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Progress Report

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Title	:	Ecology of Dengue and Other Arboviruses of Malaysia
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Abstract

The program objectives are to determine the roles that arboviruses play in producing infection and/or disease in man, with special reference to their ecology and the clarification of the non-urban cycles of dengue. The survey to date has investigated a great variety of areas of differing ecology throughout Malaysia. Improved and intensified methods of collection have allowed sampling of great numbers of mosquitoes for virus isolation. Predominant species, species composition and habits of mosquitoes in various areas have been elucidated. Extensive collection of animals and human sera has provided information on the incidence and distribution of arbovirus infection in man and animals, while the diagnostic service has provided routine surveillance of arbovirus disease.

Additional serological evidence strongly supporting a jungle cycle for dengue has been obtained in wild monkeys and forest-dwelling aborigines, while no evidence has been found to demonstrate significant involvement of any other animals. Bats show evidence of group B infection, but this is probably not due to dengue virus.

The discovery of Zika virus, previously known in Africa only, in Aedes aegypti and serological evidence of Zika infection in man and wild monkeys is significant.

A new arbovirus, Seletar, is described and a large number of unidentified virus isolates from mosquitoes, ticks, and sentinel animals are being investigated.

A serological survey of residents of Djakarta, Indonesia, the first to be done, revealed the occurrence of dengue and Japanese encephalitis and possibly chikungunya infection.

A study of the Manila hemorrhagic fever epidemic of 1966 has resulted in the isolation of numerous strains of dengue and has revealed the probable presence of Zika virus infection.

It is anticipated that the projected intensive-phase studies of dengue ecology will be initiated shortly in rural and jungle sites.

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Arbovirus Research Unit

The Ecology of Dengue and Other Arboviruses of Malaysia*

Introduction

Background:

Dengue has traditionally been regarded as a mild disease. The serious nature of the disease as it was recorded in the Brisbane epidemic over 60 years ago and in the great Greek epidemic almost 40 years ago had received little attention. During the past decade, the medical and scientific world has been surprised by the frightening outbreaks of hemorrhagic dengue that occurred in the Philippines, Thailand, Malaysia, and most recently, India. There are now no doubts about the serious nature of this infection. Recurring outbreaks in Bangkok alone have resulted in the death of thousands of children since 1958. The clinical picture has been well-documented: fever with or without rash, not dissimilar to ordinary dengue, followed by sudden collapse, a hemorrhagic diathesis, coma, and often death.

The emergence of this serious form of dengue in southeastern Asia has probably become one of the most important virus problems of this decade. The future development of this disease is not only unknown and unpredictable at the present stage of our knowledge, but is also a cause for grave concern. There is no specific therapy available nor have the experimental vaccines available yet been adequately tested

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or proved. We have had essentially no information on possible endemic or enzootic cycles nor do we know anything about the genesis of the recent epidemics. The urgent need for extensive laboratory and especially field studies is clear and long term studies in the home areas of the disease are strongly indicated.

In 1962, we initiated a study of the ecology of dengue in Malaysia, based on the suspicion that the virus is active in forest areas away from normal human activity and in the absence of Aedes aegypti mosquitoes. From 1962 to 1964, more than 2200 animals representing over 50 species of 28 genera were collected principally by live trapping in urban, rural, and forest areas of differing ecology. Most of the common mammals were well-represented in the collections, which included reptiles, birds, insectivores, rodents, bats, primates, and domestic species. The only groups that were not well-represented in terms of numbers collected were the bats and birds and we are presently attempting to fill in this gap. In addition, over 25,000 live adult mosquitoes were taken. These included more than 69 species of eight genera from several areas of differing ecology.

Virus isolation attempts in suckling mice and employing the dengue challenge technique were made from the mosquitoes, and sera and tissues of the other animals. No dengue virus was isolated, from any of the material, although other viruses including some arboviruses were. During the same period, dengue viruses were isolated from sera of dengue patients in Malaysia.

The animal sera were tested for the presence of hemagglutination-inhibiting (HI) antibodies for thirteen arboviruses. Eight units of sucrose-acetone extracted antigen were used and sera were treated with acetone. The antigens included all the group B arboviruses known to occur in Malaysia and Singapore (Dengues 1, 2, 3, 4, Japanese encephalitis, tembusu, and Langat) as well as Sindbis, chikungunya, getah, and eastern equine encephalitis in group A, marituba in group C, and batai in the bunyamwera group. A serum titer of 1:20 or greater was considered positive.

Although confusing HI antibody patterns may result from repeated infection by the same virus, subsequent infection by a related virus, or by the passage of time after infection, one may recognize a meaningful and significant pattern when sera are tested against several viruses of the same group. Such a pattern emerged in our survey. Of all the animal species tested, only the monkeys and domestic animals demonstrated significant group B infection. Among these group B positive domestic animals (pigs, horses, cattle, goats), Japanese encephalitis HI antibody was present with little or no evidence of other group B antibody except among the pigs. Of 50 pigs collected in Penang while dengue disease was occurring in the human population, 82% had JE HI antibody and 58% had dengue HI antibody. The JE antibody was highest in titer except in two animals where it was equal to the dengue titer. In neutralization tests, only 20% of the animals tested had dengue antibody in low titer. It was reasonable to conclude that the majority, if not all, of the group B antibody in the pigs was due to infection with Japanese encephalitis virus.

Of 238 monkey sera tested by HI, the majority represented Macaca irus, the long-tailed macaque, and Presbytis cristatus, the silvered leaf monkey, two of the most common species in Malaya. For all species, the percentage of positives for the four dengue types ranged from 52 to 62%. The percentage for Japanese encephalitis was 56%, for Langat 45%, and for tembusu 32%. In almost all cases, HI titers were highest for the dengues and in some cases the dengue titers were high enough ($\geq$ 1:1280) to suggest current or very recent infection.

Of all the group B HI positive monkey sera, none had significant neutralizing antibody ($\geq$ 2.0 log neutralization index) for tembusu or langat viruses. Only two sera demonstrated low-titered neutralizing antibody for Japanese encephalitis virus. In contrast, 85% of all the HI group B positive sera had significant neutralizing antibody for dengue type 2 virus. Only a small sample (26) of the

HI group B positives have been tested so far for neutralizing antibody for all four dengue types. Of these, all had significant neutralizing antibody for one or more types. On the basis of these results, it was quite reasonable to conclude that the majority of the group B antibody demonstrated was due to infection by one or more of the dengue viruses.

Additional serological evidence of dengue virus activity was obtained from a series of 215 sera taken from forest-living aborigines in Malaysia in 1965 and 1966. These sera, which were tested by HI only, showed minimal group A activity and broad group B activity. Of the 49 sera taken in 1965, all were positive for one or more group B viruses, while of 166 sera from 1966 only eleven showed no group B activity. The majority of the negatives were from children less than ten years of age. On the basis of highest serum titer obtained, dengue type 3 was the most active virus, while dengue types 2, 4, and 1 appeared less active or less recent in activity. Again on the basis of level of serum titer obtained, activity of Japanese encephalitis, tembusu, and Langat viruses appeared to be considerably less than for the dengues. The overall pattern suggests that one or more of the dengue viruses was responsible for the majority of the group B antibodies demonstrated. In comparison with 151 normal human sera taken in an urban area, Penang, in 1964, we found less group B and/or dengue activity than among the forest aborigines, although dengue virus, again, appeared to be responsible for the majority of the group B antibody demonstrated.

In conclusion, we felt that the serological findings suggested almost conclusively that a cycle of dengue virus, involving monkeys and unknown forest mosquitoes has been active in the forests of Malaysia. Rural dengue, which is mild and often unrecognized is probably maintained by Aedes albopictus transmission among susceptible humans with occasional re-introductions from the forest. Aedes albopictus undoubtedly transmits dengue in suburban and urban areas also, where

it occurs, but A. aegypti is responsible for epidemic dengue, which is often severe in nature. Although we feel that the serological results are convincing, we recognize that the final proof lies in demonstrating that the virus can be isolated in these areas from forest animals and mosquitoes. Thus, one of the principal objectives of the current study is to isolate dengue virus from sentinel monkeys, wild monkeys, and mosquitoes in forests of Malaysia.

The demonstration of a jungle cycle for dengue will be of considerable importance in helping to explain the appearance of epidemics, in establishing effective surveillance methods for control activities, and in the clarification of the dengue virus complex.

The recognized arboviruses presently known to occur in Malaysia, as demonstrated by recovery of virus in nature, include three members of serogroup A, 8 members of group B, one of the Bunyamwera group, two of the Ketapang group, one closely related to Wad Medani virus, and one ungrouped virus (Table I). In addition, several viruses have been recently isolated by us and by Smith in Sarawak from man, other vertebrates, and arthropods. Some of these may represent new viruses. Of the identified viruses, only Japanese encephalitis virus and the dengue viruses are certainly known to produce overt disease in man. However, there is serological evidence indicating human infection with another five: Sindbis, Getah, Bebaru, Ketapang, and Bakau. There has been no evidence of natural human infection with Tembusu, Batai, Langat, and Wad Medani. Experimental human disease, however, has been produced with Langat virus.

Many of the more than 150 recognized arboviruses of the world are known only by one or more isolations in nature and in some cases by limited serological surveys. Except for Japanese encephalitis and dengue, this more or less applies to the arboviruses of Malaysia. The majority have been isolated from arthropods in jungle areas and/or in urban areas.

We have had essentially no information on the cycles or distribution of these viruses. It is likely that some or all of these are zoonoses and may be responsible for some of the fevers commonly diagnosed as PUO's (pyrexias of undetermined origin) in Malaysia. With changing conditions and relationships, they may be responsible for epidemics in the future. There is a clear need for field studies of the ecology of these viruses and the roles they may play in producing infection and/or disease in man.

Facilities and Personnel

The Arbovirus Research Unit is a continuing project of the UC ICMRT. Therefore, two facilities are being operated concurrently: the base laboratory in San Francisco (Hooper Foundation) and the field laboratory (from July 1965) in Kuala Lumpur (University of Malaya Faculty of Medicine). This arrangement allows the professional staff to alternate tours of duty without significant interruption of the research program. The base laboratory, in addition, provides services, such as preparation of antigens and antisera, for the field laboratory, thus freeing the staff in Malaysia from time-consuming laboratory procedures that can be done best in San Francisco at this time. The Malaysian laboratory is concerned primarily with field studies, virus isolation, preliminary characterization of virus isolates, serological screening, and diagnostic service; while the detailed characterization of viruses and serology are performed in San Francisco.

In addition to the personnel listed below, auxiliary services are provided by the general staffs of the Hooper Foundation in San Francisco and the Department of Bacteriology of the University of Malaya Faculty of Medicine in Kuala Lumpur.

Personnel

San Francisco

J. Ralph Audy, M.B., B.S., Ph.D.
Program Director, ICMRT

Albert Rudnick, Ph.D.(1)

Eulalia Venzon, M.D.(2)

Elene Dukellis(3) (From Aug., 1966)

Administrative Assistant

Margot Segal (10%)

Laboratory Technicians

Bruce O. Jang

Roberta Williams

Mac J. Faber (From Feb., 1967)

Kirk Hoffner (Part-time)

Douglas Pile (Part-time; from Jan., 1967)

Typist-Clerk

Lucille Valentine (75%)

(1) Senior investigator, Arbovirus Research Unit

(2) International Research Fellow, NIH

(3) Assistant Specialist in Virology

(4) In charge of investigations in Malaysia

Kuala Lumpur

T. J. Danaraj, F.R.C.P.E.
Dean, Faculty of Medicine

Nyven J. Marchette, Ph.D.(4)

Richard Garcia, Ph.D.

Duncan W. MacVean, D.V.M.

Laboratory Assistants

Meer John Jai Hind Jeffery

Santha Kumari

Harry Lee Chong Seng (From July, 1966)

Annie Keong Sneo Cha (From June, 1966)

Teh Siew Khim (From March, 1966)

Collectors

Ahmad bin Abdul Hamid

Nagiah Vengitasamy

Peh Boon Seng (Temporary; March-May, 1966)

Ang Eng Chin (Temporary; March-May, 1966)

Driver - Collectors

Chan Wah San

Tee Kem Tho

Musa bin Long (From May, 1967)

Laboratory Attendant

M. Loganathan (from June, 1966)

Animal Attendant

Ibrahim bin Yaacob

Typist-Clerk

Connie Wong Hup Mooi

Objectives

The specific objectives of the current program are:

1) Continuation of the current survey in Malaysia in an attempt to delineate an area or areas of known dengue virus activity away from normal human activity.

2) Initiation of intensive ecological studies of urban and jungle areas of known dengue virus activity in an attempt to demonstrate the vectors and vertebrates, other than man and Aedes aegypti, involved in natural dengue cycles.

3) Studies of other arboviruses of Malaysia with particular reference to the roles they may play in producing infection and/or disease in man.

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We wish to acknowledge the generous assistance and cooperation received from Professor T. J. Danaraj, Dean of the University of Malaya Faculty of Medicine, and Dr. J. Ralph Audy, Director of the Hooper Foundation. We are also grateful to Dr. Ungku Omar, Director of the Institute for Medical Research, Kuala Lumpur, and his staff for their continued cooperation, and to Mr. Chong Sue Kheng, Director of the Veterinary Research Institute, Ipoh. We are especially grateful to the many physicians and hospital and public health personnel throughout Malaysia, who have given their time, interest, and effort unsparingly to provide information and materials for these studies.

Field Program - General

In addition to the permanent collecting stations at Ulu Lui (dipterocarp forest), Pacific Tin (fresh water peat swamp forest), and Telok Gong (mangrove swamp forest), numerous collecting trips have been made to a variety of habitats

throughout mainland Malaysia (Western Malaysia). These included most of the major urban centers and towns, coastal fishing villages, remote primary forest areas, miscellaneous mangrove swamp forests, agricultural and rural areas, forest aborigine villages, high mountain forest, rubber, coconut, and oil palm plantations, and a remote heronry in mangrove swamp. In addition, Dr. Garcia conducted a brief mosquito survey in Kuching, Sarawak in cooperation with Dr. C. E. Gordon Smith of the Microbiological Research Establishment, Porton, England.

Special cooperative arrangements have been made recently to obtain specimens in addition to our own collecting program. 1) Lord Medway, University of Malaysia ornithologist, is kindly providing filter paper bird blood samples from all birds collected in the Migratory Animal Pathological Survey (MAPS) in Malaysia. 2) Cooperative monkey collecting expeditions are being conducted with Dr. Morris Goodman's group from the Department of Anatomy, Wayne State University School of Medicine, Detroit. 3) Ticks are being obtained from a new tick-collecting program organized at the Institute for Medical Research in cooperation with Dr. Harry Hoogstraal of the U.S. Naval Medical Research Unit in Cairo.

Entomology

The entomological program was resumed in August 1965 with the arrival in Kuala Lumpur of Dr. Garcia. Prior to his arrival, Mr. Jeffery received ten months of intensive training in mosquito identification in the Department of Entomology of the Institute for Medical Research. He is now proficient in rapid and accurate identification of Malayan mosquitoes and is able to provide expert assistance to Dr. Garcia both in the laboratory and in the field.

From August 1965 through April 1967, mosquitoes have been collected from an extensive range of habitats and localities throughout Western Malaysia. The

majority of the female mosquitoes have been processed for virus isolation. Table 2 presents the total number of female mosquitoes collected and processed for virus isolation from areas of differing ecology. The area types listed are mostly well-defined ecologically with the exception of the rural and urban areas. These types were included as such, however, for ease of reporting. In a final analysis, it will be necessary to break down the rural and urban listings into several additional categories.

A variety of methods has been employed for collection of live adult mosquitoes. While many methods have been tried, the following have been the most productive.

1) Diurnal collection of resting mosquitoes in houses and other structures by mouth- and battery-operated aspirators.

2) Diurnal and nocturnal human-biting collections.

3) Domestic animal-biting collections.

4) Malaise traps. These are particularly useful in evening collections at domestic animal sheds. Traps are placed near the stalls to collect mosquitoes approaching and leaving the area.

5) Use of the "D-vac" vacuum insect net with dry ice. This method has proved particularly useful in mangrove swamp forests. Dry ice is placed at various sites. Periodically, each site is visited and mosquitoes found hovering near the dry ice are collected with the "D-vac" net by suction.

6) Small modified Magoon-type bait traps with monkeys, chickens, or dry ice, or a combination of animal and dry ice. This trap can be raised into the forest canopy and is large enough to hold two monkeys.

7) Aspiration of mosquitoes resting in crab-holes.

The collected mosquitoes include the majority of species known to occur in Malaysia. Use of new methods has provided very large collections of certain

mosquitoes, such as Uranotaenia and Aedes (Cancraedes), that were previously collected in relatively low numbers. The use of battery-operated aspirators has significantly increased the efficiency of collection of resting mosquitoes and mosquitoes in flight.

Collection of freshly-engorged blood samples from mosquitoes is continuing and special efforts are being made to obtain samples from those mosquitoes whose feeding habits are poorly known. These will be tested to determine host preferences.

Table 3 shows the mosquitoes collected and processed for virus isolation in mangrove swamp forest. [Male mosquitoes and female mosquitoes not processed for virus isolation are not included in this table or similar tables in this report]. The predominant species were Aedes (Cancraedes) spp., Uranotaenia lateralis, and Aedes butleri followed by Aedes amesii, Culex (Lophoceraomyia) spp., Anopheles baezai, and Aedes albopictus. The first two species listed were taken principally from crab-holes. Comparison of this table with the several following tables of mosquitoes will show a rather striking difference in predominant species and species composition in the various areas of differing ecology.

Table 4 shows the mosquitoes collected and processed for virus isolation in freshwater peat swamp forest. Mansonia annulata was the predominant species. A further analysis would show a difference in species composition and numbers between the fringe of the forest and the interior. Collections taken in the forest fringe consist of a greater variety of species and lower numbers of specimens, while the collections in the interior consist largely of Mansonia annulata. This difference between the forest fringe and interior can be seen for all types of areas investigated, but it is most striking in the freshwater peat swamp forest.

Table 5 shows the mosquitoes collected and processed for virus isolation in rubber estates. Aedes albopictus was the predominant species. The collection of

Culex pipiens quinquefasciatus (fatigans) represented house mosquitoes taken in nearby dwellings. This species does not normally occur in the rubber. It is interesting to note that in the rubber estates examined, no Aedes aegypti were recovered.

Table 6 presents the mosquitoes collected and processed for virus isolation in secondary forest and scrub areas. Aedes albopictus was the predominant species.

Table 7 presents the mosquitoes collected and processed for virus isolation in rural and disturbed areas. Culex gelidus and Mansonia uniformis were the predominant species taken. This category requires further breakdown for final analysis since the localities included are not as uniform as in other categories.

Table 8 shows the mosquitoes collected and processed for virus isolation in urban, town, village and adjacent areas of human activity. This category includes a variety of habitats affected by man and his activities. Culex gelidus and Culex tritaeniorhynchus were taken in the greatest numbers. The list may be modified, however, according to type of specific habitat as well as method of collection. For example, collections taken in human dwellings in urban areas consisted largely of Culex pipiens quinquefasciatus and Aedes aegypti. Daytime human biting collections taken outside dwellings consisted principally of Aedes albopictus. House collections in some coastal fishing villages included Culex sitiens as a principal species. Many such examples could be listed.

Table 9 shows the mosquitoes collected and processed for virus isolation in lowland dipterocarp forest. The predominant species taken were Armigeres (Leicesteria) spp. followed by Aedes albopictus and Culex pseudovishnui.

Table 10 shows the mosquitoes collected and processed for virus isolation in Gunong Benom, a primary rain forest area in central Malaysia. Survey of this

area was started recently and requires prolonged and difficult expeditions because of the terrain and its isolation. It was chosen because of its isolation and minimum human disturbance. Monkeys taken in this area have shown the presence of dengue antibody. The predominant mosquitoes were species of Aedes (Finlaya).

In addition to mosquitoes, ticks have been taken in number from a variety of domestic and wild animals for virus isolation attempts. These are presented in Table 11.

Other biting arthropods have been collected as time permits. A collection of tabanids now includes over twenty species. Table 12 shows the miscellaneous arthropods collected and processed for virus isolation.

Miscellaneous studies in relation to efficiency of mosquito collecting methods and mosquito ecology are being performed.

The D-Vac insect sweep net has proved especially useful for collection of mosquitoes in mangrove swamps. A comparative study with the D-vac net and dry ice placed on the ground as an attractant versus human biting collections showed striking increases in the number of specimens and number of species taken by D-Vac.

A comparative study of the use of small bait traps with monkeys, dry ice, and a combination of the two showed an apparent synergistic effect in mosquito attraction when both were used together. The mosquitoes were attracted in significantly greater numbers and there was also a significant increase in mosquito engorgement.

A study of mosquito populations in crab-holes in mangrove swamp has been made. Great numbers of Uranotaenia lateralis and Aedes (Cancraedes) spp. adults were taken from crab-holes by means of a battery-operated aspirator. Many of the specimens were freshly engorged.

A study of biting and ovipositing behaviour of Aedes albopictus in a rubber estate (Effingham Estate) has been made. This will be compared with similar studies on this species in a forest fringe habitat, where it is found in abundance also. The study has shown that Aedes albopictus will bite man from dawn to dusk with a peak in late morning. Observations indicate that the species primarily feeds at ground level in contrast to the canopy.

Oviposition was studied by the use of artificial bamboo containers placed at various elevations in the rubber trees. Some eggs were deposited as high as 25 ft. but the majority were found in containers closest to ground level. The efficiency of bait traps, baited with monkeys and/or dry ice for capturing A. albopictus was tested. A. albopictus will enter the bait traps, feed, and remain in the trap.

Bait trap studies in fresh-water swamp forest compared an empty trap with ones baited with monkeys, dry ice, and both monkey and dry ice. Four positional traps were rotated each day for four days and resulted in the capture of over 19,000 mosquitoes, with the combination bait being most successful. Similar studies were conducted at the forest fringe, where the number of mosquito species is greater than inside the swamps.

A bait trap study comparing number of monkeys used as bait in attracting mosquitoes showed an increase by monkey weight. It was concluded that several monkeys in a trap provide each monkey with a greater chance of infection by mosquito bite than a solitary monkey (as used on some of our sentinel monkey platforms).

Vertebrate Collections

Blood and/or tissue samples have been collected from a great variety of vertebrates in several areas of differing ecology throughout Western Malaysia.

Dr. Peter Ward of the University of Singapore Department of Zoology kindly provided us with bird bloods from Singapore and Dr. Jerry Theis (UC ICMRT) provided us with domestic animal sera from Singapore. Dr. J. Strauss of the U.S. Army Medical Research Unit supplied domestic animal serum specimens from various parts of Malaysia.

Table 13 lists the vertebrates collected in Malaysia and Singapore from August 1965 to April 1967 compared with those collected in the preliminary survey (1962-1964). It may be seen that bats and birds, which were not collected in number during the preliminary survey, are now being taken.

Sentinel Animal Program

Because of the high incidence of dengue-neutralizing antibodies demonstrated in wild monkeys in the preliminary survey, a special effort has been made to station sentinel monkeys in the forests to detect dengue activity. Young monkeys, obtained from a dealer, and shown to be free of arbovirus group A and B antibody are being used. An elevatable platform of wire mesh, with a shelter, self-feeding container and water trough was designed for one or two monkeys and has proved satisfactory. The platforms have been placed at various elevations from near ground level to the forest canopy. Small samples of blood have been taken twice weekly from each sentinel monkey for virus isolation attempts and once every four weeks to check for serological conversion.

Of 204 monkeys that were tested, 34 were selected and used as sentinels over the past 14 months. Some deaths have occurred from attacks by wild animals and others from undetermined causes. Several of the sentinels, however, have survived the full period. The sentinels were stationed routinely in mangrove swamp forest, fresh-water peat swamp forest, and dipterocarp forest. On a periodic basis, sentinels have been used in miscellaneous areas.

Sentinel chickens have also been used to a lesser extent in various collecting sites. These have been placed in the small modified Magoon-type bait trap.

None of the sentinel animals tested to date for HI antibodies to twelve arboviruses in groups A, B, and Bunyamwera have converted. However, four agents have been isolated from two monkeys stationed in dipterocarp forest, one agent from a monkey stationed in mangrove swamp forest, and one agent from a chicken at Gunong Benom. These are reported in a following section.

After the recent isolation and identification of Zika virus, the sentinel monkeys were checked for Zika antibody and one monkey, negative to all other group B agents tested, was found to have had Zika antibody throughout the period that it was used.

Normal Human Serum Survey

It is difficult to obtain normal human sera in Malaysia, especially from children, as opposed to sera from hospitalized patients. Nevertheless, an effort to obtain normal human serum samples from various parts of Malaysia was initiated during the past year in an attempt to assess the incidence and distribution of arbovirus infection in the population.

Through the outstanding cooperation of health authorities, physicians, and public health personnel, a series of over 1600 specimens was obtained through April, 1967. Table 14 lists the locations of serum samples collected. In addition, a series of normal human sera from residents of Djakarta, Indonesia have been studied in cooperation with Dr. H. T. Poey-Oey of the University of Indonesia Faculty of Medicine.

Diagnostic Service

A diagnostic service for arbovirus infections was established in the Kuala Lumpur laboratory in August, 1965. This was made possible through the kind cooperation of the Virology Division of the Institute for Medical Research, which was the only laboratory in the Federation providing this service. The Institute now refers to us all specimens submitted to them for arbovirus studies. In addition close contact is maintained with physicians and hospitals throughout Malaysia by personal visits, telephone calls, correspondence, lectures, and distribution of a newsletter describing our activities. In this manner, we believe the interest of the local clinicians in arbovirus disease has been stimulated and maintained and routine surveillance of arbovirus disease in the human population has been made possible.

In addition, a close liaison has been established with the Veterinary Research Institute in Ipoh and the same diagnostic service has been made available to them.

Acute and convalescent specimens have been received from many parts of Malaysia including Sabah and Sarawak. Clinical diagnoses range from pyrexias of undetermined origin to encephalitis and suspected hemorrhagic fever.

The number of specimens examined varied from about 25 to 75 per month. Results showed that Japanese encephalitis infections occur sporadically throughout the year. Occasional cases of dengue, some with minor hemorrhagic manifestations, have been detected sporadically. No dengue epidemics occurred in Malaysia during the past year even though epidemics were occurring in neighboring Thailand and Singapore. Special efforts were made to survey the border areas for evidence of epidemic activity with negative results.

One febrile case from Johore Bahru, possibly due to infection with chikungunya virus, was detected. Further tests will determine the validity of the results. If this infection is confirmed, it will represent the first known case of chikungunya in Malaysia.

One possible case of tembusu infection has been tentatively diagnosed.

Virus Isolation and Characterization

A total of 35 virus strains have been isolated in Malaysia during the current study (to April 1967). This brings the number of virus isolates being investigated in the San Francisco laboratory to 83. Table 15 lists the virus isolates, their source and locality, and identifications where completed.

Mosquito Isolates

Aedes aegypti

Five agents (SM-1, SM-3, SM-11, SM-12, and SM-14), previously isolated from Singapore, have all been confirmed as strains of dengue type 2.

Two strains of dengue (X-1 and X-34), previously isolated in Bangkok are still being held for final identification due to low priority in the laboratory schedule. These strains are also being studied in Dr. W. McD. Hammon's laboratory at the University of Pittsburgh.

P6-740 from Bentong has been identified as a strain of Zika virus in group B.

Zika virus was first isolated in 1947 from the blood of a sentinel monkey stationed in the Zika Forest of Uganda, East Africa. It was again isolated the following year from a pool of Aedes (Stegomyia) africanus collected from the

same forest. Subsequently a number of strains of the virus were recovered from A. (Stegomyia) africanus taken in and above the Uganda forest. One isolation has been made from a human suffering from a mild illness, but this was considered the result of a laboratory infection.

Although Zika virus has not previously been isolated from anywhere except Uganda, human serological surveys have suggested its presence in other parts of Africa, India, Malaya, Borneo, Thailand, North Vietnam and the Philippines.

The isolation of Zika virus reported here is, to our knowledge, the first from Malaya and Southeast Asia and the first from a species other than A. africanus. It was recovered from a pool of 29 A. aegypti collected in July 1966 from shop houses in the town of Bentong in west-central Malaya. The isolation was made in the Arbovirus Research Laboratory in Kuala Lumpur where known strains of Zika virus have never been kept. Re-isolation from the original mosquito suspension maintained at -60°C was successful when attempted four months later. Identification of the isolate was made in our San Francisco laboratory and has been referred to Dr. Jordi Casals at the Rockefeller arbovirus laboratory at Yale for confirmation.

This is the only isolation of Zika virus obtained from the inoculation into suckling mice of nearly 300 pools of 33,000 Aedes mosquitoes (including over 1200 A. aegypti and 4500 A. albopictus) collected from a variety of habitats in Malaya during the past two years. Over 200,000 other mosquitoes of many species have also been tested for virus isolation, but to date all have been negative for Zika virus. A large collection of serum samples from humans, domestic animals, monkeys and other vertebrates are now being tested to determine the prevalence of Zika antibody in different vertebrate species in Malaya. Preliminary results suggest the presence of Zika antibody in man and in wild monkeys. These

preliminary serological results, along with the location of Bentong in the central part of the country, suggests that Zika may be a zoonosis in Malaya with a jungle cycle among primates and unknown forest mosquitoes. It suggests that Zika may have a cycle similar to that proposed for dengue. Since Zika virus is much easier to work with than the dengue viruses, it is possible that further information on the distribution and ecology of Zika may be more readily obtainable and may provide clues to the jungle vectors of dengue.

The addition of Zika virus to the several other closely-related group B arboviruses in southeastern Asia will require re-evaluation of results of previous serological surveys, and possibly diagnostic studies, made in the area.

Table 16 presents a summary of a cross comparison of P6-740 and other closely-related group B viruses. Table 17 shows the results of neutralization tests in mice comparing P6-740 with Zika, West Nile, dengue 1, dengue 4, yellow fever (17-D), and Uganda S viruses.

Aedes albopictus

Strain SM-18, previously isolated in Singapore, has been re-confirmed as a strain of dengue type 2. This strain was the first dengue virus isolated from naturally-infected Aedes albopictus mosquitoes.

The isolation of E-24 virus from Kuala Lumpur is presently being reviewed. Its validity could not be ascertained by re-isolation. By HI test, its hyper-immune serum did not react with our complete range of arbovirus antigens (see Table 18).

Aedes sp.

P6-1346 was recently isolated from a pool of unidentified Aedes sp. collected in Kota Bharu, Kelantan. Attempts to identify it have not yet been made.

Culex fuscocephalus

P6-1368 from Tumpat, Kelantan is being processed for identification and will be compared with P-581 previously isolated from the same species in the Philippines.

Culex gelidus

The six isolates (P6-410, P6-412, P6-462, P6-578, P6-1458, and P7-211) from this vector species all behave similarly to Japanese encephalitis in mice. Three strains, P6-410, P6-462, and P6-578, have been identified as Japanese encephalitis in a comparison with closely-related group B viruses.

Culex sinensis

E-145 virus isolated in a forest area in Selangor has been identified as a strain of Sindbis virus.

E-140 virus has not been identified yet due to difficulty in adapting it, but resembles E-145 in its behavior in suckling mice.

Culex vishnui group

Four viruses (P6-553, P6-856, P6-1333, and P6-1462) have not been identified yet, but resemble Japanese encephalitis virus in their behavior in mice.

Culex sp.

E-210 virus was isolated in fresh-water peat swamp forest from an unidentified Culex species, which may be new to science. The virus has been identified as a strain of Bakau in the Ketapang group. Its isolation has been validated by successful re-isolation from the original mosquito suspension.

Table 18 shows the results of cross-neutralization tests in mice with Bakau and Ketapang viruses.

Mansonia uniformis

P5-350 from Puchong, an area of secondary forest, has not been identified yet. Its hyperimmune serum failed to react in an HI test with our complete range of arbovirus antigens. It has been successfully re-isolated.

Tick Isolates

Amblyomma testudinarium

P7-275 from Kelantan is a new isolation, which has not yet been processed for attempted identification.

Argas puisilus

Two strains, P6-1361 and P6-1362, were isolated from separate pools of the same collection taken from Scotophilus temmincki bat roosts in Koterah, Kelantan. Stock suspensions and immune sera are being prepared for attempts to identify them.

Boophilus microplus

SM-214 strain was isolated from a pool of B. microplus ticks collected from cattle in Singapore in 1961. Isolation was made by intracerebral inoculation of and brain passage in suckling mice. The virus was successfully re-isolated from the original tick suspension. P5-127 virus was isolated similarly from a pool of B. microplus ticks collected from cattle in Kepong, Malaysia in 1965.

Comparison of SM-214 with our complete range of arboviruses, prior to obtaining Wad Medani and IG-673, (see Table 19) revealed no cross-relationships.

Subsequently, Dr. Jordi Casals showed that it was very closely-related, if not identical, to Wad Medani and IG-673 viruses by complement-fixation tests. Wad Medani virus was previously isolated from Rhipicephalus sanguineus ticks collected from sheep in Egypt and IG-673 virus from Hyalomma ticks in India.

Subsequent cross-neutralization tests in suckling mice showed that SM-214 and P5-127 are strains of the same virus and differ from Wad Medani and IG-673, which also showed differences from each other. Results of the neutralization tests are shown in Table 20. These agents lose titer during incubation at 37°C, resulting in low neutralization indices. The tests were repeated several times with and without incubation and resulted in figures comparable to those presented in the table.

In conclusion, we feel that SM-214 may be considered a new arbovirus, to be named Seletar for the district of Singapore where the ticks were collected. P5-127 is a strain of Seletar. On the basis of our results, Seletar, Wad Medani, and IG-673 viruses will form the new Wad Medani group of the arboviruses.

In 1965, SM-214 virus was inoculated into two calves, which were subsequently observed for signs of illness at the Veterinary Research Institute in Ipoh. No signs of illness occurred during the first several weeks when the animals were under routine surveillance. Several months later, one of the calves died with massive subcutaneous hemorrhages. At the time, it was assumed that there was probably no relationship to the experimental inoculation.

In Singapore, imported police dogs of considerable value have been contracting a fatal, hemorrhagic disease since 1963. The problem has been intensively investigated by British military authorities without success to date. These dogs apparently become infested, not uncommonly, with Rhipicephalus sanguineus ticks acquired locally. Over 75 dogs have died and the problem is

considered a serious one.

Since Wad Medani virus was isolated from R. sanguineus ticks and because of the hemorrhagic manifestation exhibited in the experimental calf, it was conceivable that Seletar virus may be related to the hemorrhagic disease in dogs. As a result, experimental infection of pups has just been initiated. No results are yet available.

Haemaphysalis semermis

P7-239 and P7-249 from Belera, Trengganu are recent isolates requiring further work.

Haemaphysalis sp.

P5-123, isolated from a pool of Haemphysalis ticks taken from Rattus rajah in Kuala Trengganu, has been identified as a strain of Lanjan virus. Successful re-isolation was made from the original tick suspension. The newly-named Lanjan virus, unrelated to other known arboviruses, is represented by two strains, TP-94 and TP-123, previously isolated from Dermacentor ticks from rodents taken at Bukit Lanjan, Selangor. Results of cross-neutralization tests comparing P5-123 and TP-94 strains are shown in Table 21.

Vertebrate Isolates

Ten agents (P-106, P5-150, P-303, P-132, P-378, V-323, V-294, V-364, R-751, P6-725) isolated from tissue or serum samples from miscellaneous vertebrates are in various stages of processing in the laboratory. None has been identified and it is likely that none of these are arboviruses.

Five agents (N-316, P-73, P-223, P-225, and SF-37) isolated from tissues of Rattus jalorensis appear to be strains of the same virus.

No similar virus has been isolated from the many other rodent species tested in the same manner. It is probably not an arbovirus since it is resistant to treatment with ether and sodium desoxycholate. SF-37 is not heat-stable, but Mg^{++} increases its heat stability at 50°C. It is not stable at pH 3.0. It produces a flaccid paralysis and occasional death in rats, it kills infant and adult mice by the intracerebral and intraperitoneal routes, and it is negative in rabbits by the intracerebral, intraperitonel, subcutaneous, and corneal routes. It produces hind leg paralysis and death in guinea pigs and hamsters. Cell destruction is complete in 2 days in hamster kidney cell cultures. By electron microphotography, it appears to be an intranuclear agent about 25 mμ in size. A sucrose-acetone extracted antigen does not agglutinate a variety of mammalian and avian red blood cells, but it has a low titer in complement-fixation. Infected mouse brain was negative for rabies inclusion bodies by the fluorescent-antibody technique. Further work is in progress, but it seems likely that these agents may belong to the PICODNA group of viruses.

One agent (R-340) isolated from the serum of a Rattus norvegicus, collected in Penang, was identified as a strain of dengue type 2. Since no antibody for dengue could be demonstrated in several R. norvegicus sera tested, the question of the validity of this isolation was raised and is being investigated.

P5-293 was isolated from the brain of a horse with encephalitis in Ipoh by the Veterinary Research Institute and referred to us. It has been identified as a strain of Japanese encephalitis. Table 22 shows the results of a neutralization test with P5-293 virus.

Sentinel Animal Isolates

One agent isolated from the blood of a sentinel chicken (P7-284) in primary rain forest (Gunong Benom) has just been received in the San Francisco laboratory.

Two agents have been isolated from the blood of each of two sentinel monkeys stationed for longer than one year in dipterocarp forest.

One agent (P7-174) has been isolated from the blood of a sentinel monkey stationed in mangrove swamp forest.

None of the above viruses has been identified yet, but sera, taken subsequent to isolation from these animals, have not shown development of HI antibody to any of the arboviruses that we use in our routine tests.

Human isolates

Sixteen viruses previously isolated from the sera of patients in Penang, Kuala Lumpur, and Petaling Jaya have all been confirmed as strains of dengue type 2.

A member of the staff of the U.C. Medical Center was referred to us when she returned from Colorado with a history of tick-bite followed by development of fever. A-342 virus was isolated from an acute blood specimen and was readily identified as a strain of Colorado Tick Fever. The patient was referred to Dr. Emmons of the California State Virus Laboratory, who subsequently also isolated the virus from washed blood cells of a fourth blood specimen taken over 30 days after onset of the disease.

Two agents (Chin I and Chin II) were isolated from the throat swab of a case of herpangina in an American-Chinese child in Malaya and from a throat swab of the mother, who was febrile at the same time. Isolations were made in Malaya and tentatively identified as Coxsackie A strains. Pathology in suckling mice was typical of Coxsackie A infection. The viruses were subsequently referred to the California State Virus Laboratory and identified as strains of Coxsackie A-8.

R-876 virus was isolated by blind passage in suckling mice from an acute-phase serum of a case of febrile fits in Penang. The virus is desoxycholate sensitive, but re-isolation from the original material was unsuccessful. Comparison with a wide range of arboviruses has revealed no relationships. The isolate may not be valid and is being compared with known mouse viruses and other unknown virus isolates.

Two agents (P5-312 and P6-525) from the acute-phase sera of dengue cases in Johore Bahru and Petaling Jaya have been identified as strains of dengue, but have not been typed yet.

Three additional agents (P6-1358, P6-1359, and P6-1457) were isolated recently from febrile patients, the latter one from an aborigine at the Gombak Aborigine Hospital.

Philippine Hemorrhagic Fever Epidemic of 1966

Dr. Venzon arrived in San Francisco in January 1967 as an International Postdoctoral Fellow of the National Institutes of Health for one year. She had begun collecting serum specimens in Manila early in 1966 from patients with undifferentiated fevers for her proposed work in San Francisco. Soon after her collections were initiated, hemorrhagic fever appeared. As a result, the majority of her material came from cases diagnosed clinically as hemorrhagic fever. Her work is briefly summarized below.

During 1966, an epidemic of mosquito-borne hemorrhagic fever occurred in the city of Manila and neighboring areas. Over 5000 cases were admitted to San Lazaro Hospital, the principal infectious diseases hospital. This study is based on 399 cases representing approximately 7% of those admitted to San Lazaro Hospital and others from Philippine General Hospital and the Manila Sanitarium.

79% of the cases studied were hemorrhagic fever of varying degrees of severity while 21% were undifferentiated fever syndromes clinically. Age distribution was from one year to 51 years with 60% in the 5-14 year age bracket. Specimens consisted of 166 serum pairs (acute and convalescent) and 233 single acute or convalescent sera. Of these, 227 were collected early, within 4 days of onset of illness. These, with 13 5th day specimens with suggestive serology, were inoculated in suckling mice for attempted virus isolation. To date, surviving mice from 56 of 212 attempts, subsequently challenged with a lethal dose of dengue virus, have shown significant resistance suggesting the presence of dengue virus. In addition to these 56 probable dengue isolates, six agents have been demonstrated on the basis of development of illness and death in the mice. This represents a virus recovery rate of approximately 29% so far.

All sera have been tested for the presence of hemagglutination-inhibiting antibodies to the following viruses: Dengue 1, 2, 3, 4, Japanese encephalitis, tembusu, Zika, Langat, Sindbis, getah, chikungunya, and bunyamwera. 58% of the paired specimens showed a 4- fold or greater rise in titer for the group B viruses, mostly with highest titers for one or more dengues. Presumptive positive diagnoses ($\geq$1/1280 serum titer but no demonstrable rise) of group B infection (principally dengue) were made for 24% of the late single and paired sera. A serological diagnosis could not be made for those cases with single specimens taken earlier than the fourth day of disease.

One case demonstrated a striking rise in antibody titer (1/5120 to $\geq$1/600,000) for Zika virus, recently isolated in Malaysia. Antibodies were found in one case for getah and in 8 cases for chikungunya, but these probably were not related to the current illnesses. No sera were positive for Sindbis or bunyamwera antibodies. Approximately 11% of the sera were completely negative for all the antigens tested.

Further work will include completion of virus isolations, identification of isolates, and further serological testing by complement-fixation and neutralization procedures.

In summary, the 1966 Manila hemorrhagic fever epidemic was principally associated with one or more types of dengue virus. Preliminary serological results suggest that the principal dengue types involved were 4 and 2, but the final conclusion will depend on the identification of the isolated virus strains.

Serology

Animals

In the preliminary survey (1962-1964), it was found that dengue antibody occurred commonly in wild monkeys (62%) and was not significant in any other animals tested. Japanese encephalitis (JE) antibodies were common in domestic animals (pigs, cattle, horses, and goats) and also occurred in a few wild monkeys, one bandicoot, and a wild boar. Tembusu antibody appeared to be cross-reacting JE in most cases. Only a few animals showed the presence of group A antibody: Sindbis in a few ducks, Rattus bowersi, and one Presbytis melalophos monkey (the positive monkey was from the same area in which Sindbis virus, E-145 strain, was isolated from Culex sinensis mosquitoes); chikungunya-related antibody in monkeys, pigs, and cattle. Batai antibody was demonstrated in cattle, goats, a few monkeys, and one chicken.

In the current survey, 1101 sera of 1476 collected have been tested by HI. Broad group B antibodies were found in most domestic animals (pigs, horses, carabaos, dogs, chickens, and cattle), as well as in talking mynas and low-titered antibody in several species of bats. The positive bats included Cynopterus brachyotus, C. horsfieldi, Eonycteris spelaea, Hipposideros diadema, and

Tadarida plicata. Only a few of each species was positive except E. spelaea of which 11/47 had low-titered antibody.

Dengue antibody was common, as before, in all species of wild monkeys. It was also demonstrated in 3/49 Tadarida plicata bats in low titer and in one dog.

Zika antibody was seen in several wild monkeys, but its incidence appears to be lower than for dengue.

JE antibody was common in pigs, horses, cattle, and carabaos and was also seen in dogs and one Rattus rajah.

Tembusu antibody was demonstrated in cattle, pigs, dogs and in some birds (talking myna and the white-headed munia).

Langat antibody with little or no cross-reaction was observed in low titer in 3/119 yellow-vented bulbuls (Pycnonotus goiavier).

Bunyamwera antibody was seen only in cattle.

Sindbis antibody was found in cattle, carabao and the talking myna.

Getah antibody was common in domestic pigs (33/113) with relatively high titers. It was also demonstrated in some cattle, carabao, yellow-vented bulbuls, and in 7/34 Presbytis obscurus monkeys.

Chikungunya antibody was found in pigs, cattle, chickens, Presbytis obscurus monkeys, and two bats (C. horsfieldi and E. spelaea). It was most common in pigs, but usually low-titered and probably representing cross-reacting Getah in most cases.

Normal Humans

Of the 1725 normal human sera listed, 931 have been tested by HI. As expected, the incidence of group B antibodies was high and widespread in all areas sampled. The majority of the group B antibody appears to be dengue with a lower incidence of JE.

In the general population, exclusive of the aborigine peoples, there was evidence of low activity of Sindbis, bunyamwera (batai), getah, and umbre viruses: Sindbis - 1/87 in Bentong; Umbre - 2/81 in Kampong Kuala Pahang; and getah - 33 of the total tested. The getah antibody, however, was usually very low-titered and was probably not specific.

Although no illness ascribed to chikungunya virus has yet been conclusively demonstrated in Malaysia, the incidence of chikungunya antibody, often in relatively high titer, was not uncommon: 11/87 in Bentong, 14/81 in Kampong Kuala Pahang, 12/66 in Kuala Lumpur, 2/60 in Malacca, 13/64 in Mentakab, 13/151 in Penang, 10/43 in Segamat, and 13/72 in Seremban. Most of the chikungunya antibody was found in persons over 30 years of age with exceptions. However, the young age groups are not as well-represented in our collection as the older groups. In Kampong Kuala Pahang, antibody was found in a 9-year-old. These factors, together with the relatively high titers demonstrated, suggest that chikungunya infection may be occurring currently in the human population.

A series of 224 sera taker from forest-living aborigines in 1965 and 1966 were tested by HI only. All except eleven (from children under 10 years of age) were positive for broad group B activity. There was minimal group A activity. The overall pattern suggested that one or more of the dengue viruses was responsible for the majority of the group B antibody. In comparison with 151 sera taken in Penang City, where dengue is recognized, there appeared to be serological evidence of more dengue infection in the forest aborigines. Bunyamwera antibody was found in

2/33 sera from Ulu Aring. Sindbis antibody was found in 10 sera; getah in 9 sera, and chikungunya in 13 sera. Marituba (group C) antigen was also included in tests of the aborigines' sera and was completely negative.

Indonesian sera:

A group of 76 sera from Indonesians, resident in Djakarta, showed a high incidence of group B arbovirus infection. It appeared likely that most of the group B positives resulted from dengue infection and fewer from Japanese encephalitis infection. There was also a significant incidence of chikungunya antibody indicating the existence of group A infection.

Summary

The program objectives are to determine the roles that arboviruses play in producing infection and/or disease in man, with special reference to their ecology and the clarification of the non-urban cycles of dengue. The survey to date has investigated a great variety of areas of differing ecology throughout Malaysia. Improved and intensified methods of collection have allowed sampling of great numbers of mosquitoes for virus isolation. Predominant species, species composition and habits of mosquitoes in various areas have been elucidated. Extensive collection of animals and human sera has provided information on the incidence and distribution of arbovirus infection in man and animals, while the diagnostic service has provided routine surveillance of arbovirus disease.

Additional serological evidence strongly supporting a jungle cycle for dengue has been obtained in wild monkeys and forest-dwelling aborigines, while no evidence has been found to demonstrate significant involvement of any other animals. Bats show evidence of group B infection, but this is probably not due to dengue virus.

The discovery of Zika virus, previously known in Africa only, in Aedes aegypti and serological evidence of Zika infection in man and wild monkeys is significant.

A new arbovirus, Seletar, is described and a large number of unidentified virus isolates from mosquitoes, ticks, and sentinel animals are being investigated.

A serological survey of residents of Djakarta, Indonesia, the first to be done, revealed the occurrence of dengue and Japanese encephalitis and possibly chikungunya infection.

A study of the Manila hemorrhagic fever epidemic of 1966 has resulted in the isolation of numerous strains of dengue and has revealed the probable presence of Zika virus infection.

It is anticipated that the projected intensive-phase studies of dengue ecology will be initiated shortly in rural and jungle sites.

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Table 1 - Known Arboviruses of Malaysia and Singapore

as Determined by Virus Isolation (June 1967)

A	B	Bunyamwera	Ketapang	Wad Medani	Ungrouped
Bebaru	Dengue 1	Batai	Bakau	Seletar	Lanjan
Getah	Dengue 2		Ketapang		
Sindbis	Dengue 3				
	Dengue 4				
	Japanese encephalitis				
	Langat				
	Tembusu				
	Zika				
3	8	1	2	1	1

Total 16

Table 2 - Total Mosquitoes Collected and Processed for Virus Isolation from Areas of Differing Ecology (August 1965 - April 1967)

Area Type	Number of Females
Mangrove Swamp Forest	41,536
Freshwater Peat Swamp Forest	67,830
Rubber Estates	3,198
Secondary Forest and Scrub	410
Rural disturbed areas	6,672
Urban, Town and adjacent areas of human activity	86,170
Lowland Dipterocarp Forest	4,355
Primary undisturbed Rain Forest	1,472
Total	211,643

Table 3 - Mosquitoes Collected and Processed for Virus Isolation

in

Mangrove Swamp Forest

(August 1965 - April 1967)

Species	Number of Females
Aedes (Cancraedes) spp.	19,582
Uranotaenia spp.[1]	14,374
Aedes (Aedes) spp.[2]	5,029
Aedes (Skusea) spp.[3]	976
Culex (Lophoceraomyia) spp.[4]	481
Anopheles (Anopheles) spp.[5]	371
Aedes (Stegomyia) albopictus	296
Aedes (Rhinoskusea) spp.	159
Armigeres (Armigeres) moultoni	138
Aedes (Mucidus) aurantius	30
Culex (Culex) spp.[6]	59
Armigeres (Armigeres) malayi	11
Armigeres (Armigeres) durhami	10
Aedes indonesiae	9
Mansonia (Mansonioides) bonneae/dives	7
Aedes (Stegomyia) w-albus	3
Aedes masculinus	1
Total	41,536

1 Principally lateralis.
2 Principally butleri and fewer lugubris.
3 Principally amesii and fewer fumidus.
4 Includes cinctellus.
5 Principally baezai, with some umbrosus group.
6 Principally sitiens with some annulus.

Table 4 - Mosquitoes Collected and Processed for Virus Isolation
in
Freshwater Peat-Swamp Forest
(August 1965 - April 1967)

Species	Number of Females
Mansonia (Mansonioides) annulata	56,931
Coquillettidia hodgkini	3,739
Mansonia (Mansonioides) bonneae/dives	2,417
Anopheles (Anopheles) umbrosus group	2,162
Culex (Culex) pseudovishnui	732
Heizmannia spp.	725
Armigeres sp. 'banting'	279
Coquillettidia nigrosignata	247
Culex (Lophoceraomyia) spp.	121
Aedes (Finlaya) poicilius	96
Anopheles spp.	83
Armigeres (Armigeres) sp. no. 1	59
Coquillettidia crassipes	51
Armigeres (Armigeres) malayi	34
Mansonia (Mansonioides) uniformis	29
Culex sp. 'near nilgiricus'	23
Culex (Culex) gelidus	21
Aedes (Stegomyia) albopictus	19
Aedes (Paraedes) ostentatio	9
Armigeres (Armigeres) subalbatus	5
Aedes (Finlaya) flavipennis	2
Miscellaneous	46
Total	67,830

Table 5 - Mosquitoes Collected and Processed for Virus Isolation

in

Rubber Estates

(August 1965 - April 1967)

Species	Number of Females
Aedes (Stegomyia) albopictus	2,672
Aedes spp.	94
Aedes (Stegomyia) w-albus	26
Armigeres (Armigeres) subalbatus	22
Culex (Culex) pipiens quinquefasciatus*	384
Total	3,198

* In adjacent dwellings.

Table 6 - Mosquitoes Collected and Processed for Virus Isolation

in

Secondary Forest and Scrub

(August 1965 - April 1967)

Species	Number of Females
Aedes (Stegomyia) albopictus	356
Mansonia (Mansonioides) dives	20
Aedes sp. no. 1	8
Aedes (Stegomyia) w-albus	5
Aedes (Aedes) spp.	5
Armigeres (Armigeres) subalbatus	4
Armigeres (Armigeres) spp.	3
Aedes (Stegomyia) albolineatus	2
Armigeres (Leicesteria) flavus	2
Mansonia (Mansonioides) uniformis	2
Heizmannia spp.	2
Aedes sp.	1
Total	410

Table 7 - Mosquitoes Collected and Processed for Virus Isolation

in

Rural and Disturbed Areas Including an

Aborigine Village (August 1965 - April 1967)

Species	Number of Females	Viruses
Culex (Culex) gelidus	3,429	
Mansonia (Mansonioides) uniformis	1,949	1 (P5-350)
Anopheles (Anopheles) spp.*	314	
Culex (Culex) vishnui group**	272	
Aedes (Aedimorphus) vexans	247	
Aedes (Banksinella) lineatopennis	178	
Culex (Culex) fuscocephalus	85	
Culex (Culex) annulus	76	
Culex (Culex) pipiens quinquefasciatus	47	
Aedes (Stegomyia) albopictus	43	
Mansonia (Mansonioides) bonneae/dives	19	
Aedes (Finlaya) poicilius	7	
Mansonia (Mansonioides) annulifera	6	
Total	6,672	

* Principally hyrcanus, umbrosus, and barbirostris groups in order.

** Principally tritaeniorhynchus.

Table 8 - Mosquitoes Collected and Processed for Virus Isolation
in
Urban, Town, Village and Adjacent Areas
of Human Activity (August 1965 - April 1967)

Species	Number of Females	Viruses
Culex (Culex) gelidus	35,809	6
Culex (Culex) vishnui group[1]	22,405	4
Culex (Culex) pipiens quinquefasciatus	8,298	
Mansonia (Mansonioides) uniformis	7,958	
Culex (Culex) fuscocephalus	3,826	1
Anopheles spp.[2]	3,628	
Aedes (Stegomyia) aegypti	1,458	1
Aedes (Stegomyia) albopictus	1,257	
Aedes (Banksinella) lineatopennis	340	
Culex (Culex) sitiens	151	
Aedes (Aedes) butleri	150	
Mansonia (Mansonioides) bonneae/dives	133	
Armigeres (Armigeres) durhami/subalbatus	126	
Mansonia (Mansonioides) annulifera	120	
Aedes spp.	156	1
Culex (Culiciomyia) nigropunctatus	58	
Aedes (Aedimorphus) vexans/caecus	53	
Coquillettidia nigrosignata	42	
Culex (Culex) bitaeniorhynchus	40	
Aedes sp. no. 1	39	
Culex (Culex) sinensis	30	
Culex (Culex) spp.[3]	26	
Culex (Lutzia) fuscanus	13	
Coquillettidia crassipes	11	
Coquillettidia hodgkini	11	
Coquillettidia ochracea	11	
Aedes (Skusea) spp.	9	
Aedes (Stegomyia) w-albus	4	
Aedes (Paraedes) ostentatio	4	
Aedes indonesiae	3	
Culex sp.	1	
Total	86,170	13

1 Principally tritaeniorhyrchus.

2 Includes hyrcanus group, separatus, and barbirostris group in subgenus Anopheles, and kochi, philippinensis, vagus, subpictus, and karwari in subgenus Cellia.

3 Includes annulus.

Table 9 - Mosquitoes Collected and Processed for Virus Isolation
in
Lowland Dipterocarp Forest
(August 1965 - April 1967)

Species	Number of Females
Armigeres (Leicesteria) spp.	3,400
Aedes (Stegomyia) spp.	430
Culex (Culex) pseudovishnui	401
Heizmannia spp.	68
Aedes (Finlaya) spp.	38
Mansonia (Mansonioides) dives	8
Culex (Lophoceraomyia) spp.	4
Armigeres (Armigeres) subalbatus	3
Coquillettidia crassipes	2
Anopheles (Anopheles) noniae	1
Total	4,355

Order of Predominance of Species of:

Leicesteria	Stegomyia	Finlaya	Heizmannia
annulitarsis	albopictus	albotaeniatus	macdonaldi
dolichocephalus	desmotes	niveus	predominant
dentatus	pseudalbopictus	saxicola	
omissus			
digitatus			
flavus			
pectinatus			
balteatus			
traubi			
maiae/jugraensis			
inchoatus			
magnus			

Table 10 - Mosquitoes Collected and Processed for Virus Isolation

in

Primary Undisturbed Rain Forest

(Gunong Benom - April 1967)

Species	Number of Females
Aedes (Finlaya) spp.	1,155
Culex (Culex) pseudovishnui	179
Culex (Culex) spp.	136
Armigeres (Armigeres) spp.	2
Total	1,472

Table 11 - Ticks Collected and Processed for Virus Isolation

(August 1965 - April 1967)

Species	Number	Viruses Isolated
Ixodidae		
Ixodes granulatus	88	
spp.	7	
Ixodid larvae	150	
Haemaphysalis bispinosa	128	
koningsbergeri	19	
papuana	97	
semermis	104	2
spp.	192	1
Dermacentor auratus "A"	161	
auratus "B"	73	
auratus "?"	2	
spp.	111	
Rhipicephalus sanguineus	94	
Boophilus microplus	469	1
Amblyomma geoemydae	3	
testudinarium	7	1
spp.	4	
Argasidae		
Argas spp.	511	2
Total	2,220	7

Table 12 - Miscellaneous Arthropods Collected and Processed for Virus Isolation (August 1965 - April 1967)

Culicoides spp.	2,408
Culicoides type "A"	62
Tabanus sp. no. 1	44
Tabanus effilatus	25
Chrysosoma spp.	69
Basilia hispida (new species)	6
Total	2,614

Table 13 - Vertebrates Collected in Malaysia and Singapore
(1962 - 1967)

Species	Common Name	Number (1962-4)	Number (1965-7)
Amphibia			
Rana tigrina	Frog		1
Reptilia			
Geoemyda spinosa	Turtle	1	
Najah hannah	Cobra		1
Snake (?)			1
Gekko monarchus	Gecko	1	
Hemidactylus brooki	"	15	
frenatus	"	70	
garnoti	"	11	
platyurus	"	77	
Gehyra mutilata	"	8	
Varanus salvator	Monitor lizard	3	1
Aves			
Ardeidae			
Nycticorax nycticorax	Black-crowned night heron		49
Scolopacidae			
Numenius phaeopis variegatus	Whimbrel		1
Columbidae			
Geopelia striata	Zebra dove		2
Streptopelia chinensis	Spotted-necked dove		1
sp.	Turtle dove		1
Treron vernans	Green pigeon	1	
Psittacidae			
Loriculus galgulus	Hanging parakeet		1
	Parakeet		2
Cuculidae			
Phaenicophaeus curvirostris	Chestnut-breasted malcoha		1
Strigidae			
Otus bakkamoena	Collared scops owl		2
Caprimulgidae			
Caprimulgus macrurus	Long-tailed night jar		3
Trogonidae			
Harpactes duvauceli	Red-rumped trogon		1
erythrocephalus	Red-headed trogon		1
Alcedinidae			
Ceyx rufidorsus	Red-backed kingfisher		2
Halcyon concreta	Chestnut-collared kingfisher		1
coromanda	Ruddy kingfisher		1
pileata	Black-capped kingfisher		3
Picidae			
Micropternus brachyurus	Rufous woodpecker		2
Pichus sp.	Green woodpecker		3
Eurylaimidae			
Calyptomena viridis	Green broadbill		1
Dicruridae			
Dicrurus remifer	Large racket-tailed drongo		4
Corvidae			
	Crow		7

Table 13 - Vertebrates Collected in Malaysia and Singapore (continued)

Species	Common Name	1962-4	1965-7
Timaliidae			
Alcippe castaneiceps	Chestnut-headed nun babbler		5
nipalensis	Mountain nun babbler		1
porocephala	Malay nun babbler		1
Malacopteron cinereum	Lesser red-headed babbler		1
magnarostre	Brown-headed babbler		6
Napothera brevicaudata	Streaked wren babbler		3
Pomatorhinus hypoleucos	Large scimitar babbler		1
Stachyris chrysea	Golden tree babbler		1
leucotis	White-eared tree babbler		2
maculata	Red-rumped tree babbler		1
nigriceps	Gray-throated tree babbler		1
Trichastoma malaccense	Short-tailed babbler		1
Pycnonotidae			
Criniger bres	Scrub bulbul		2
ochraceus	Brown white-throated bulbul		5
phaeocephalus	Crestless white-throated bulbul		1
Hypsipetes criniger	Hairy-backed bulbul		2
erythrocephalus	Mountain streaked bulbul		3
Pycnonotus brunneus	Red-eyed brown bulbul		6
erythropthalmos	Lesser brown bulbul		1
goiavier	Yellow-vented bulbul		120
plumosus	Olive-brown bulbul		14
simplex	White-eyed brown bulbul		4
sp.	Crested bulbul		1
Aegithinidae			
Chloropsis cyanopogon	Lesser green leafbird		1
Turdidae			
Copsychus pyrropygus	Orange-tailed shama		1
saularis	Magpie robin		2
Enicurus ruficapillus	Chestnut-naped forktail		1
Sylviidae			
Acrocephalus arundinaceus	Great reed warbler		2
Orthotomas cucullatus	Mountain tailor bird		1
Muscicapidae			
Culicicapa ceylonensis	Gray-headed flycatcher		1
Muscicapa grandis	Niltava		2
hyperythra	Thicket flycatcher		1
solitaris	White-gorgeted flycatcher		2
Rhipidura albicollis	White-throated fantail		3
Motacillidae			
Anthus novaeseelandiae	Richard's pipit	10	
Sturnidae			
Gracula religicsa	Talking myna		4
Sturnus tristis	Common myna		2
Nectariniidae			
Arachnothera affinis	Gray-breasted spider hunter		1
longirostris	Little spider hunter		1
magna	Streaked spider hunter		1
Dicaeidae			
Prionochilus maculatus	Yellow-throated flower-pecker		1

Table 13 - Vertebrates Collected in Malaysia and Singapore (continued)

Species	Common Name	1962-4	1965-7
Ploceidae			
Lonchura maja	White-headed munia		1
punctulata	Spotted munia		1
Passer montanus	Tree sparrow	10	1
Ploceus philippinensis infortunatus	Baya weaver		1
Mammalia			
Insectivora			
Echinosorex gymnurus	Moon rat	15	3
Hylomys suillus	Pig-tailed shrew	3	
Suncus murinus	House shrew	120	30
Dermoptera			
Cynocephalus variegatus	Flying lemur	1	1
Chiroptera			
Pteropodidae			
Balionycteris maculata	Spotted-winged fruit bat		1
Chironax melanocephalus	Black-capped fruit bat		2
Cynopterus brachyotis	Common short-nosed fruit bat	2	55
horsfieldi	Larger short-nosed fruit bat		15
Eonycteris spelaea	Cave fruit bat	2	47
Macroglossus lagochilus	Common long-tongued bat		1
Pteropus hypomelanus	Lesser flying fox	5	
Emballonuridae			
Taphozous melanopogon	Black-bearded tomb bat		8
Rhinolophidae			
Hipposideros diadema	Diadem horseshoe bat		11
galeritus	Common roundleaf horseshoe bat		3
Rhinolophus affinis	Brown horseshoe bat		8
philippinensis			1
refulgens	Glossy horseshoe bat		8
stheno	Lesser brown horseshoe bat		28
spp.			18
Molossidae			
Tadarida plicata	Free-tailed bat		29
Vespertilionidae			
Eptesicus verecundus	False serotine bat		1
Scotophilus temminckii	House bat		32
Tylonycteris robustula	Bamboo bat	5	
Primates			
Tupaia glis	Common tree shrew	104	17
minor	Lesser tree shrew	8	
Macaca irus	Long-tailed macaque	165	12
nemestrina	Pig-tailed macaque	2	3
Nycticeous coucang	Slow loris		1
Presbytis cristatus	Silvered leaf-monkey	73	
melalophos	Banded leaf-monkey	4	2
obscurus	Dusky leaf-monkey		34
Pholidota			
Manis javanica	Scaly anteater	1	

Table 13 - Vertebrates Collected in Malaysia and Singapore (continued)

Species	Common Name	1962-4	1965-7
Rodentia			
Sciuridae			
Callosciurus caniceps	Gray-bellied squirrel	7	
hippurus	Horse-tailed squirrel	1	
nigrovittatus	Black-banded squirrel	19	5
notatus	Common red-bellied squirrel	163	23
prevosti	White-striped squirrel	3	1
Iomys horsefieldi	Red-tailed flying squirrel		3
Lariscus insignis	Striped ground squirrel	1	6
Petaurista elegans	Spotted giant flying squirrel		1
petaurista	Red giant flying squirrel		1
Ratufa bicolor	Black giant squirrel		1
Rhinosciurus laticaudatus	Shrew-faced ground squirrel	9	3
Sundasciurus lowii	Short-tailed little squirrel		1
tenuis	Slender little squirrel	5	1
Muridae			
Bandicota indica	Greater bandicoot	1	
Chiropodomys gliroides	Bamboo tree mouse	5	
Mus musculus	House mouse	22	
Rattus annandalei	Singapore rat	48	18
argentiventer	Ricefield rat	32	6
bowersi	Gray giant rat	58	3
canus	Gray tree rat	8	
cremoriventer	Dark-tailed tree rat	21	8
edwardsi	Mountain giant rat	5	
exulans	Little house rat	44	4
jalorensis	Malaysian wood rat	206	35
muelleri	Swamp giant rat	108	3
norvegicus	Norway rat	51	10
rajah	Rajah spiny rat	53	27
rattus diardi	Malaysian house rat	247	102
rattus roa		65	
sabanus	Long-tailed giant rat	108	43
surifer	Red spiny rat	91	1
whiteheadi	Little spiny rat	92	5
Artiodactyla			
Sus scrofa	Wild boar	1	1
Domestic Animals			
Carabaos			38
Cats		4	
Cattle		3	464
Chickens		20	334
Dogs		10	28
Ducks		10	97
Geese		2	
Goats		10	217
Horses		18	11
Pigs		50	250
Sheep			30
Totals		2,321	1,476

Table 14 - Normal Human Sera Collected
(to April 1967)

State	Locality	Number of Sera
Johore	Segamat	43
Kedah	Alor Star	78
	Gurun	2
	Kuala Ketil	2
	Sungei Lallang	1
	Sungei Patani	116
Kelantan	Kota Bharu	26
	Ulu Aring (aborigines)	33
Malacca	Malacca	60
Negri Sembilan	Seremban	72
Pahang	Bentong	87
	Kampong Koi (aborigines)	9
	Kampong Kuala Pahang	80
	Kuala Lipis	20
	Kuantan	113
	Mentakab	64
	Raub	7
	Sungei Lembing	72
Penang	Georgetown	151
Perak	Batu Gajah	53
	Ipoh	67
	Kampar	1
	Kampong Ayer Denak (aborigines)	56
	Kampong Timah Tanjong	7
	Kampong Tunku Hussein	1
	Silibin	2
	Ulu Gruntong (aborigines)	25
	Miscellaneous (aborigines)	1
Perlis	Jejani	1
	Kangar	5
Selangor	Bukit Manchang (aborigines)	77
	Kuala Lumpur	177
	Semenyat	1
	Ulu Gombak (aborigines)*	44
Trengganu	Kuala Barang	11
	Kuala Trengganu	26
	Total	1,649
Indonesia	Djakarta	76
	Total	1,725

* Temporary residence - originate in various parts of Malaysia.

Table 15 - Virus Isolates Under Study*
(to April 1967)

Source	Number	Locality	Identification
Mosquitoes			
Aedes aegypti	SM-1	Singapore	Dengue 2
	SM-3	Singapore	Dengue 2
	SM-11	Singapore	Dengue 2
	SM-12	Singapore	Dengue 2
	SM-14	Singapore	Dengue 2
	X-2	Bangkok	Dengue
	X-34	Bangkok	Dengue
	P6-740	Bentong, Pahang	Zika
Aedes albopictus	E-24	Kuala Lumpur, Selangor	----
	SM-18	Singapore	Dengue 2
Aedes sp.	P6-1346	Kota Bharu, Kelantan	----
Culex fuscocephalus	P6-1368	Tumpat, Kelantan	----
Culex gelidus	P6-410	Johore Bahru, Johore	Japanese encephalitis
	P6-412	Johore Bahru, Johore	----
	P6-462	Seremban, Negri Sembilan	Japanese encephalitis
	P6-578	Malacca	Japanese encephalitis
	P6-1458	Kuantan, Pahang	----
	P7-211	Kuala Gula, Perak	----
Culex sinensis	E-140	Ulu Mandul, Selangor	----
	E-145	Ulu Bendol, Selangor	Sindbis
Culex vishnui group	P6-553	Malacca	----
	P6-856	Kuala Lipis, Pahang	----
	P6-1333	Tumpat, Kelantan	----
	P6-1462	Kuantan, Pahang	----
Culex sp. 'near *nilgiricus*'	E-210	Pacific Tin, Selangor	Bakau
Mansonia uniformis	P5-350	Puchong, Selangor	----
Ticks			
Amblyomma testudinarium	P7-275	Kelantan	----
Argas pusilus	P6-1361	Keterah, Kelantan	----
	P6-1362	Keterah, Kelantan	----
Boophilus microplus	SM-214	Singapore	Seletar
	P5-127	Kepong, Selangor	Seletar
Haemaphysalis semermis	P7-239	Belera, Trengganu	----
	P7-249	Belera, Trengganu	----
Haemaphysalis sp.	P5-123	Kuala Trengganu, Trengganu	Lanjan
Vertebrates			
Rattus rattus diardi	P-106	Paroi, Negri Sembilan	----
	P5-150	Sekinchang, Selangor	----
Rattus rattus roa	P-303	Pulau Aur	----
Rattus jalorensis	N-316	Temerloh, Pahang	----
	P-73	Sungei Buloh, Selangor	----

* Exclusive of Philippine virus isolates.

Table 15 - Virus Isolates Under Study (continued)

Source	Number	Locality	Identification
Vertebrates			
Rattus jalorensis	P-223	Rantau Panjang, Selangor	----
	P-225	Rantau Panjang, Selangor	----
	SF-37	Rantau Panjang, Selangor	----
Rattus norvegicus	R-340	Georgetown, Penang	Dengue 2*
Rattus and Tupaia spp.	P-132	Bukit Lanjan, Selangor	----
Suncus murinus	P-378	Kuala Lumpur, Selangor	----
	V-323	Georgetown, Penang	----
Chicken	V-294	Georgetown, Penang	----
	V-364	Georgetown, Penang	----
Sentinel chicken #203	P7-[illegible]	Gunong Senar, Pahang	----
Pig	R-751	Georgetown, Penang	----
	P6-725	Sungei Buloh, Selangor	----
Horse	P5-293	Ipoh, Perak	Japanese encephalitis
Sentinel monkey #19	P6-898	Ulu Lui, Selangor	----
#19	P6-950	Ulu Lui, Selangor	----
#5	P7-171	Ulu Lui, Selangor	----
#5	P7-177	Ulu Lui, Selangor	----
#141	P7-174	Telok Gong, Selangor	----
Human	A-342	San Francisco, California	Colorado Tick Fever
	Chin I	Kuala Lumpur, Selangor	Coxsackie A-8
	Chin II	Kuala Lumpur, Selangor	Coxsackie A-8
	R-155	Georgetown, Penang	Dengue 2
	R-254	Georgetown, Penang	Dengue 2
	R-347	Georgetown, Penang	Dengue 2
	R-396	Georgetown, Penang	Dengue 2
	R-456	Georgetown, Penang	Dengue 2
	R-480	Georgetown, Penang	Dengue 2
	R-590	Georgetown, Penang	Dengue 2
	R-592	Georgetown, Penang	Dengue 2
	R-875	Georgetown, Penang	Dengue 2
	R-876	Georgetown, Penang	----
	R-887	Georgetown, Penang	Dengue 2
	R-888	Georgetown, Penang	Dengue 2
	R-939	Kuala Lumpur, Selangor	Dengue 2
	R-968	Georgetown, Penang	Dengue 2
	R 972	Georgetown, Penang	Dengue 2
	R-982	Petaling Jaya, Selangor	Dengue 2
	R-1134	Georgetown, Penang	Dengue 2
	P5-312	Johore Bahru, Johore	Dengue 1 or 3
	P6-525	Petaling Jaya, Selangor	Dengue
	P6-1358	Petaling Jaya, Selangor	----
	P6-1359	-----	----
	P6-1457	Ulu Gombak, Selangor	----
		Total	83

* Possible contaminant.

Table 16 - Summary of HI Cross-Comparison of P6-740 Virus with Group B Viruses

	P6-740 Antigen		P6-740 Immune Serum	
	Ht/Ho*	Ratio	Ht/Ho	Ratio
Dengue 1	<10/80	>1/8	<10/80	>1/8
Dengue 2	<10/80	>1/8	20/80	1/4
Dengue 3	10/80	1/8	20/80	1/4
Dengue 4	10/80	1/8	20/80	1/4
Tembusu	10/640	1/64	<10/80	>1/8
J E	20/640	1/32	20/80	1/4
Langat	<10/160	>1/16	20/80	1/4
Zika	80/160	1/2	160/80	2/1
M V E	20/--		--	
S L E	10/--		--	
Y F (17D)	<10/--		--	
Ilheus	20/--		--	
Ntaya	20/--		--	
Uganda S	<10/--		--	
West Nile	20/--		--	

* Heterologous/Homologous

Table 17 - Comparative Neutralization Tests in Mice with P6-740 and Zika viruses

	Virus	
Serum	Zika	P6-740
Zika	<3.0*	>4.3
P6-740	3.0	4.3
West Nile	0	0
Dengue 1	0	0
Dengue 4	0.8	0.5
Y F (17D)	0.3	0
Uganda S	0	0

* Neutralization Index

Table 18 - Viral Antigens Used in Hemagglutination-Inhibition and Complement-Fixation Tests

Group	Antigens
Routine Use	
A :	Chikungunya, Getah, Sindbis.
B.:	Dengue 1, 2, 3, 4, Japanese encephalitis, Tembusu, Langat, Zika.
Bunyamwera :	Bunyamwera.
Non-routine Use	
A :	Bebaru, Eastern Encephalitis.
C :	Marituba.
Bunyamwera :	Batai.
Ketapang :	Bakau, Ketapang.
Simbu :	Sathuperi.
Turlock :	Umbre.
Miscellaneous :	Colorado Tick Fever, Lanjan, Wad Medani, IG-673, Seletar.

Table 19 - Cross-comparison of E-210 with Ketapang and Bakau viruses by Neutralization Test in Mice

Sera	Viruses		
	E-210	Bakau	Ketapang
E-210	>5.2*	4.2	1.1
Bakau	>5.2	4.5	1.7
Ketapang	<1.2	<0.7	4.5

* Neutralization Index.

Table 20 - Cross-Neutralization Tests with Wad Medani, IG-673, SM-214, and P5-127 Viruses

Sera	Viruses			
	Wad Medani	IG-673	SM-214	P5-127
Wad Medani	1.4*	0.6	0.7	0.5
IG-673	0.7	1.6	0.8	0.7
SM-214	0.3	0.7	1.4	1.8
P5-127	0.4	0.8	1.5	2.5

* Neutralization index.

Table 21 - Cross-Neutralization Tests in Mice Comparing TP-94 and P5-123 strains of Lanjan Virus

Sera	Viruses	
	TP-94	P5-123
TP-94	2.0*	2.2
P5-123	2.4	2.1

* Neutralization index.

Table 22 - Neutralization Test in Mice with P5-293 Virus

Sera	Neutralization Index
P5-293	3.7
Japanese Encephalitis	3.8
St. Louis Encephalitis	1.0
West Nile	2.0
Murray Valley Encephalitis	2.1

Table 23 - Stock Arboviruses Maintained in the San Francisco Laboratory

Group	Virus	Strain
A	Bebaru	AMM-2354
	Chikungunya	African
		TH-35
		T-185
	Eastern Equine Encephalitis	
		T-172
	Getah	AMM-2021
		Sagiyama (Mag 132)
	Mayaro	
		Uruma
	O'nyong Nyong	Gulu
	Semliki Forest	
	Sindbis	P-886
	Western equine encephalitis	Olitsky
B	Dengue 1	Hawaiian
		Chia
		S-601
	Dengue 2	New Guinea C
		Trinidad 1751
		TH-36
		S-843
	Dengue 3	H-87
		P-301
		P-482
	Dengue 4	H-241
		A-5002
	Japanese encephalitis	Nakayama
	Langat	TP-21
	Murray Valley Encephalitis	
	Ntaya	
	St. Louis Encephalitis	Webster
	Tembusu	AMM-1775
		AMM-1673
	Uganda S	
	West Nile	#26
	Yellow Fever	17-D
		French Neurotropic
	Zika	
C	Caraparu	
	Marituba	
	Oriboca	
Bunyamwera	Batai	AMM-2222
	Bunyamwera	
	Wyeomyia	
California	California	BFS-283
	Trivittatus	

Table 23 - Stock Arboviruses Maintained in the S.F. Laboratory (continued)

Group	Virus	Strain
Simbu	Sathuperi	G-11155
Anopheles A	Anopheles A	
Turlock	Turlock	S-1954
	Umbre	G-1424
Ketapang	Bakau	AMM-2325
	Ketapang	AMM-2549
Ungrouped	Anopheles B	
	Chandipura	I-653514
	Colorado Tick Fever	Florio
	Lanjan	TP-94
		TP-123
	Wad Medani	EgAr 492
		IG-673