^2 (481,351 square miles) and the human population is about 5,673,000 (1970 census). The population density is about 4.5 inhabitants per Km^2 (11.8 inhabitants per square mile).

A total of 4,590 serum specimens from males and females between 3 and 20 years old were studied in this survey by the hemagglutination-inhibition (HI) test. This means that about 1 in every 1,200 inhabitants had been studied. The serum samples were collected during 1971-72 in 16 selected areas of Angola.

In each area about 300 sera were chosen, half collected from the urban population and half from the people living in the rural areas.

The following antigens were used: group A (Sindbis, chikungunya, Semliki); group B (yellow fever, Zika, West Nile, Banzi, Ntaya, dengue 1, dengue 2); Bunyamwera; group C (Marituba, Oriboca); Tahyna; Sicilian sandfly fever, and Rift Valley fever.

The hemagglutination-inhibition test was performed according to the technique of Clarke and Casals. Some of the sera with HI antibodies were later examined by the mouse neutralization test.

This survey is now finished and the results will be published very soon. Some of the results obtained can be seen in the Tables 1, 2, and 3.

(Armindo R. Filipe)

Table 1

Summary of the results with HI test obtained for different antigenic groups in the studied areas

	Zone	Total nº. of sera studied	Total of positive sera in each antigenic group						
			A	B	Bun	Cal	C	SFF	RVF
	North of Angola								
1	Cabinda	301	3	122	4	0	1	0	0
2	Maquela Zombo	253	10	73	0	0	1	0	0
3	Carmona	226	17	94	1	0	1	0	0
4	Catete	288	80	184	21	0	1	0	5
5	Malange	302	4	51	2	0	0	0	3
6	Henrique de Carvalho	272	3	11	0	0	0	0	0
	Total	1 642	117	535	28	0	4	0	8
	South of Angola								
7	Porto Amboim	303	2	19	0	0	0	0	0
8	Novo Redondo	300	1	12	0	0	0	0	0
9	Silva Porto	301	0	17	0	0	0	0	0
10	Huambo	298	20	48	0	0	0	0	0
11	Lobito	304	2	12	1	0	0	0	0
12	Benguela	289	2	9	2	0	2	0	0
13	Sá da Bandeira	243	1	12	0	0	0	0	0
14	Moçamedes	295	11	26	4	0	0	0	0
15	Cuanhama	462	3	5	1	0	1	0	0
16	Cuamato	153	0	2	1	0	3	0	0
	Total	2 948	42	162	9	0	6	0	0

Table 2

Summary of the results with the HI test for antigenic group

Antigenic group	North of Angola			South of Angola		
	Negative	positive		Negative	positive	
		total	%		total	%
A	1525	117/1642	7.1	2906	42/2948	1.4
B	1107	535/1642	32.6	2786	162/2948	5.5
Bunyamwera	1614	28/1642	1.7	2939	9/2948	0.3
California	1642	0/1642	0	2948	0/2948	0
C	1638	4/1642	0.3	2942	6/2948	0.2
Sandfly Fever	1642	0/1642	0	2948	0/2948	0
Rift Valley Fever	1634	8/1642	0.5	2948	0/2948	0

Table 3

Results of hemagglutination-inhibition (HI) and Neutralization (N) test with group A viruses (Chikungunya and Semliki Forest)(*) and Bunyamwera virus on sera from children and teenagers of Catete.

Zone	Male - Female	age group	total of studied sera	Antigen - Virus: CHIK HI	CHIK N	SEM HI	SEM N	BUN HI	BUN N
Catete (urban)	M	9-10	26	7(**)	2/2(***)	4	1/2	8	6/8
		11-12	47	10	2/2	2	2/2	1	1/1
		13-14	22	8	2/2	0	-	0	-
	F	9-11	26	6	-	2	-	0	-
		11-12	18	8	-	1	-	1	1/1
		13-14	7	3	-	0	-	1	1/1
Total	-	-	146	42	6/6	9	3/4	11	9/11
Catete (rural)	M	9-10	31	9	4/6	0	-	5	3/5
		11-12	35	9	3/3	0	-	0	-
		13-14	15	4	1/1	1	1/1	0	-
	F	9-10	26	1	1/1	0	-	5	4/5
		11-12	16	3	2/3	2	-	0	-
		13-14	12	3	2/2	1	-	0	-
		⩾ 15	7	2	-	0	-	0	-
Total	-	-	142	31	13/16	4	1/1	10	7/10

(*) No sera with antibodies against sindbis virus were found

(**) No. of positive sera by HI test

(***) No. of positive sera /total tested

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

Virological Studies

1.1 Human Blood Samples

119 blood specimens collected from humans with fever in Kedougou, Bandia and Dakar were processed for virus isolation.

Two isolates were made from laboratory workers :

Koutango virus was isolated from the blood of an entomologist working on transmission experiments. Clinical picture was as follows : sudden onset with fever (101° F), headache, stiffness, retroorbital pain. On the 3rd day, a maculo-papular rash developed. From the blood taken on the 2nd day after the onset, Koutango virus was isolated. Serological studies showed a broad group B conversion : the patient had been immunized against yellow fever in the past. There is some evidence that contamination occurred by aerosol.

Wesselsbron virus was isolated from the blood of a laboratory technician girl who has prepared a SA antigen 5 days before. She had fever (103° F) headache, joint pain, but no rash. She completely recovered after 3 days. Wesselsbron virus was isolated in suckling mice and by plaquing in cell culture (PS cell line).

Strain SH 17783, isolated from the blood of an entomologist working in the field has been identified as Zika virus. Cultured kidney cells from infected suckling mice yielded Zika virus even after 7 serial cultures.

1.2 Wild Vertebrates Samples

76 specimens were processed for virus isolation without success.

1.3 Arthropods

1.3.1. Mosquitoes - 3590 mosquitoes were processed in 582 pools. Seven isolates were identified to Zika virus : 6 from Aedes of the furcifer - taylori group, 1 from Aedes luteocephalus, all being collected in Kedougou.

1.3.2. Ticks - 24 ticks were collected. No virus was isolated. Extensive work on ticks has been done in the past showing that cattle ticks are heavily infected. Numerous strains of Jos, Congo, Dugbe and Bhanja virus were isolated.

Serological Studies

2.1 Human sera

2.1.1. Senegal - Serological survey along the Senegal River (Department of Dagana and Matam) showed a high level of arboviruses activity especially group B viruses : more than 80 % had broad group B reaction. In the group A, chikungunya (45 %) and Sindbis (13 %) seemed to be active too.

2.1.2. Chad - 201 sera collected in Chad (Moyen Chari) were examined for HI, CF and neutralizing antibodies (Plaques reduction test in microplates) 125 concerned children, 76 adults.
74 % of children had HI antibodies, 10 % CF antibodies and 54 % neutralizing antibodies for YF virus. All the adults had HI antibodies for YF virus 17 % had CF antibodies and 96 % neutralizing antibodies. These results point out a high group B viruses activity in Chad.

2.2 Wild Vertebrates sera

100 sera from bats (Pterofus rufus) caught in Madagascar have been studied for HI arbovirus antibodies : 12.5 % were Sindbis positive, 12.5 % Wesselbron positive and 9.4 % West Nile positive. 4 (4.2 %) had antibodies for yellow fever virus but they were WN positive too. It may be recalled that 2 strains of virus isolated from this type of bats by Dr Coulanges in Madagascar, have been identified as Dakar - bat virus.

(Ch. Jan, G. Le Gonidec and Y. Robin of the Institut Pasteur ; J. Coz and M. Cornet of the ORSTOM, Dakar)

REPORT FROM THE VIRUS RESEARCH LABORATORY
UNIVERSITY OF IBADAN, IBADAN
NIGERIA

Chikungunya outbreak in Ibadan

In Ibadan, late in May 1974, one isolate of chikungunya virus was made from heparinized blood of a 16-month-old female reporting with fever (103°F) at the Children's Outpatient Clinic of the University College Hospital, Ibadan. The further isolation of two more strains of Chikungunya virus between 8th and 17th June 1974 from children at the Children's Outpatient, led to a more intensive surveillance for the virus. Two centers were used for this surveillance. Heparinized blood samples were collected from all children with temperatures of 100°F and above.

A total of 500 samples have so far been collected and 9 isolations provisionally identified as Chikungunya virus have been made. Follow-up studies on the patients and a general serological survey in Ibadan are on.

It is interesting to note that chikungunya fever outbreaks were previously reported in Ibadan in 1964 and 1969. It does appear to follow a five-year cycle.

(Akinyele Fabiyi)

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUT PASTEUR AND ORSTOM
YAOUNDE, UNITED REPUBLIC OF CAMEROUN

This report summarizes our results for the year 1973 and the period running from January 1 to June 30 1974. Investigations have been actively continued on arboviruses with special reference to yellow fever.

Since 1970 four cases of jungle yellow fever have been confirmed by histopathology from two "departments" in Cameroon. Three of these cases occurred near the town of Ayos (Nyong and Mfoumou department) a tropical rain forest, and one case in the vicinity of Ndop in the northern part of the Western Cameroon, a mountain-rain forest.

1. Virological studies

1.1. Human blood samples

25 human blood specimens have been collected from febrile patients with a rash, and two other blood specimens from men dead with a hepato-nephritis syndrome. No virus has been isolated from these materials.

1.2. Wild vertebrates samples from the Ayos surroundings

328 specimens were processed for virus isolation. Three strains were isolated from these materials: YV 821 isolated from Crocidura species; YV 992 isolated from Mus situlosus; YV 694 a group B virus from Cercopithecus nictitans. These three strains are still under identification. The first two (YV 821 and YV 992) do not show any relationship to reference materials maintained in the regional reference laboratory in Dakar and have been sent to YARU for further study.

1.3. Arthropods

1.3.1. Mosquitoes collected in the Ayos surroundings

309 pools of mosquitoes were inoculated into suckling mice. One strain of a subtype of Sindbis (Y 251) was isolated from Culex telesiella.

1.3.2. Ticks

Ticks were collected at the Yaounde abattoirs and processed in 157 pools. Two strains of Dugbe were isolated from Amblyoma variegatum. Three strains of C.H.F. - Congo were isolated, two from Amblyoma variegatum and another from Boophilus decoloratus. A strain of a subtype of Sindbis (Y 251) was isolated from Amblyoma variegatum.

2. Serological studies

119 human sera from Ayos surroundings were collected after fatal cases of YF confirmed by histopathology at the Pasteur Institute in Yaounde. Only

children's sera can be usefully interpreted (77 sera): 69/77 (89%) have no antibody against the group B viruses of our collection; 2/77 (2.5%) have antibodies against Zika. HI and N antibodies to YF only were detected in the sera of two children. HI, CF and N antibodies to YF only were detected in the sera of two children. HI, CF and N antibodies to YF were detected in the sera of three children. Thus, apparently, 3.8% of these children (unvaccinated) have had a recent exposure to YF virus.

(G. Le Gonidec)

REPORT FROM THE VIROLOGICAL SECTION OF THE DUTCH MEDICAL RESEARCH CENTRE/NATIONAL PUBLIC HEALTH LABORATORY SERVICES OF KENYA AT NAIROBI.

Arbovirus isolation work in Kenya up till ultima 1971 was reported in "Transactions Roy. Soc. Trop. Med. Hyg.", 1974, V.68, p.114; work on Aedes(Aedimorphus) dentatus in relation to its possible role as a vector of yellow fever virus in "Bull. Ent. Research," 1973, V.62 p.597.

During one week in May 1972 and 10 days in may 1973 mosquitoes were collected on human baits on the shore of Lake Naivasha in Central Kenya. A total of 9817 mosquitoes was collected. The species best represented in the collections, in order of the numbers collected were: M.uniformis, M.africana, Ae.dentatus group, C.rubinotus, C.nakuruensis, An.coustani and Ae.cumminsi. Of other species less than one hundred specimens came to bite. From a pool of 3 Ae.cumminsi and a pool of 12 C.rubinotus virus was isolated.

In the nineteen-forties staff members of the Rockefeller Yellow Fever Laboratory at Entebbe found neutralizing antibodies in primates in the coastal strip of Kenya, especially in bush babies. We collected mosquitoes on human baits in an area around Msambweni, a coastal village 50 km south of Mombasa. The collections were done on three occasions: June - July 1972, November 1972 and November-December 1973. In November 1972 part of the collections were done inside a forest. On all three occasions blood was collected from patients with fever attending a clinic at Msambweni.

In November and December 1973 the collections were extended to Mrima, a village 50 km to the South of Msambweni. The area around Mrima is more forested than the area of Msambweni. Several species of non-human primates, including Colobus, baboon and bush babies are well represented. The area looks like one that could favour yellow fever virus circulation. Ae.simpsoni is present and it is known that at the coastal strip, this species is not reluctant to bite humans. However, Ae.africanus has never been reported from the coast.

A total of 20697 mosquitoes were collected. The species best represented in the collections, in order of the numbers collected, were: M.uniformis , An.coustani group, An.funestus, M.africana, C.thalassius-tritaeniorhynctus group, An.gambiae, C.antennatus, Ae.pembaensis and C.sitiens. Of other species less than one hundred came to bite. Almost ninety percent of the An.funestus and 35 percent of the An.gambiae were collected in somewhat open places in the forest. Only 4 specimens of Ae.simpsoni were collected near Mrima and one specimen of Ae.aegypti near Msambweni. From two pools of M.uniformis, one pool of M.africana and one pool of An.funestus virus was isolated. The An.funestus had been collected in the forest. No virus was isolated from 255 sera taken from patients with fever.

Sera from 7 Colobus monkeys, 2 vervets, 1 blue monkey, 1 baboon, and 6 bush babies (4 Galago crassicaudatus and 2 Galago senegalensis), collected near Mrima were negative for yellow fever antibodies in the H.I. test. From organs (spleen, liver, kidney, brain) no virus was isolated.

Sera collected in 1971 from 115 school children in a village not far from Mrima, where 4 adults had died of an acute illness with jaundice were tested in the East African Virus Research Institute at Entebbe. All were negative for yellow fever antibodies.

The only case in Kenya accepted by most experts to have been a case of yellow fever, contracted the infection probably in or near Langata - Ngong Forest, not far from Nairobi. In July-August 1973 and March-April 1974 a total of 2916 mosquitoes were collected on human baits in or at the border of the forest. The species best represented in the collections, in order of numbers collected were Ae.dentatus, C.fatigans, C.zombaensis, Ae.cumminsi, Ae.deboeri and An.coustani. Only one Ae.aegypti came to bite. From a pool of 5 Ae.cumminsi virus was isolated.

On several occasions in 1973 a total of 494 ticks were collected from cattle in a slaughter house 20 km from Nairobi. No virus was isolated from tick pools.

The virus strains isolated from mosquitoes are being identified at the Pasteur Institute at Dakar.

Nairobi, July 1974.

D. METSELAAR.

REPORT FROM ARBOVIRUS RESEARCH UNIT,

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG

An epidemic of West Nile fever in South Africa, Feb.-May 1974

Following exceptionally heavy rains early in 1974 the largest epidemic of WN ever observed in S. Africa occurred in the arid Karoo region where rainfalls of 40 ins. were recorded in an area averàging 10-15 ins. per annum. WN is enzootic over the inland plateau region of South Africa, including the Karoo, where Culex univittatus is believed to be the enzootic vector as well as that of Sindbis (SIN) virus. In this area sporadic infection of man by both viruses is an annual summer occurrence but a large epidemic by either virus has never before been observed. In the present outbreak in which at least about 30,000 cases probably occurred, most infection was urban, perhaps partly because the rural areas are sparsely populated and partly because urbanization with the associated small agricultural holdings have favoured the vector and avian host. Numerous cases were reported from the country towns of Williston, Pofadder, Laingsburg, Beaufort West, Kakamas, Prieska, Brandvlei, Keimos and Upington involving an area of some 50,000 square miles. Upington was particularly severely affected with about 2/3rds of its 30,000 population infected.

Infection apparently started early in February, peaked in mid-March, and continued until May. For various reasons it was not until April that field observations at Upington were possible and unfortunately these were of short duration because of the onset of cold weather. In these studies we pursued four objectives, viz.,

diagnosis; identification of the epidemic vector; human attack rate in Upington; post-epidemic immune rate in wild birds.

Diagnosis

In April 58 bloods were collected from sick or recently recovered human beings. Forty-three were regarded as possibly acute phase sera and inoculated into infant mice. Six strains of WN virus were isolated, representing a 20% isolation rate if only those sera free of WN antibody are considered. All sera were tested for antibodies to SIN, chikungunya, Banzi, Wesselsbron, WN, dengues 1 and 2, yellow fever, Germiston, Bunyamwera and Rift Valley fever viruses. Twenty-four sera reacted with WN, 16 of them at high titre. Seventeen sera reacted with SIN, 6 at high titre. Other reactions were negligible or clearly due to overlap. Convalescent phase sera were obtained from 4 of the patients yielding WN virus and antibody tests on these revealed that each had developed WN antibodies during convalescence. A diagnosis of WN was made with the possibility of some SIN infections, and this agreed with the isolations then being made from mosquitoes.

Epidemic vector

Because of the sporadic nature of human infection in South Africa it has been difficult to identify the main vector infecting man. Initially we tended to disregard univittatus because of its low feeding rate on man and concluded that Culex theileri was mainly responsible as this mosquito feeds readily on man and fairly well on birds. (Because of low viraemia levels in man a man-mosquito-man cycle is probably unimportant). However, the low isolation rate recorded from theileri over many years combined with its poor vector

capability had more recently led us to conclude that univittatus was the real culprit and it seems this viewpoint has been vindicated by recent observations in Upington.

During April, among other mosquito species, 1325 univittatus and 4889 theileri were collected in Upington. Tested in 52 and 53 pools, respectively, the univittatus yielded 33 strains of WN and 8 of SIN and the theileri 4 strains each of WN and SIN. In terms of infection rates per 1000 mosquitoes these isolations represent rates as follows:-

	WN	SIN
univittatus	38,99	6,48
theileri	0,83	0,83

Considering these infection rates and the relative known transmitting abilities of univittatus and theileri it was concluded that only univittatus could have been responsible for the majority of the 20,000 cases in Upington, even if allowance be made for a 20-fold greater feeding rate by theileri on man. On the same basis it seemed that univittatus probably caused $\pm$ 2,500 cases of SIN infection in Upington.

Attack rate in man in Upington

Six weeks after the conclusion of the epidemic 282 sera were obtained from randomly selected donors in Upington. These were tested for HI and N antibodies to WN, SIN and other arboviruses. If only those sera positive in both tests and in which the HI titre was 1/80 or higher are considered, 56 sera (20%) gave evidence of recent

SIN infection and 180 sera (66%) of recent WN infection. These immune rates are in accord with the number of cases observed. From previous antibody surveys in this area we know that immune rates before the epidemic were unlikely to be higher than 5%.

Immune rate in wild birds

For such a large epidemic to develop and be maintained it seems essential that avian infection would be at an abnormally high rate since there can be no significant feed-back of virus from man to a vector. With this aspect in mind 346 bloods were collected from wild birds in Upington 6 weeks after the epidemic and these are at present being tested for antibodies to SIN and WN. Tests so far indicate that immune rates will indeed be higher than the 5-10% we have previously observed in South Africa under enzootic conditions.

(B. M. McIntosh)