ECOLOGICAL INVESTIGATIONS PROGRAM
ARBOVIRAL DISEASE SECTION
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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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IMPORTANT NOTICE: 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 this exchange must be authorized directly by the person or agency which submitted the text.

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REPORT OF THE CHAIRMAN OF THE SUBCOMMITTEE ON ARBOVIRUS INFORMATION EXCHANGE

The Arthropod-borne Catalogue.

Accompanying this issue (No. 14) of the Information Exchange is what has been termed a Special Report on the Catalogue, comprising a synopsis of data extractable from the Catalogue, mainly by means of the marginal key-sort method.

This represents the third Special Report. The first, including 139 viruses, was made following the first revision of the Catalogue and was distributed with Issue Number 8 of the Infoexchange, September 1963. The second, including 160 viruses, was made following the second revision of the Catalogue and was distributed with Issue Number 12 of the Infoexchange, September 1965. The accompanying third Special Report was prepared following the first quarter (April 1966) issue of Catalogue material and includes 182 viruses. Since the preparation of this report, registrations of nine "new" viruses have been received, making a total of 191. Registration cards of seven of these new viruses were distributed with the second quarter issue of Catalogue material and two more will be distributed with the third quarter (October) issue of Catalogue material.

The distribution of the Catalogue is shown in the Special Report. There have been no additions since this report was prepared.

To date, 1,980 abstracts from Biological Abstracts, 1,479 from Bulletin of Hygiene and Tropical Diseases Bulletin, and 213 personal communications have been coded and issued to recipients of the Catalogue, making a total of 3,672.

Proposed International Advisory Committee.

With the approval of the American Committee on Arthropod-borne Viruses (ACAV), under which this Subcommittee serves, the recipients of the Catalogue were circularized last November for opinion on appointment, with the assistance of the WHO, of an International Advisory Committee to review and make recommendations for the improvement and future conduct of the Catalogue. At the time it was anticipated that a meeting of this proposed Advisory Committee would be held in Moscow and report to the Directors of the Arthropod-borne Virus Regional Reference Laboratories, who met there last July under the auspices of the WHO in connection with the International Microbiological Congress. Arrangements for the meeting of this Advisory Committee did not materialize, and consequently the ACAV has taken no action in the appointment of members.

The Question of Publication of the Catalogue.

Another matter which was presented to and discussed by the ACAV at a meeting in Washington last March was the publication of the Catalogue. Upon recommendation of the ACAV, contributors to the Catalogue have been canvassed for opinion. The replies were generally favorable. The question will be further discussed at a meeting of the ACAV scheduled for this month (October) in Washington, and will be presented to the open conference to be held 9:00-12:00 a.m., November 1, in connection with the meeting of the American Society of Tropical Medicine and Hygiene at San Juan, Puerto Rico. It is hoped that all those who may be interested will attend.

REPORT OF THE SUBCOMMITTEE ON LABORATORY ACQUIRED INFECTIONS

The Subcommittee on Laboratory Acquired Infections has summarized in tabular form reports from a survey that it has conducted this past year. The new information was put into context with earlier information collected by the American Public Health Association. In spite of the inadequacies of the questionnaire method, certain conclusions can be drawn from the data. The Committee invites further reports from laboratories and also would welcome comments concerning the presentation of the information.

A working document based on replies to the questionnaire on Lab-acquired arbovirus infections was studied by the Subcommittee at a meeting in Washington in April. A full report from which the following table was taken is being prepared for submission at the annual meeting of the American Committee on Arthropod-borne Viruses to be held in November in San Juan. It will subsequently be made available for distribution to recipients of the Information Exchange. At that time, publication of the unrestricted information in a more widely accessible form will be considered.

The Communicable Disease Center has proceeded with its program to produce human gamma globulins wich will contain antibody to agents that induce laboratory infections. The ACAV Committee on Laboratory Acquired Infections is acting as a medium for transmitting information to CDC on individuals who have high titer antiserum to specific arboviruses and who might act as donors. Dr. Work will report on the CDC program at the meeting in San Juan.

SUMMARY OF CASES OF LABORATORY-ACQUIRED INFECTIONS WITH ARBOVIRUSES

Virus	ACAV Cat. No.	Committee Obtaining Information			Totals
		APHA/CLIA*	ACAV/SLI		
			Total No. of Cases	Cases Not Previously Recorded†	
Apeu	52	1	1		1
Bunyamwera	7	4			4
Chikungunya	89	13	12	6	19
Colorado Tick Fever	74	7	6	1	8
Dengue		4	4	2	6
Eastern EE (EEE)	100	2			2
Germiston	13	3	3		3
Japanese E (JBE)	119	1	1		1
Junin	77	2	3	3	5 (1)**
Kunjin	67	2	1		2
Kyasanur Forest Disease	45	9	60	56	65
Louping Ill	80	19	3	2	21
Machupo	37	1	1		1 (1)
Marituba	50		1	1	1
Mayaro	97	1	3	2	3
Mucambo	148	2	2		2
Omsk Haemorrhagic Fever	156	2	1	1	3
Oriboca	51		1	1	1
Oropouche	24		2	2	2
Piry	28	1	3	3	4
Powassan	94	1	1		1
Rift Valley Fever	6	27	2	2	29 (1)
Rio Bravo	72	5	5		5
St. Louis Encephalitis	102	1			1
Spondweni	4	2	2		2
Tick Borne Encephalitis:					
Absetterov	155		1	1	1
CEE		2	1	1	3
Hypr	154		4	4	4
Omerzu		2	2		2
RSSE	152	4	2	1	5 (2)
Slovenia BS			2	2	2
Stillerova ?			1	1	1
Venezuelan EE (VEE)	112	91	46	26	117 (1)
Vesicular Stomatitis	142	38	1		38
Wesselsbron	3	2	2	2	4
West Nile	1	3	8	8	11
Western EE (WEE)	61	6			6 (2)
Yellow Fever	75	15	1	1	16 (1)
Zika	93	1			1
18 AR 1742 (Unidentified)			1	1	1
TOTALS		274	190	130	404 (9)

*American Public Health Association Laboratory Section Committee n Laboratory Infections and Accidents, S. E. Sulkin (Chairman), R. M. Pike and M. L. Schulze.

†Number of cases for which information first became available through ACAV questionaire.

**Figure in paranthesis refers to number of deaths.

EDITORIAL NOTE

Of the six internationally quarantinable diseases, four are arthropod-borne. Only one of these is an arbovirus disease - yellow fever. But in many respects yellow fever is of the greatest potential and actual importance, one reason being that it is transmitted by Aedes aegypti; the mosquito which has biologically adapted most successfully to association with the urban proclivities of man. During the past year yellow fever has erupted in a greater number of cases in more widely distributed situations than has been recognized for many years.

In reviewing this, the fourteenth issue of the Arbovirus Information Exchange, which contains a greater number of reports on a greater variety of arbovirus subjects than ever before, it is apparent that the magnitude of what has happened with yellow fever this past year has not come through. It is therefore considered to be of sufficient importance to bring this to the attention of the participants. Not only is this pertinent to what expert advisors to many governmental health authorities need to know, but it illuminates needs for better epidemiological reporting, development of surveillance commensurate with augmented arbovirus facilities and modern techniques, and actually, for further field and laboratory investigations to supply solutions to previously unsolved or newly emerging problems.

Several times in the past half century, it has been assumed that enough was known about yellow fever to make its elimination as a human disease amenable to application of simple public health measures of Aedes aegypti control or vaccination Several times some new feature of the virus, the vector or change in behavior of the human host has brought the problem of yellow fever back again as a subject which requires further special attention of specially qualified medical scientists.

In this issue is a map of the occurrence of yellow fever in Africa and South American in 1965, prepared by the World Health Organization, on the basis of official reports. It should be emphasized that this is a minimum documentation because it is based on official information. Scientific information and information from scientists expands this considerably, and brings what knowledge is available to the point where it is the subject of this note.

From mid-October to mid-December, 1965, Senegal in West Africa was the site of a major epidemic of yellow fever. Following the extensive vaccination campaigns begun in the late 1930's, Senegal had recorded only sporadic cases of yellow fever until 1965. In 1960 the periodic vaccination campaigns were discontinued and by

1965 there was a growing population of non-immune young children. The epidemic centered around the city of Diourbel, 125 kilometers east of Dakar. Although the official number of reported cases is 238, the actual number was much higher. Serological surveys carried out in Senegal suggest that at least 5,000 human infections occurred during the epidemic. Aedes aegypti mosquitoes were prevalent in the Diourbel region breeding in domestic water containers. The method of introduction of virus into the epidemic region is unknown, although it is suspected that it accompanied migratory workers from Portuguese Guinea where epidemic yellow fever had been reported earlier. To prevent spread of epidemic yellow fever from the Senegalese hinterland into Dakar, prophylactic vaccination of children with mouse brain vaccine (because sufficient 17D was not available) was followed by reports of many cases of encephalitis and a number of deaths.

The shift in public health practice following change in the status of former colonial territories has resulted in decrease or cessation of routine vaccination against yellow fever. This has led to accumulation of non-immunes over vast areas in Africa, susceptible to exposure to potential or actual intrusion of yellow fever virus from a sylvan or human transported infection.

Rescheduling of priorities, wrought by political changes, and the population explosion has separated more rural residents from the routine vaccination practices of public health programs. This is what led to the spill of jungle yellow fever into residents of Paraguay, Brazil, Argentina and Bolivia, even though Aedes aegypti has been eradicated from southern South America.

Dr. Vilches' informative report on page 22 about the yellow fever outbreak in Argentina gives some insight into what was obviously occurring in adjacent and other areas of South America where as competent observation or capable action was not as readily available. The search for, and resultant difficulty, in obtaining adequate supplies of 17D vaccine exemplified the serious problem of vaccine availability in the face of geographically extensive yellow fever. In fact, Argentinian health authorities had a difficult time filling their needs. The Rio de Janeiro laboratory had previously been tapped for contributions to Portuguese Guinea and Senegal and the subsequent needs of Brazil soaked up their reserves. Following generous PAHO support from the Bogota laboratory reserve, Argentina finally had to obtain the remainder of their need by purchase at substantial expense from resources abroad.

There are two available prophylactic vaccines for yellow fever. 17D must be carefully refrigerated and administered immediately to prevent loss of potency Scratch applied mouse neurotropic vaccine in gum arabic is used at ambient temperature for longer

periods without significant loss of potency. The mouse brain vaccine has been associated with a significant number of cases of encephalitis particularly in children so that there is a question regarding use for primary vaccination of children. Post vaccinal encephalitis following 17D is extremely rare, most frequently occurring with subsequent recovery in children less than one year of age. However, a case of fatal encephalitis in a three year old American child twelve days following vaccination with 17D - virus isolated from brain tissue that showed histological lesions of viral encephalitis - occurred in North Carolina in October 1965.

Obviously, neither vaccine is the ideal for use in many tropical areas where the risk of yellow fever is greatest. The problem of prophylactic vaccination of those most exposed to yellow fever is therefore not yet solved.

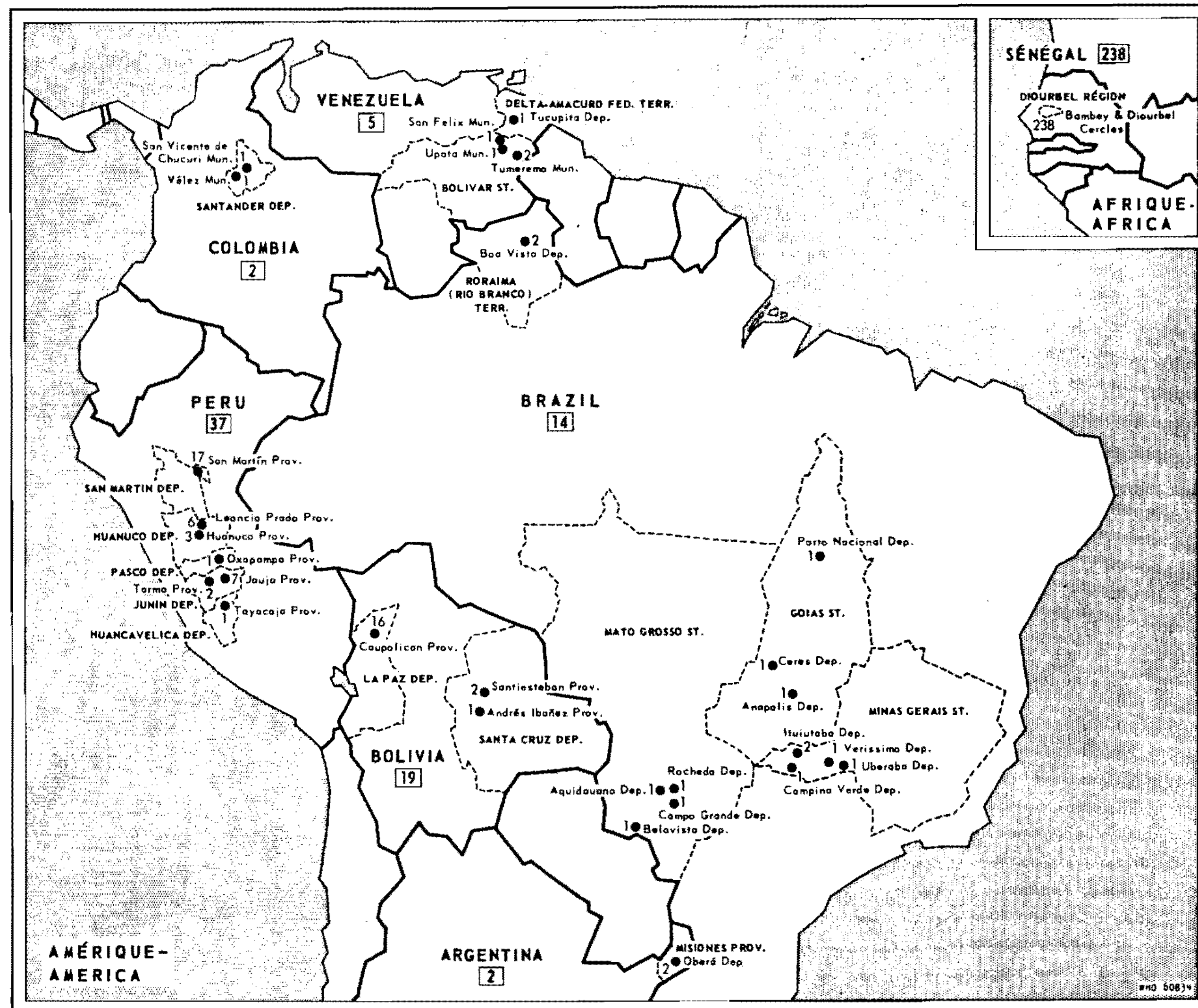
Maps of the Endemic Yellow Fever Areas of the World, based largely on the mouse neutralization test survey with relatively few sera collected more than a quarter century ago, and more recent official reports of cases, is the presently used authoritative reference for quarantine and vaccination requirements, and for a variety of other public health purposes. In the age of airborne safaris and jet propelled international transportation, it is remarkable that so much faith is placed in the classic diagrams.

With recently developed techniques and concepts, a more accurate and timely official documentation of endemic yellow fever areas of the world might be sought through collaboration of arbovirus laboratory personnel and facilities that have joined already existing resources in recent years. These facilities are capable of obtaining significant up-to-date information on the immune status of exposed populations and evidence of recent activity of yellow fever virus. They can participate in an international field and laboratory surveillance that has become of obvious urgency following the eruptions of yellow fever in vast areas of Africa and South America in the past year.

Such a surveillance system might be accomplished through the developing network of WHO Reference and Collaborating Laboratories, or by a collective effort of laboratories and professionals in the areas most directly concerned. Whatever the means, it is now clear and urgent that the outstanding needs for a safe vaccine procedure useful in remote areas, up-to-date definition of endemic areas and effective continuing surveillance for yellow fever should occupy priority attention of participants in this world wide exchange of information on arthropod-borne viruses, and the diseases they cause.

Telford H. Work, M.D.
Editor

FIÈVRE JAUNE 1965 — YELLOW FEVER 1965



From the RELEVE EPIDEMIOLOGIQUE HEBDOMADAIRE WEEKLY EPIDEMIOLOGICAL RECORD.

Cas notifiés (chiffres provisoires) Cases notified (provisional figures)

REPORT FROM THE VIRUS DISEASES UNIT
WORLD HEALTH ORGANIZATION, GENEVA, SWITZERLAND

The World Health Organization called a meeting of Directors of Arbovirus Reference Centres in Moscow from 19 - 22 July 1966.

The programme of production and distribution of reagents was discussed. The International Reference Centre at New Haven reported that a large number of polyvalent arbovirus immune reference ascitic fluids had been prepared or are in preparation. Certain broad grouping immune ascitic fluids are presently available for immediate distribution by the International Centre to the Reference Centres and other arbovirus laboratories.

A large number of immune ascitic fluids specific for single arbovirus types have also been produced, but a comprehensive testing for cross relationships to other arboviruses has not been completed for many. These specific reference immune substances will be available in minimal quantities in response to documented requests. Larger amounts of polyvalent immune substances will be available.

Some of the reference centres and collaborating laboratories have prepared non-infectious antigens for WEE, EEE, VEE, Sindbis, Semliki Forest, Middelburg, chikungunya, Japanese encephalitis, West Nile, RSSE, and KFD viruses. Non-infectious antigens for other viruses are in preparation.

Arbovirus laboratories are encouraged to request some of these inactivated antigens for evaluation of titer and practical utilization, reporting their findings to the laboratory source of the antigens and to WHO.

The directors agreed that the initiation of a reporting system which would reflect the occurrence of arbovirus disease and arbovirus activity in human and non-human hosts was desirable. The prime purpose of such a reporting system should be to inform every reference centre of the findings made in their zone of responsibility by other centres concerning the activity of new viruses or new forms of activity of already known arboviruses; also, to try to obtain information on the geographical and quantitative extension of the zone of activity of the more important arboviruses.

Two somewhat different forms which will provide detailed information on individual cases of disease resulting from different arboviruses and a more comprehensive assessment of certain recognized arbovirus diseases on a regional scale will be prepared by the secretariat and circulated among directors of reference centres for their comments.

The group discussed the information obtained from a canvas by the Virus Unit on the availability of immune substances in different laboratories in the world. It was agreed that human immune globulin was the most promising substance in providing effective protection of laboratory workers if given soon after accidental infection.

Specific offers of immune gamma globulin within the next year were made by some directors of reference centres and other arbovirus laboratories. As soon as these materials become available, WHO will notify the regional centres of points where emergency requests can be directed and will facilitate requests for prophylactic immune substances in emergency situations. To avoid wastage of limited supply, documentation of the circumstances requiring the material will be necessary before release will be considered.

REPORT FROM MEDICAL ZOOLOGY DEPARTMENT
UNITED STATES NAVAL MEDICAL RESEARCH UNIT NUMBER THREE (NAMRU-3)
CAIRO, EGYPT

Arrangements are presently underway to establish a new Virus-vector Laboratory aimed especially to investigate biological inter-relationships between tick-borne viruses and their hosts. A secondary objective is to make preliminary isolations of viruses from various tick species to furnish specialists in viral characterization with comparative research material in this field. The new laboratory is headed by Dr. Makram N. Kaiser. Also underway in the Medical Zoology Department is an extensive collaborative investigation between NAMRU-3 and the Rocky Mountain Laboratory, partly supported by NIH funds, on the biochemistry of ticks at different stages in their life cycle. The purpose of this study is to determine the internal environment in relation to survival of pathogens in ticks and to assist in development of media for tissue culture investigations.

(Harry Hoogstraal and Makram N. Kaiser)

REPORT FROM THE U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3
FIELD FACILITY, ADDIS ABABA, ETHIOPIA

Through an agreement signed by the United States and Ethiopian Governments on December 30, 1965, the U.S. Naval Medical Research Unit No. 3 (NAMRU-3) has established a Field Facility in Ethiopia. The mission of the daughter unit will be to conduct a program of basic and applied research on tropical diseases prevalent in Ethiopia and adjacent countries. The Facility's program will be directed by Dr. Jack R. Schmidt, formerly Head of the NAMRU-3 Virology Department in Cairo. Base laboratories are located on the grounds of the Imperial Central Laboratory and Research Institute in Addis Ababa, and a collecting station has been set up in Gambela, situated in the western lowlands about 100 miles east of the Sudan border. A major part of the Facility's research effort will be devoted to arthropod-borne diseases, e.g. arbovirus infections, malaria, onchocerciasis and other filariases. It is hoped that the Facility will serve as a base of operations not only for Navy scientsts but also for other investigators interested in diseases prevalent in this part of Africa. Persons interested in conducting collaborative work at the NAMRU-3 Field Facility are invited to contact Dr. Schmidt for further information.

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE ENTEBBE, UGANDA

During 1965 nearly 16,000 mosquitoes from the Entebbe area, including 5,500 Aedes africanus (Theobald) were processed for virus content without the isolation of a single virus strain. Sentinel mouse studies with 200 litters were also unprofitable. In contrast, a small collection of 136 ticks from Karamoja yielded two agents from Amblyomma ledpidum Donitz. These two agents appear to be clearly viruses and preliminary studies show that they are unrelated to the 5,640 African arboviruses in our collection.

Isolation studies with bat viruses have continued to yield Dakar Bat and Bukalasa Bat viruses from salivary glands and saliva of Tadarida condylura (A. Smith) and T. pumila (Cretzschman) respectively. Five hundred specimens from Tadarida species were processed with negative results. A virus (BP 846) has been isolated from the salivary glands of a Rhinolophus hildebrandtii eloquens (K. Anderson) collected on the slopes of Mount Elgon. BP 846 is ether sensitive, maintained in Aedes aegypti and unrelated to any African arboviruses in our collection. It is, therefore, considered to be a hitherto undescribed arbovirus.

In tissue culture, attempts have been made to grow 28 different arboviruses in a variety of cell systems. Primary baby hamster kidney cells appear to give the best results, and the primary isolation of chikungunya and o'nyong-nyong viruses from acute human sera has been made with this cell line.

Study of the behaviour of Aedes simpsoni (Theobald) has started again. Blood meals have been collected from wild fed mosquitoes collected in the Kampala area where this species does not bite man. Precipitation studies show that the host is mammalian.

(M.C. Williams)

REPORT FROM THE VIROLOGICAL SECTION OF THE DUTCH MEDICAL RESEARCH CENTRE NAIROBI, KENYA

The Research Centre was officially opened on the 18th of March 1966. Part of the work of the Virological Section will be devoted to arboviruses. Isolation attempts from mosquitoes were initiated. An area near Mombasa was chosen for the first experiments, because the climate of the warm and humid coastal region was considered to be more promising than that of the plateau-area of Kenya.

A number of gravid mice were transported to an existing field laboratory and the litters inoculated with suspensions of the collected mosquitoes. Collections were performed either on human bait or by means of nets in the daytime resting places. The experiment lasted for five weeks. Twenty-nine mosquitoe species were represented in a total number of 11,559 specimens. Of fourteen species the numbers collected were small and these were not used for isolation attempts. The remaining fifteen species were suspended in 69 pools and inoculated.

Two virus strains were isolated from different pools of Aedes pembaensis. Their behaviour in baby mice suggests that they might be identical. They are being investigated in the Nairobi Laboratory. The table summarizes the results of the experiment.

(D. Metselaar)

TABLE

Mosquito species	Number collected	Number of pools	Number of isolations
Mansonia (Mansonioides) africana	17	2	0
" " uniformis	16	1	0
Aedes (Stegomyia) aegypti	11	1	0
" " simpsoni	5	1	0
" " metallicus	13	1	0
" (Aedimorphus) argenteopunctatus	8	1	0
" " albocephalus	467	7	0
" (Skusea) pembaensis	7605	22	2
Eretmapodites subsimplicipes	25	1	0
Culex (Culiciomyia) cinereus	9	1	0
" (Culex) sitiens	1230	10	0
" " fatigans	166	5	0
Anopheles (Anopheles) coustani coustani	82	4	0
" (Cellia) gambiae	1854	10	0
" " funestus	29	2	0
Total, 15 species	11537	69	2

REPORT FROM ARBOVIRUS RESEARCH UNIT
SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH
JOHANNESBURG, SOUTH AFRICA

Results we have obtained on the vector capability of *Culex fatigans* with Sindbis and West Nile viruses are of interest in that they showed an apparent difference in susceptibility between two colonies of this species. Colony A originated from material collected in Johannesburg and had been maintained with random mating in the laboratory about five years before the present tests. In 1964 the colony died out from undetermined causes and colony B was then started from material collected on the outskirts of Johannesburg and was also maintained by random mating. It had been in the laboratory for nine months before being tested.

In the tests an attempt was made to work quantitatively by infecting mosquitoes with 10-fold graded doses of virus and thereafter testing for infectivity rates (proportion infected on 21st day to number tested for virus), and also for transmission rates (proportion of mosquitoes transmitting to those engorging on susceptible chickens). The transmission rate was determined only with the mosquito group infected with the highest viral dose in each instance. Transmission was attempted between the 18th-21st days. With the Reed and Muench method an infectivity threshold was calculated which was the virus dose required to infect 10% of the mosquitoes.

Th Table shows the results obtained with both colonies. It will be noted that with Sindbis virus the infectivity threshold rose from 2.8 logs with colony A to 4.5 logs with colony B and with West Nile virus it rose from 3.0 logs to 5.9 logs. The lower vector capability of colony B is also apparent when the transmission rates obtained with both viruses are compared. Despite lower infecting doses colony A showed higher transmission rates.

Unfortunately the results are not strictly comparable as slightly different methods of infecting the two colonies were used and the reproducibility of the results is unknown. However, the differences are so marked that it would seem likely that genuine differences in viral susceptibility are involved. If so, the applicability of transmission experiments with laboratory-maintained colonies to other colonies and feral populations of the same species is open to question. Craig (Bull. Wld. Hlth. Org. 1964, 31, 469) has recently discussed the genetic aspects of this problem.

Table. Comparison of susceptibility of two laboratory colonies of <u>Culex fatigans</u> to Sindbis and West Nile viruses.

	Colony A				Colony B			
Virus	Virus dose	Infectivity rate	Infect. threshold	Transmission rate	Virus dose	Infectivity rate	Infect. threshold	Transmission rate
Sindbis	4.7	18/5[a]	2.8[b]	3/4[c] (75%)	4.8	4/29	4.5	2/32 (6%)
	3.7	8/6			3.8	0/25		
	2.7	2/13			2.8	0/25		
	1.7	0/21						
W. Nile	5.5	7/0	3.0	4/5 (80%)	6.8	18/6	5.9	5/13 (2[illegible]%)
	4.5	-[d]			5.8	0/7		
	3.5	2/7			4.8	1/14		
	2.5	0/6			3.8	0/14		

a - numerator = No. mosquitoes infected, denominator = No. mosquitoes not infected.

b - virus dose required to infect 10% of mosquitoes.

c - numerator = No. mosquitoes transmitting, denominator, number feeding. (only mosquitoes feeding on highest virus dose tested)

d - No. mosquitoes fed in the group.

REPORT FROM THE CAMEROON PASTEUR INSTITUTE

I. Isolation.

During the last two years, we have used two principal sources:

- febrile patients
- mosquitoes

As far as human diseases are concerned, in 1964 we inoculated the serums of feverish patients entering the hospital. Since the beginning of 1965, we have been paying particular attention to children under five years old under treatment at the Maternal and Child Protection Centre in Yaounde.

With regard to mosquitoes, systematic day and night captures were carried out, using human 'bait' and also nets, in the region of Yaounde. The insects are grouped according to the species, date and place of capture. The females are kept at temperatures of -65° up to the moment of preparing the inoculum.

In 1964

233 serums were inoculated: no strain was isolated
11,741 mosquitoes in 234 pools

Nine strains were isolated:

Strain	
YM 21-64	
YM 43-64	
YM 59-64	identified with virus NTAYA or a closely related virus.
YM 158-64	
YM 179-64	
YM 3-64	
YM 20-64	virus MIDDLEBURG
YM 175-64	virus SPONDWENI

YM 50-64 a virus which cannot be placed in a group, differing from the arboviruses of the Dakar Pasteur Institute and from the collection of the East African Virus Research Institute at Entebbe.

In 1965

338 serums inoculated
1 strain isolated: YS 190-65 from a young European girl of

13 years infected with exanthematic fever. The first results of identification direct the diagnosis towards the Bunyamwera group. Actual identity appears to be Ilesha virus.

7,579 mosquitoes in 117 pools
4 strains were isolated:

YM 25-65 YM 52-65	virus BUNYAMWERA
YM 31-65 YM 34-65	in process of identification

Table Summarizing the Isolations Beginning with the Mosquitoes

No. of batch	Species and Number	Place of origin	Strain	Date of capture	Date of inoculation
YM 3/64	479 Mansonia africans	Akonolinga	Middelburg	3/12/64	3/13/64
YM 20/64	14 Eretmapodites gr. chrysogaster 1 E.gr. inornatus 10 Aedes gr. tarsalis 3 A. kummi 3 A.gr. palpalis 1 Mansonia pseudocono-pas	Nkolbisson	Middleburg	4/13-14/64	4/16/64
YM 21/64	32 Culéx spp.indét.	Nkolbisson	Ntaya	4/13-14/64	4/16/64
YM 43/64	2 Culex albiventris 24 Culex spp.indét.	Nkolbisson	Ntaya	5/26/64	5/27/64
YM 50/64	15 Eretmapodites gr. chrysogaster	Okola	?	5/21/64	6/2/64
YM 59/64	4 Culex albiventris 29 Culex spp. indét.	Nkolbisson	Ntaya	6/2/64	6/6/64
YM 158/64	27 Culex nebulosus	Ofoumselek	Ntaya	9/25/64	9/30/64
YM 175/64	31 Eretmapodites gr. chrysogaster	Ofoumselek	Spondweni	10/16/64	10/22/64
YM 179/64	19 Culex cinerus 4 C. telesilla 1 C. moucheti 1 C. tigripes 11 C. albiventris	Ofoumselek	Ntaya	10/16/64	10/22/64
YM 25/65	52 Aedes gr. domesticus	Nkolbisson	Bunyamwera	from 10/14/64 to 3/12/65	3/18/65
YM 31/65	17 Eretmapodites leucopus	Nkolbisson	?	from 10/26/64 to 4/14/65	4/20/65
YM 34/65	133 Culex telesilla	Nkolbisson	?	4/2 & 4/6/65	4/20/65
YM 52/65	4 Aedes gr. tarsalis 1 A. simulans 2 A. capensis 1 A. mutilus 1 A. kummi	Centrale electrique de Yaounde	Bunyamwera	4/30/65	5/22/65

II. Serological Survey.

Two distinct serological inquiries took place, one in the region of Bouar in the Central African Republic in collaboration with the Pasteur Institute of Bangui. The findings will be reported by that Institute. Another inquiry took the whole of Cameroon for its subject, 3,000 serums being collected in different areas distinguished by geographical and climatic features.

At first only the reaction of inhibition of hemagglutination was used.

The following reference strains were used in the preparation of antigens:

Chikungunya, Sindbis, O'nyong-nyong, Middelburg, West Nile, Yellow Fever, Zika, Uganda S, Ntaya, Spondweni, Bunyamwera.

The complete results will be published.

Fundamental differences exist between the North (dry savanna zone) and the South (rain forest).

In the southern forests, the Bamileke country (a humid mountainous area) and the wooded savanna, the activity of the arbovirus, similar in these three regions, is weak. There are few traces of group A virus. In group B the proportion of yellow fever antibodies is most important (vaccination campaigns), the other group B viruses only rarely intervening.

In the north of Cameroon: consisting of the high plateaux in the Adamawa region and desert-like savanna, a recent epidemic caused by a group A virus (probably O'nyong-nyong) left its serological mark (60 to 80% of positive serums). The incidence of yellow-fever is low (from 1 to 12% of positive serums) except near urban centres where the percentages are considerably higher (vaccinations).

The other group B viruses occur more frequently than in the south, in particular the Zika virus: from 20 to 60% of positive results being obtained according to the area. The intervention of the virus Bunyamwera is weak but certainly occurs.

(H. Brottes, J.J. Salaun and A. Rickenbach)

REPORT FROM ARBOVIRUS RESEARCH PROGRAM
UNIVERSITY OF IBADAN, NIGERIA

Late in 1965 virus AR 7920 was isolated from the blood of a goat at the Ibadan abattoir and identified as related to the Congo-Semunya-Nakiwogo agents by CF testing with antisera obtained from Entebbe.

Subsequently a serological review of agents stored for identification revealed that six unrecognized isolations had been made previously in the Ibadan laboratory. One of these was AR 1392 from _Hyalomma truncatum_ obtained from cattle in September 1964, among the first 10 tick pools inoculated for virus isolation attempts. Two agents were obtained from these samples. One proved to be serologically identical with many subsequent isolates, of which AR 1792 became the prototype. The other was stored as apparently unrelated to other isolates from Nigeria, or to any agent for which antiserum was then available. Two more tick isolates, one from _Boophilus decoloratus_, collected on sheep in December 1964 and another from _Hyalomma rufipes_ from cattle in June 1965, as well as three isolates from blood of cattle at the slaughterhouse, were found to be antigenically similar to Congo by CF testing. There are thus seven isolates of Congo-like agents among the 463 agents obtained and identified during 18 months of arbovirus surveillance in Nigeria.

Agents Semunya and Nakiwogo of the Congo group (Annual Report 1963-64, East African Virus Research Institute) had been registered in the arbovirus catalog in 1961 but later were withdrawn by the authors at Entebbe because of failure to grow in mosquitoes. The isolation of similar agents in Nigeria from ticks and cattle blood strongly suggests the tick as a vector of these agents and supports their inclusion as arboviruses. The prototype isolate in Nigeria is AN 7620 from goat blood, although the first isolation was made actually more than a year earlier from ticks as AR 1392.

REPORT FROM THE INSTITUTO NACIONAL DE MICROBIOLOGIA
BUENOS AIRES, ARGENTINA

Outbreak of Sylvatic Yellow Fever, Corrientes and Misiones Provinces.

During the last week of February, 1966 materials from cases of a disease compatible with a diagnosis of yellow fever were received from the northeast tip of Argentina, a region where the

two provinces of Misiones and Corrientes border with both Brazil and Paraguay. Within 24 hours the diagnosis was confirmed by histopathological examination of the liver specimen from one of the lethal cases. A group of workers from the Institute moved then to the affected area and verified that:

1) There were three foci of human cases of a febrile disease likely to be sylvatic yellow fever (see tables). They were located at a distance between each other of approximately 60 to 90 miles. All cases were within the Uruguay basin, i.e. S.E. of the Misiones range (height 200-800 m):

a) The northernmost focus was in the Department of San Pedro, Misiones province. All cases were located within an area of 24 sq. miles south and east of the town of San Pedro. This was a typical subtropical forest area either unexploited or devoted to lumbering. It is 22 miles distant from the Brazilian border (Pepiri-Guazu river) near the point of highest altitude in the area (about 600-800 meters). The earliest case reported in this area occurred on January 31.

b) The second focus within the province of Misiones was about 90 miles southwest of San Pedro, in the Department of Obera; the cases were distributed within an area of 150 sq. miles mainly to the west, northwest and southwest of the town of Obera, which is the second in importance within the province. This area is partly covered with forest with many clearings devoted to agricultural production such as tea, yerba mate and tung. Retrospective analysis of case histories revealed the occurrence in this area of cases compatible with diagnosis of YF back in December 1965 and early January 1966.

c) The third focus was in the northeast of the province of Corrientes (Department of Santo Tome) within the forest marginal to the Uruguay river and to its affluents Ciriaco and Garavi Brooks, which are surrounded by gallery forest.

Whereas all of the male cases in the other two areas were either agricultural workers or lumbermen, five of the twelve reported cases in this focus were privates of the coast guard detachments covering the Uruguay river.

2) Diagnosis was confirmed by histopathology of liver in eight cases, by complement fixation in 20 cases and by virus

isolation in four cases. The isolated viruses were identified by cross-complement fixation and cross-neutralization using anti-17D antisera produced in our laboratory and reference YF anti-serum provided by Dr. J. Casals (Yale's Rockefeller Foundation Laboratory).

3) A serological survey of about 200 unvaccinated people within the forest detected YF-complement fixing antibodies in only three persons living in areas very near to the reported cases.

4) The primate population at the time of our visit to the area was very scanty. Many reports from the local people gave indications of abnormal silence of the forest monkeys (A. caraya, A. guariba, Cebus apella vellerosus) in late December and January, followed by observation of dead specimens. This could not be directly verified by us, but is in agreement with the reduction in number of the monkey flocks, usually abundant and noisy.

5) Entomological studies were made in March 1966 in the areas of San Pedro and Obera. They showed a low density of culicineae with very scanty Haemagogus sp., Aedes leucocelaenus and Sabethes chloropterus, the known vectors of sylvatic yellow fever. Fifty-nine pools of mosquitoes collected near San Pedro (making a total of 1,412 specimens) were inoculated into baby mice. Only one viral isolate was obtained from a pool of Aedes serratus, which is not YF virus; it has not yet been identified.

6) Administration of 17D strain YF vaccine to the human population of whole area under risk was initiated in the last days of February with emphasis in the three epidemic foci. The last notified case, which was later confirmed, had its first symptoms on March 11, 1966. By March 25, 1966, more than 450,000 people had been vaccinated, and the remaining sparse population was being traced for vaccination and blood sampling for the serological survey.

7) On only one occasion had a yellow fever case been confirmed in the region. This was a lethal case with characteristic liver lesions which occurred in 1948 in the locality of Cerro Azul, a few miles southwest of Obera (Blaksley and Del Ponte); surveys from 1940 to 1950 have shown the occasional presence of YF neutralizing antibodies (Bejarano).

GEOGRAPHICAL DISTRIBUTION OF NOTIFIED CASES

BY CLINICAL CLASS AND LABORATORY CONFIRMATION

Location		Clinically typical			Clinically atypical º	Total
		Lethal	Moderate or severe	Mild		
San Pedro, Mis.		6	3	0	5	14
Oberá, Mis.		9	5	4	17	35
Santo Tomé, Corr.		3	0	7	3	13
Total		18	8	11	25	62
Total confirmed ºº	HP	8/8	-	-	-	8/8
	Virus isol.	1/1	2/2	1/4	0/2	4/9
	CF	-	6/6	11/11	2/9	19/26

º Clinical suspicion of YF based mainly on febrile disease within epidemic environment, with or without other elements of the classical YF picture.

ºº Number confirmed/number studied. HP = histopathology of liver CF = convalescent serum sample with CF titer higher than 1/8.

AGE AND SEX DISTRIBUTION OF CASES

Age group	Clinically typical and/or confirmed cases		Atypical and unconfirmed cases		Total
	Male	Female	Male	Female	
0 - 9 years	0	0	4	0	4
10 - 14 years	2	1	2	0	5
15 - 19 years	6	1	4	2	13
20 - 39 years	18	3	4	3	28
over 40 years	7	1	4	0	12
TOTAL	33	6	18	5	62

Argentine Hemorrhagic Fever Studies in Pergamino County, 1965 and 1966.

A cooperative study with the Center for Medical Education and Clinical Research (CEMIC) was initiated in 1965 to study the clinical and physiopathological aspects of the disease (with the support of an NIH grant) as well as the virology, epidemiology and ecology of it and related subjects in the county of Pergamino. No cases had been reported there in 1963 or before.

During the 1964 epidemic season (March to September with a peak in June-July), more than 3,300 cases were notified in the whole epidemic area, which was larger, and extended further North than in previous years. One hundred and twenty four cases were reported in Pergamino, most of them in the southern half of the county. Systematic attempts to confirm the etiological diagnosis in each case were not made in 1964.

1965 was a year of low general incidence of AHF. Thirty-nine cases were reported in Pergamino. All of them were thoroughly studied and 18 were confirmed serologically or by viral isolation. All confirmed cases (17 males and one female) were older than 17 years, and lived and worked in the southern half of the county. Viral isolates of human origin were: eight from blood, one from urine (out of 12 attempts), and several from necropsy material obtained from a deceased patient (cerebral cortex, corpus striatum, kidney, lung, adrenals and several lymph glands). No isolates could be obtained from pharyngeal swabs, in spite of repeated attempts with material from twelve confirmed cases.

Virus was isolated from everyone of the small wild rodent species prevalent in the agricultural areas (*Akodon azarae* 7+/33, *Akodon obscurus* 3+/14, *Calomys laucha* (including *C. musculinus*) 4+26, *Oryzomys flavescens* 1+/59, and *Mus musculus* 2+/30, as well as from two out of four specimens of *Lepus europaeus* captured in the field. No isolates were obtained from 18 *Cavia sp.* specimens.

Systematic studies on ecological grids and line traps placed around the home and working place of cases, showed that the population density of small rodents was low everywhere, in contrast with the tremendous numbers of Cricetidae (mainly of the geni Akodon and Calomys) observed in 1964 until late July, when a sudden decrease occurred.

Acari collected from field rodents were pooled according to place of origin and species, fasted for three days (to allow for digestion of recent blood meals), repeatedly washed for two hours with buffer solution pH 4(to destroy any surface contaminating virus), centrifuged, ground in BSS calf serum mixture pH 7, and

inoculated into baby mice. Out of 19 pools, only one (consisting solely of Eubrachilaelaps rotundus) gave origin to a viral isolate which proved to be Junin virus. In spite of the precautions taken, the significance of this finding as to the role played by acari in the trasmission of the disease is still disputable. Active transmission experiments are being planned with laboratory bred rodents and acari of the incriminated species. Preliminary experience with Mus musculus albinus (laboratory mice) indicate that the disease can be transmitted by Ornitonissus bacoti (not yet found among the field rodents in the area of AHF) from artificially infected baby mice to susceptible baby mice, by bite. Baby mice can be infected by intracutaneous route and by oral route with Junin virus (recently isolated) in dilutions similar to those infective by intracerebral route. Orally infected baby mice excrete the virus in the urine at varying time from the 7th to the 19th day after administration.

In 1966, the number of cases has been considerably higher than in 1965. One hundred and eight cases have been reported up to June 30 (more than 9/10 of them after April 15). Out of 47 patients whose etiological study has been completed, 35 have been confirmed either serologically or by viral isolation. Seven Junin isolates were obtained from blood and one (out of 15 attempts in confirmed cases) from urine. Specimens from the remaining patients, including two necropsies and 28 spinal fluids, are under study.

Another rodent species has been added to those known to be infected in nature: Junin virus was isolated from one specimen of Rattus rattus captured in a grain storage shed. Thirty-two previous specimens had been studied with negative results.

The population of small field rodents began to increase in the southern ("dirty") part of the county during the second half of April, just before a sudden increase in the number of human cases. Ecological grid studies (capture, ear tagging and capture of small mammals in four square grids of about 6.8 hectares, i.e. 16 acres, with 286 traps each, set for rotating periods of two weeks each) show a definite predilection of small rodents for weed "reserves" during the low level periods, and for corn growing areas (which in contrast with wheat or sunflower, contain many gramineous weeds) during periods of higher rodent population. The significance of weeds and other edaphic and botanical factors is presently being studied by marking the captured and released rodents with radioactive gold (Au 198) needles, and by following their whereabouts for several days by means of a scintillation counter.

A serological survey among 300 permanent inhabitants of the county, detected two sera with CF titers of 1/8 and 1/16 in men with no history of AHF or a febrile acute disease in the last two

years (ages 50 and 54 years). Seven hundred and ninety-eight migrant workers were also surveyed: seven of them, with no previous history of AHF, had serum CF titers of 1/4 or 1/8.

Note. Pergamino County is located in the north of Buenos Aires province. Its head is Pergamino city (pop. about 70,000), which is 145 miles WNW of Buenos Aires. The rural population is approximately 30,000 distributed within 1,100 sq. miles.

REPORT FROM THE INSTITUTO DE MICROBIOLOGIA Y PARASITOLOGIA UNIVERSIDAD DE BUENOS AIRES BUENOS AIRES, ARGENTINA

Tacaribe Virus Infection of The Guinea-Pig

It has been demonstrated that Tacaribe virus is not pathogenic for adult guinea-pigs and is not known to be pathogenic for any other adult laboratory animal. In addition, guinea-pigs immunized with Tacaribe virus are resistant to Junin virus infection, although this animal is the most susceptible host to the latter.

The nature of the protective effect observed is not yet well understood. The purpose of this study is to ascertain whether the resistance to the challenge virus is due to a real inmunization or to a viral interference.

Guinea-pigs were inoculated with live Tacaribe virus by i.m. route. For a period of 48 days after inoculation, infectious virus was isolated from lymph nodes but not consistently. Virus is present in serum on the 7th day and in liver on the 13th day.

There is not immunity against challenge with Junin virus in the first three days, partial resistance appears after seven days and complete immunity afterwards.

The complement fixing antibodies against Tacaribe and Junin viruses are detected on the 13th day; the antibody titer is higher against the homologous antigen.

(Ceclia E. Coto, Edith Rey and A.S. Parodi)

TABLE 1

Virus isolation from lymph nodes, sera, urine and different organs of guinea-pigs, inoculated with 0,2 ml. of Tacaribe virus (see text)

Days after inoculation	Lymph nodes	Serum	Liver	Brain	Kidneys	Lungs	Urine
3	1/2(+)	2/3	0/1	0/2	0/1	0/1	0/1
7	2/2(x)	2/3	1/1	0/2	0/2	-	-
13	-	0/1	1/1(xxx)	-	-	-	-
16	1/2(xx)	0/2	0/2	0/2	0/2	0/1	0/1
30	0/2	0/2	0/1	0/2	0/2	-	-
48	1/1	0/1	0/1	0/1	0/1	-	-

(+) Positive isolation / Number of experiments

(x) Inguinal and axilar lymph nodes positive

(xx) Axilar lymph node negative - Inguinal lymph node: mice with convulsions but recovered.

(xxx) Mice with convulsions but recovered.

TABLE 2

Appearance of immunity against Junin virus in guinea-pigs infected with Tacaribe virus.

Days after the inoculation with Tacaribe virus	(x) Guinea-pigs inoculated with Tacaribe virus and Challenged with Junin virus	Normal guinea-pigs infected with Junin virus only (control)
3	4/4	4/4
5	1/6	5/5
7	2/5	5/5
9	1/4	5/5
15	0/5	5/5
30	0/10	10/10
48	0/2	2/2

(x) $\dfrac{\text{number of animals dead}}{\text{number of animals inoculated}}$

T A B L E 3

Appearance of C.F. antibodies against Junin and Tacaribe antigens at various days after infections with Tacaribe virus.

Days after inoculation with Tacaribe virus	C.F. titer (Junin antigen)	C.F. titer (Tacaribe antigen)
6	< 4/4	< 4/4
7	< 4/4	< 4/4
11	< 4/4	< 4/4
13	4/16(x)	4/16(x)
15	8/8	32/16(x)
19	32/16 (x)	64/16(x)
24	no done	64/64
48	16/16(x)	32/16(x)

(x) the antigen was diluted only to 1/16.

T A B L E 4

Relationship among virus recovery, C.F. antibodies and resistance against Junín virus infection in guinea pigs inoculated with 0,2 ml. of a 10^{-1} suspension of Tacaribe virus (titer in mice $10^{-7.3}LD_{50}$) in the posterior leg muscle.-

days after Tacaribe inoculation	Virus recovery from a pool of [illegible] guinea-pigs					C.F. titer(x)		Challenge with a $100LD_{50}$ of J.V.	
	inguinal lymph node	axilar lymph node	brain	kidneys	serum	Junin antigen (x)	Tacar. antigen	Immunized animals	Non immun. an. (Contr.)
7	+	+	N	N	N	<4/4	<4/4	0/5	5/5
16	(+)	N	N	N	N	32/16	32/16	0/5	5/5
30	N	N	N	N	N	32/16	32/16	0/5	5/5

N = negative

\+ = positive

(+)= mice with nervous symptoms but recovered.

(x)= $\frac{\text{serum dilution}}{\text{antigen dilution}}$

REPORT FROM THE INSTITUTO DE VIROLOGIA DE CORDOBA, ARGENTINA

Manfredi Area Studies

Since the last report in Infoexchange Issue Number 12, a fourth strain of virus was isolated from mosquitoes Aedes sp. caught in February 1966, one year later than the three previous strains. All four viruses are identical by complement fixation (CF) test. The prototype strain Cba AR 420 antigen, was negative in CF with the following immune sera: Group A, EEE, WEE, VEE, Mayaro, Una, Aura, Pixuna, Mucambo, Group B, SLE, Ilheus, Bussuquara and Yellow Fever. It was positive with Bunyamwera Group, Maguari and Guaroa immune sera. A block CF test showed that the strain Cba AR 420 is closely related to Maguari virus. These isolations confirmed previous serological Bunyamwera group activity evidence in this Province (Am. J. Trop. Med. and Hyg. 14, 1073-1078)

Junin Virus Studies

In 1963, Hemorrhagic Fever was recognised in the southeast of Cordoba Province by Junin virus isolation. Vanella (El Dia Medico, 34, 290; La Semana Medica, 125, 1502) reported 71 human cases during 1964 on clinical basis.

In 1965 there were 25 confirmed and five doubtful cases in man. Virus was isolated from one of them. The others had serological confirmation. The morbidity ratio was 60/100.000 and the mortality rate 6%. The first case occurred in February. There were three in March, five in April, seven in May, seven in June, one in August, two in September and four in October. All of them occurred in rural areas located in the southeast of Cordoboa Province, on Presidente Roque Saenz Pena County and General Roca County, 60 Km or less from the Buenos Aires Province limit.

From November 1964 to April 1966, 550 wild rodents live trapped in rural areas in this zone, were examined for virus isolations using newborn mice as hosts. They were 330 *Calomys laucha*, 96 *Akodon azarae*, 25 *Akodon obscurus*, 9 *Oryzomys flavescens*, 84 *Mus musculus* and six rodents without classification. Fifty Junin virus strains were isolated, 46 from *C. laucha* and four from *A. azarae*. Positive rodents were captured through the year, in June, July and October 1965, and January, February and April 1966.

Dr. Jorge Crespo from the Museo Argentino de Ciencias Naturales, Buenos Aires, is advising us in the study of rodent population. The relative density index (r.d.i.) is calculated on the basis of the number of trap nights and captured rodents. As shown in Figure 1, there is an increase in fall and early winter, and there seems to be a relationship between this variation and the monthly incidence of human cases.

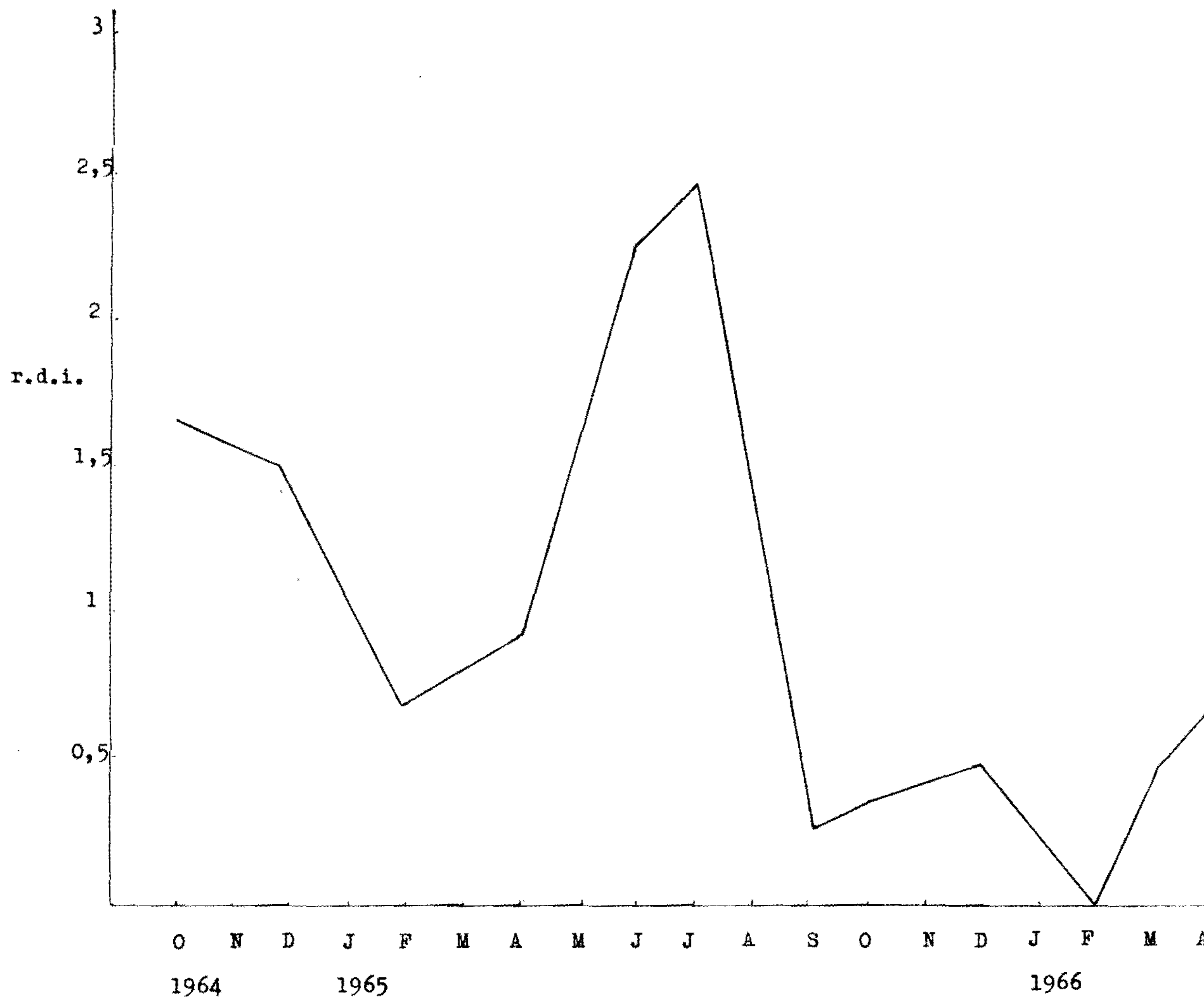
Calomys callosus is not trapped in the southeast, but it is present in the central and north region of Cordoba Province. Here, 359 rodents examined by virus isolation attempts were negative. They included 125 Calomys (both laucha or callosus), 71 Akodon sp. 34 Phyllotis griseoflavus, 16 Oryzomys flavescens, 3 Mus musculus, 16 Rattus sp. and 94 rodents without classification.

Schistolaelaps mazzai, Eubrachilaelaps rotundus, Haemolaelaps glasgowi, Gigantolaelaps mattogrossensis, Mysolaelaps microspinosus and Eulaelaps stabularis are parasites of the wild rodents and are now under study for isolation attempts.

Recently a recapture program of wild rodents in an infected area was initiated.

(M.S. Sabattini, L.E. Gonzalez, N.R. Bianchini, and B.R. de Ferradas)

Figure 1: RODENT POPULATION IN THE SOUTHEAST OF CORDOBA PROVINCE



REPORT FROM THE BELEM VIRUS LABORATORY
BELEM, PARA, BRAZIL

The Guama Ecological Research Area (APEG), IPEAN, Belem

In January 1966 Dr. Jose Maria Conduru, Director of the Instituto de Pesquisas e Experimentacao Agropecuarias do Norte (IPEAN), issued a series of official announcements establishing an ecological research reserve of 310 hectares and two smaller annexed reserves, the Mocambo Forest Reserve (6 hectares) and the Capoeria do Black (5 hectares) on the property of IPEAN. In addition, a Commission for the Coordination of Research Activities was formed to review and coordinate research at the Guama Ecological Research Area. The Commission now comprises the following members: Jose Maria Conduru (President), Djalma Batista (Director of INPA, National Research Council), Dominiciano de Souza Dias (Faculdade de Filosofia, Rio Claro), Philip S. Humphrey (Smithsonian Institution), Joao Murca Pires (University of Brasilia and IPEAN), John P. Woodall (Director of the Belem Virus Laboratory, Rockefeller Foundation).

The environment and facilities of APEG. The 310 hectare tract (the APEG) measures approximately 1.7 by 2 kms. and borders the Guama River which, although entirely fresh water, undergoes periodic tidal fluctuations. The humid tropical vegetation of the APEG comprises 1) relatively undisturbed varzea forest, 2) moderately disturbed forest of the terra firme, and 3) a narrow strip of relatively new second growth (Capoeira) and overgrown rubber plantation. The APEG is a part of a long strip of uninterruped tropical rain forest which lies along the north bank of the Guama.

The APEG is presently being fenced and surveyed. A network of trails is being cut at 100 meter intervals North-South and East-West to form a series of quadrates which will be further subdivided into 10 meter quadrates. There are two small field laboratories and a weather station on the property which will be posted and watchmen provided. One long trail and boardwalk will traverse the area from the north boundary to the river bank and a motorboat will be provided for easy access to the network of tidal creeds in the varzea forest.

Coordinated research program of APEG. The Coordinating Commission envisions the development of an integrated collaborative scientific and educational program which will enable many researchers representing different disciplines and points of view to interact together to arrive at larger concepts of the tropical forest environment than would be possible if the individual researchers were working independently. The Commission feels that the emphasis of this program should be on the biology of the

forest community and the processes and inter-relationships which characterize it. The Commission is looking forward to the development of facilities on the APEG which can 1) provide a uniform base for all studies which are undertaken on the area and 2) make the data amenable to automatic processing where desirable. The Commission hopes to establish in the near future an organized program for the systematic measurement of physical environmental factors in the APEG.

There are now three research programs underway at the APEG. The botany program, under the direction of Dr. Murca Pires, will number, identify, and map all trees in the APEG and carry out an inventory of root types and root adaptations. A concurrent program will study adaptations of plants to ants.

The entomology program, under the direction of Dr. Domiciano Dias, will undertake inventories of non-haematophagous arthropods in the APEG. In addition, more specialized studies will be undertaken on 1) the biology of social insects, chiefly Hymenoptera, and 2) the ecology of the forest floor, chiefly related to the decomposition processes going on in fallen trees and forest floor litter.

An epidemiology program, under the direction of Dr. John P. Woodall, is supported by the Belem Virus Laboratory (Rockefeller Foundation) and the Instituto Evandro Chagas (FSESP). This program comprises inventories of the vertebrates and haematophagous arthropods of the APEG as well as biological studies of these groups and their associated arboviruses and ectoparasites.

Submission of research proposals. All research programs proposed for the APEG must be submitted to the Coordinating Commission for review and evaluation. On being approved, research projects will be implemented through the IPEAN or one of its collaborating institutions by means of formal agreements defining terms of use of local facilities.

REPORT FROM DEPARTMENT VAN VOLKSGEZONDHEID PARAMARIBO, SURINAM

A summary of the mosquito collections for the first half of 1966 has been collated in the table which groups the collections according to locality, bait and species.

Mosquito catches during the first 6 months of 1966 according to locality, bait and species.

Herkomst	Bait				Culex				Mansoni	Aëdes											
		Totaal	I	II	III	IV	V	fa	Ve	tit	soll	tort	aeg	ser	fulv	Ano	Weyo	Pso	Tri	Hae	Sab
Ma Retr.citrus	3 Tr vallen	351	143	139	10					59											
Ma Retr.cacao	3 Tr vallen	7096	156	12	785	5808	1			16	69	160				44	26	19			
	mens	513									230	249				2	28	4			
Ma Retr.zwampbos	2 Tr vallen	3236	226		370	2397			2	2	33	115				32	17	32			
	minterval	6944	3023		58	3147				5	83	531			2	23	28	44			
	mens	94				4			1	2	61	24					2				
Domburg	3 Tr vallen	[illegible]56	4	28	140	6			2	6	5	23					10	2			
	mens	166							2	10	46	96				1	6	5			
Brownsweg	Tr+ apevallen	4			1											1				1	1
	mens	2																	2		
Matta	Tr+ apevallen	1447	161		65		1212	3			2	2	1		1						
	mens	861	13				839	1			7							1			
Tijgerkreek	mens	3			2									1							
Saramacca	Tr vallen	5					1		2		2										
	mens	31									5						13		13		
		20969	3726	179	1431	11362	2053	4	9	100	543	1200	1	1	3	103	130	107	15	1	1

Legenda:

Tr = Trinidad val met muizen
Ma Retr. = Ma Retraite (Paramaribo)
Cu fa = Culex fatigans
Mans. ve = Mansoni venezuelensis
" tit. = " titillans
Aëdes soll.= Aëdes sollicitans
" tort. = " tortilis

Aëdes aeg. = Aëdes aegypti
" ser. = " serratus
" fulv.= " fulvus
Ano = Anopheles
Weyo = Weyomyia
Pso = Psorophora
Tri = Trichosporon

Hae = Haemagogus
Sab = Sabethes

When the catches are collected, the traps are stoppered with paper and then transported to the laboratory in a closed car. In the laboratory the mosquitoes are sucked up, killed by tobbaco smoke or chloroform and then differentiated. The differentiated mosquitoes are kept in batches up to 100 individuals in a "Revco" deepfreezer at -60° C until they are used.

Pools of about 100 mosquitoes are triturated in a Bovin-Albumin (BAD) with streptomycin 250 µgr/ml and penicillin 400 E/ml. The triturated suspension is centrifuged 10.000 r.p.m. for thirty minutes. The supernatantis inoculated into a litter of baby mice of no more than two days old; each baby mouse receiving 0.02 ml intracerebrally. For each batch a separate litter is used. The rest of the supernatant is stored at -60° C. The inoculated mice are kept in a screened room and observed for three weeks. From the mice which have become ill, brain passages are made till the survival period reaches a constant value. Then the brains are harvested and the type determination begins.

So far this has been done by the Regional Virus Laboratory in Trinidad.

The following viruses have been isolated so far:

1964 Kwatta virus and Restan virus from mosquitoes.
Oriboca virus from the blood of a patient.
1965 Kwatta virus from mosquitoes.

No isolations have been made in 1966, although some sick mice indicate that some strains are under study.

(R.A. de Haas)

REPORT FROM THE TRINIDAD REGIONAL VIRUS LABORATORY PORT OF SPAIN, TRINIDAD

Activities in Bush Bush Forest were considerably reduced during 1965. Five families of sentinel mice were exposed at five stations on a weekly bais while entomological activities were limited to one day per week. VEE virus was isolated nine times from sentinel mice and four times from Culex mosquitoes (three times from C. portesi and once from Culex amazonensis). All the isolations were made in the period 24 August - 19 October. The rodent population remained at a low level. However, it is noteworthy to record that the only detectable period of rodent activity coincided or just preceded the period of virus isolations.

During the first six months of 1966 only two virus isolations were made from Bush Bush. Both were strains of Catu virus and were isolated in January from sentinel mice. Commencing March 15 young hamsters were routinely exposed as sentinels in Bush Bush. It is hoped that these supplementary sentinels may pick up low grade activity of VEE and Caraparu viruses.

The EEE surveillance program involving the bleeding, banding and releasing of birds at Brazil Village, Fort Read and Vega de Oropouche was continued in 1965 with field operations limited to one day per week. EEE neutralization tests were performed on 1,246 avian sera. Antibody rates were low, and conversions were not detected in serial bleedings of individual birds. However, in this program two strains of Ilheus virus were isolated. Both came from Brazil Village. One from serum of a Yellow-bellied Elaenia (Elaenia flavogaster) and the other from serum of the Grayish Saltator (Saltator coerulescens). The isolations were made in May and June 1965.

Serological surveys for arbovirus activity in humans were extended to the French island of Martinique and French Guiana on the mainland of South America. These surveys were done in collaboration with Dr. Roger Mille, Director of the Pasteur Institute in Martinique and Dr. Herve Floch of the Pasteur Institute in French Guiana who will report the results of the surveys.

Five agents have been isolated from the sera of dengue cases occurring in Antigua in 1964. The agents were isolated in primary African green monkey kidney cell cultures using a modification of the interference method described by Halstead and his associates. (Nature, Vol. 202, No. 4935, pp. 931-2, May 30, 1964). One of these agents (TRVL 55747) has been adapted to suckling mice. Illness in mice appeared at the eleventh mouse passage by the intracerebral route and consisted mainly of ataxia and paresis or paralysis of hind limbs. A haemagglutinin has been prepared from this agent by sucrose acetone extraction of infected suckling mouse brain. The haemagglutinin was inhibited by a Group B antiserum. Mouse immune serum prepared with the TRVL 55747 strain inhibited haemagglutinin by TRVL 55747, dengue type 3 and dengue type 4 viruses but did not inhibit haemagglutinin by dengue type 1 and dengue type 2 viruses.

As part of the cooperative Belkin project "Mosquitoes of Middle America", the following West Indian islands were surveyed for their mosquito fauna in 1965: Dominica, Antigua and Barbuda, and Tobago.

In April 1966, mosquito collection operations were terminated after one year in Arena Forest as no virus activity was encountered. Field operations were transferred to Turure Forest

Sangre Grande. This is fairly recently cut forest, but no virus activity has been noted so far.

More than 29,000 Culicoides were collected from Vega de Oropouche and inoculated into suckling mice in February - June 1966. C. furens and C. pusillus made up the bulk of the collection, but small numbers of C. diabolicus, C. trinidadensis, C. foxi and C. heliconiae were also inoculated. No virus was isolated.

Field operations in south Trinidad became a reality towards the end of May 1966. Initial studies will be limited to one week per month in the Los Blanquizales Swamp near Cedros on the south western peninsula of Trinidad. A field base in the form of a bungalow at La Brea has been provided by Texaco Trinidad Ltd. Mosquitoes and other blood suckling arthropods will be processed for virus isolation while rodent sera will be collected for serological studies.

Sentinel hamsters are also being exposed during the operations.

(L. Spence, T.H.G. Aitken, A.H. Jonkers, C.B. Worth, and E.S. Tikasingh)

REPORT FROM THE VIROLOGY DEPARTMENT INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS (IVIC), CARACAS, VENEZUELA

Three hundred ninety-five sera of Waika Indians collected by Dr. J. Neel, Department of Human Genetics, Medical School, University of Michigan, Ann Arbor, M. USA, in February 1966 were tested (A. Saturno) for the presence of HI antibodies, against antigens of arbovirus groups A, B, and C. Inhibition was found against A in 41 and against B in 243 out of 395 sera, and against C in 18 out of 232 sera.

The method of preparation of antigens with Tween 80-Ether suggested by Mussgay (Nature, in press) was tested (A. Saturno). It was found to be much faster, cheaper and better than the conventional method for the preparation of antigens of Mayaro, WEE, Bunyamwera, all strains of YF, St. Louis, Dengue, Ilheus, and Caraparu.

A comparison of the light and temperature sensitivity of Cocal, Indiana and New Jersey sero-types of VSV viruses were carried out by H. Thormar. The inactivation rate is about the same, but the growth of Cocal virus is more sensitive to heat than to those of Indiana and New Jersey types.

The transmission and multiplication of seven strains of yellow fever virus by and in Aedes aegypti mosquitoes are being investigated (O. Suarez and G.H. Bergold). Transmission occurs readily after intrathoracic infection of the mosquitoes with all strains tested (French Neurotr., JSS, TRVL, IVIC I, IVIC II, and Manera) except with 17D from Colombia and Porterfield.

Apart from other animals, ten Calomys mice most probably of the species laucha (according to Dr. M. Kuns) were captured end of June North of Puerto Paez and West of the Orinoco (Edo. Apure) of which five could be kept alive. Since this is a vector of Junin and BHF in southern South America, they are presently being tested for the presence of virus and antibodies of the Tacaribe virus group.

Nariva, Pacuy, and Tacaribe virus (kindly supplied by Dr. Leslie Spence) were purified and demonstrated with the electron microscope (G.H. Bergold and K. Munz). Two strains of Guaroa virus producing different sized plaques were cloned but no morphological differences in size and structure of the particles could be found.

(G.H. Bergold)

REPORT FROM THE VIRUS SECTION
INSTITUTO NACIONAL DE HIGIENE
CARACAS, VENEZUELA

Work has been started using several strains of VEE virus from different parts of the country.

1. This work consist of:

   a) Preparation of immune serum from each strain
   b) Growth of plaques for each VEE strain in dilutions from 10^{-1} to 10^{-10}
   c) Immunological study by reduction of plaques in each strain using serum for the homologous and the other strains.

This research is being made in order to qualify immunological VEE variations in Venezuela.

2. In the Arbovirus Laboratory, research is continuing on, for viral interference phenomena, using blood from dengue cases of the Venezuelan epidemic outbreaks. In all these cases, it was not possible to isolate the virus in mice of our colony.

Therefore, these animals are considered not sensitive to the dengue virus of our epidemic outbreaks of 1964, 1965 and 1966.

The possible presence of dengue virus in KB blood cultures, by inoculating it to the mouse, is being studied in our efforts to isolate the virus.

3. Studies are being carried out on sera from healthy Venezuelan people living in the rural area in connection with the endemiology of VEE.

REPORT FROM THE ARBOVIRUS LAB, INSTITUTO NACIONAL DE SALUD BOGOTA, COLOMBIA

In the "San Vicente de Chucuri" project, 8,799 mosquitoes, distributed by species into 265 pools, were captured in San Vicente during a visit there from the 10th to the 27th June, 1965. Ten VEE virus strains were isolated from that material (Table I).

The mosquito catches were done at ground level using human bait. During dusk and evening catches a Shannon trap was also used.

From the mosquito isolates crude acuous antigens consisting of a 20% brain (baby mice) suspension in saline, were used in the tests after 3000 r.p.m. for 10 minutes.

Eighteen mice litters (1-3 days old) were exposed as sentinels each for a period of 24 hours in the jungle, and eight strains of virus recovered; five were identified as VEE strains.

Of considerable interest were the isolations of four strains of VEE virus from bats (Carollia perspicillata), 194 bats were captured of which 174 classified as Carollia perspicillata, 3 Glosophaga longirostris, 1 Artibeus lituratus palmarum; 1 Vampyrope helleri, Peters; 2 Carollia perspicilla perspicillata (Linneus); 1 Trachops cirrhosus cirrhosus (Spix) and 12 not identified yet.

The inoculum consisted of a suspension of organs (heart, liver and spleen) in 5.0 ml of saline centrifuged at 2500 r.p.m. during 5 minutes.

Specimens are processed at the same time and any killing mice before 48 hours is suspected as VEE. All specimens suspected as VEE infections are then inoculated in the "VEE room at the same time. Any group dying later than 48 hours is rejected and considered as secondary infection from cage to cage. In dubious

cases we come back to the original material. We never again use the "VEE room" before five days during which it has been emptied and thoroughly cleaned.

Thus we are confident of our findings, even if we fail to re-isolate the virus from the original material later.

Other interesting isolates are strains 49884, 49888, 49893 and 50431. The first two fall into the Guama group, none of which virus components had been known before in the area, which has been studied for some years (Graph 1).

Strains 49893 and 50431 are under study, and by the CF test they do not fall into any of the common antigenic groups. Strains 50431 though belonging to a latter visit, was included in Table I, because it comes from the same area.

Finally strain 50287 was neutralized at the highest titer (NI log 2.25) by a St. Louis antiserum, other steps are underway to define its identity.

(E. Prias-L and Carlos Bernal C.)

TABLE I

Virus Isolations from San Vicente de Chucuri 1965

Lab. No.	Animal Species	Specimens	Station	Obtention [a]		1st isolation [b]				Virus	Reisolation [b]			Interval from 1st to 2nd AV
				Date	Time	HA	HI	FC	NT		HA	HI	FC	
49883	Sentinel Mice 5	1	Peroles	June 17	24	100	1280	-	2.16	VEE	1600	80	10/16	4 months
49884	" 7	1	Tierrabuena	" 18	24	0	-	16/8	-	Guama Grp.	Under study			10 1/2 "
49886	" 10	1	"	" 19	24	200	1280	-	2.27	VEE.	1600	160	10/32	3 "
49887	" 11	1	Peroles	" 19	24	12800	1280	-	1.87	VEE	400	80	10/16	1/2 "
49888	" 13	1	Yarima	" 24	24	0	-	8/32	-	Guama Grp.	Under study			10 1/2 "
49890	" 17	1	Tierrabuena	" 25	24	800	20	-	5.8	VEE	-	-	-	Exhausted
49892	" 20	1	"	" 26	24	ND	-	-	3.7	VEE	1600	160	10/2	4 months
49893	" 21	1	Peroles	" 26	24	0	-	-	-	Under study	Negative			11 "
50281	Culex sp.	20	"	" 24	D	6400	640	-	2.87	VEE	-	-	-	Exhausted
50287	P. ferox	50	Yarima	" 25	N	800	0	-	2.25	St. Louis?	Negative			5 1/2 "
50297	"	18	"	" 26	D	3200	160	-	2.41	VEE	-	-	-	Exhausted
50298	A. Serratus	32	"	" 26	D	50	80	-	2.87	VEE	-	-	-	Exhausted
50300	P. ferox	50	Tierrabuena	" 26	D	400	10	-	2.78	VEE	-	-	-	Exhausted
50301	"	50	"	" 26	D	800	160	-	1.32	VEE	-	-	-	Exhausted
50306	A. Serratus	38	"	" 26	D	1600	80	-	3.15	VEE	1600	160	10/2	3 1/2 months
50307	A. fulvus	2	"	" 26	D	1600	640	-	3.42	VEE	-	-	-	Exhausted
50308	Mansonia sp.	17	"	" 26	D	800	20	-	4.5	VEE	Negative			Exhausted
50310	P. ferox	50	Peroles	" 26	D	800	40	-	3.20	VEE	Negative			8 months
50314	A. Serratus	50	"	" 29	D	200	10	-	3.0	VEE	Under study			7 "
50320	Carolia perspici.	1	Yarima	" 15	D	25600	80	-	2.61	VEE	Negative			7 "
50332	" "	1	"	" 15	D	400	20	-	3.56	VEE	Negative			7 "
50335	" "	1	"	" 15	D	1600	160	-	1.76	VEE	Negative			7 "
50338	" "	1	"	" 15	D	400	20	-	2.95	VEE	Negative			7 "
50431	P. ferox	50	Riofuego	Aug. 12	D	0	-	-	-	Under study	Negative			7 "

a) For bats the date of obtention is the day of exposure and the time of exposure 24 hours. D = capture at daylight. N = capture in the evening.

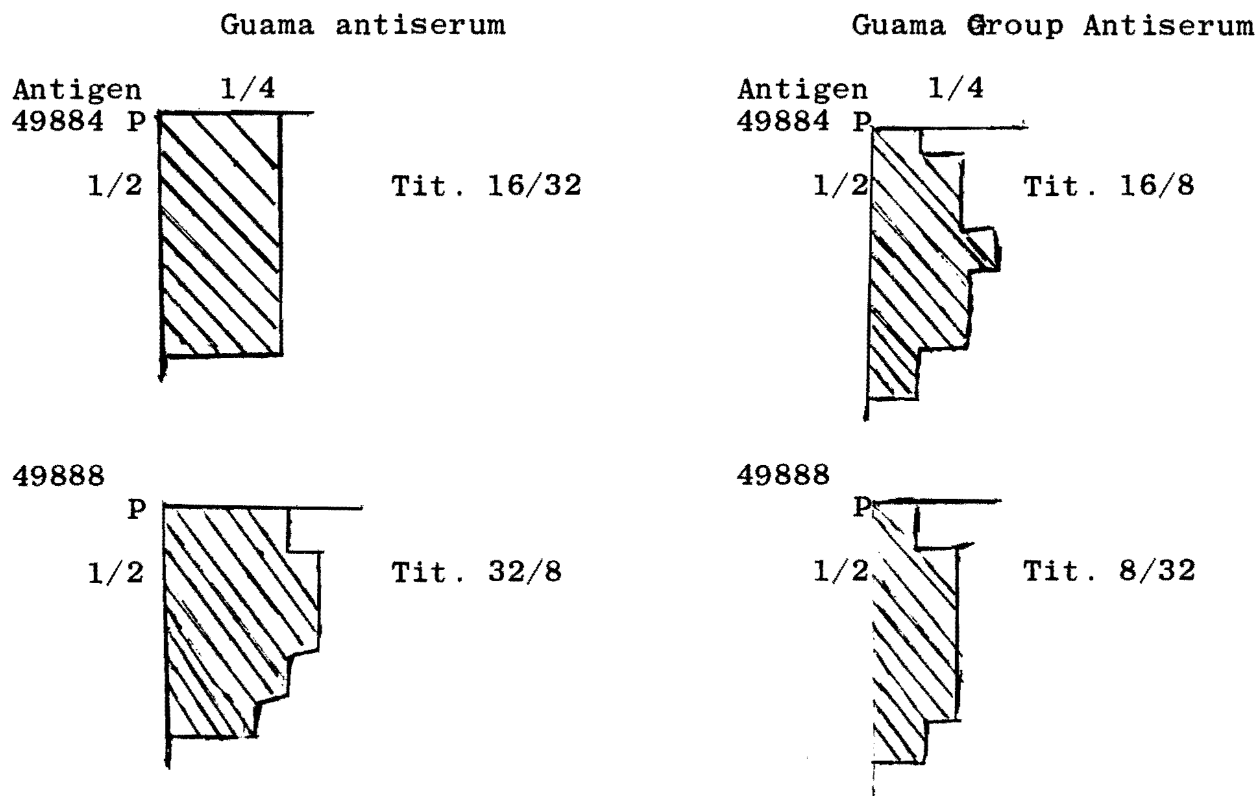
b) H.A. titers expressed as inverse of last antigen dilution producing total haemagglutination. H.I. titers expressed as inverse of last dilution of the homologous antiserum inhibiting haemagglutination in front of 8 Units of antigen. F.C. +++ titer expressed as: numerator last dilution of homologous antiserum and denomination last antigen dilution, producing or ++++ of turbidity N.T. titers expressed as log. of neutralization produced by the homologous antiserum.

c) More than once the original specimen killed mice so fact before brains control be collected. Those events are not considered in this tabulation.

NOTE: The original specimens remained at -20 ^{0} C but the power failed three times for periods of 12 to 18 hours and many suspensions appeared defrosted at the end of these accidents.

COMPLEMENT FIXATION TEST RESULTS WITH FOUR VIRUS STRAINS ISOLATED IN SAN VICENTE DE CHUCURI, COLOMBIA, 1965.

(Courtesy of Dr. C. Sanmartin)



GRAPH No. 1

The strains 49884, 49888, 49893 and 50431 were tested against the following antisera (or immune ascitic fluids) EEE, VEE, VEE, Mayaro, Una, Pixuna - Yellow Fever (JSS), Ilheus, St. Louis, DI, DII, Bussaquara- Group C (Polyvalent) - Maguari, Kairi - Guama Group (polyvalent), Guama - Anopheles A. - Anopheles B - Manzanilla, Oropouche - Melao - Tacaribe - Cocal and unclassified: Esp. Phlebotomus (Cali) - Esp. A. Neivai (Cali).

The only positive results are those appearing in the graph above.

REPORT FROM THE VIRUS LABORATORY, FACULTAD DE MEDICINA
UNVERSIDAD DEL VALLE, CALI, COLOMBIA

Field studies of arboviruses have been continued during 1965 at the Rio Raposo field station south of Buenaventura in the high rainfall forest of the Pacific Coast of Colombia. The work there is now in its fourth year.

The time expended in collecting mosquitoes has been reduced to one week of each month to avoid building up a backlog of uninoculated material and to permit use of the mouse colony for processing vertebrate tissues, while still obtaining current information on any unusual incidence of viruses in arthropods. The collection of serum and tissues from small mammals for virus isolation and serological study are continuing, but bird work was discontinued at the end of 1965 as these vertebrates had not produced any virus isolations and the results of serological testing are difficult to interpret.

Of particular interest during 1965 has been a shift in the kind of virus isolated from Anopheles neivai, one of the more common mosquitoes collected on human bait in the area. During the period from 1962 to 1964 Guaroa virus was isolated six times from this species. In 1965 Guaroa did not appear, but there were seven isolations of Anopheles A., a virus not previously recorded from western Colombia. These two viruses have been isolated only from Anopheles neivai, although many thousands of mosquitoes of other species have been processed. A third, as yet unidentified virus, has also been isolated from Anopheles neivai. Special studies on the biology and ecology of this bromeliad breeding mosquito are now being undertaken.

Guaroa neutralization tests on a sample of 81 human sera selected to represent all age groups have revealed a typical endemic pattern of infection with a graduated increase in the incidence of antibody correlated with increase in age.

By contrast with the apparent mosquito host speificity of Guaroa and Anopheles A viruses, Wyeomyia complex viruses, of which there are now 43 strains, have been isolated from various mosquito species of the genera Anopheles, Trichoprosopon, Wyeomyia, Limatus, Mansonia and Psorophora. Una virus has also been isolated from mosquitoes of several genera: Mansonia, Aedes, Psophora, and Wyeomyia. Ilheus virus was recovered only in 1964: thrice each from Psorophora ferox and Mansonia arribalzagae and once from Wyeomyia jocosa. Seventeen agents isolated from a variety of mosquito species and from Phlebotomus are not identifiable with immune sera available here and have been sent to the Yale Arbovirus Laboratory for further study.

A heady demand on the mouse colony has been occasioned in recent months by the processing of tissues from a series of about 1,000 bats collected by an ICMRT group in the vicinity of Cali and on the Pacific Coast during the summer of 1964. As yet no arboviruses have appeared, although there has been one isolation of rabies from a frugivorous bat *Artibeus lituratus*.

From August 1965 to January 1966 a trapping program for small mammals was carried out in the valley of the Rio Pichinde near Cali at an elevation of 1700 to 1900 meters in a zone of moist subtropical forest and small farms. Organs and/or sera were collected from the following animals:

Oryzomys caliginosus	189
Oryzomys fulvirostris	53
Oryzomys albigularis	16
Oryzomys alfaroi	14
Reithrodontomys mexicanus	16
Rhiphidomys latimanus	2
Rattus rattus	

An agent pathogenic for infant mice isolated from an organ pool (liver, spleen and kidney) of *Oryzomys albigularis* is now being studied. Serological examination of sera from these animals are now underway.

It had been hoped that rodents in this moist subtropical environment might turn out to be good hosts for immature stages of *Ixodes* ticks, but this proved not to be the case during the months of the study, only a few *Ixodes* having been taken.

Pregnant animals were held for parturition, and data have been accumulated on litter size and growth rates from birth to maturity. Laboratory conceptions and births have been obtained with *Oryzomys fulvirostis*.

(Pablo Barreto, Vernon H. Lee, Carlos Sanmartin and Harold Trapido).

REPORT FROM GORGAS MEMORIAL LABORATORY PANAMA, R.P.

Human Infections with Arboviruses in Panama

In order to determine the endemicity of arboviruses in man in Panama, a survey was set up in the villages along the shores of Gatun Lake, which is not far from the laboratory in Panama City.

The survey team made regular visits and blood samples were collected from the acute fever cases for virus isolation. A small number of fever cases from other areas of Panama were also examined. During a period of one year, from July 1964 to June 1965, seven strains of arboviruses were isolated from a total of 198 fever cases studied. Some of these viruses are considered rare and one of them had never been detected in man before. Isolation and identification of the virus isolates are briefly described below:

Group A viruses: Two cases of Venezuelan equine encephalitis (VEE) virus were detected, one each in Mendoza and Juan Mina. The patients had an abrupt onset with high fever and severe headache without signs of CNS involvement. Viruses were isolated from blood serum in both suckling mice and hamster kidney tissue culture systems (HKTC) and were identified as being closely related to VEE strain #3880, isolated at the Middle America Research Unit (MARU) in 1961. More than 32-fold rises in HI antibody titers were detected from both cases during convalescence.

Group B viruses: Ilheus virus was isolated from one of our staff members who was collecting amphibians and reptiles in the northwestern part of Panama in November 1964. After 20 days in the field, he returned to the city with general malaise and developed high fever and pronounced headache three days later. His blood sample drawn three days after onset of illness was inoculated into suckling mice and HKTC. All mice survived during a two-week period of observation, but cytopathogenic effects were deteced in HKTC. Infected fluid was then inoculated into a group of suckling mice and a virus strain was subsequently isolated and identified as being closely related to Ilheus virus strain BT 3875. Rise in CF antibody titers was detected in the patient's blood during convalescence. This isolate represents the first recognized human infection with Ilheus virus in Panama, where previous isolates have been obtained solely from a number of species of mosquitoes and birds.

Another virus in group B, Bussuquara was isolated from a febrile adult male resident of Arenosa, about 50 miles from Panama City in October 1964. No other signs and symptoms were recorded. The viral agent was established on the third mouse brain passage with an incubation period of 3-4 days. This new strain proved to be indistinguishable from Bussuquara (BeAn 4116) virus by cross HI and cross CF tests. A special effort was made to procure a convalescent phase serum specimen from the patient who moved from his old residence. This second sample was obtained about one year after his illness and was compared with the acute-phase serum by HI test. Significant rises in antibody titers were shown when tested with Bussuquara and some other group B arboviruses as well. This represents the first time this virus

has been isolated from man. From the time it was first recovered from a sentinel monkey in Brazil in 1961, other isolates have been obtained from wild rodents, sentinel mice and mosquitoes in Brazil, Colombia and Panama.

Phlebotomus fever group viruses: Two natural infections of Chagres virus were recovered in July 1964. The first case was the wife of one of G.M.L. staff members living in Las Cumbres, 15 miles from Panama City. She developed a sudden onset of mild fever with anorexia and nausea which persisted for three days. Profuse perspiration was experienced intermittenly with a temperature up to 102° F. The second case was a febrile 16 year-old female resident of Gerro Cama, about 50 miles northwest of Panama City. No other signs and symptoms were recorded. Blood samples from these two cases, obtained 12 days apart, were used for virus isolation attempts. Isolations from both specimens were successful in suckling mice but attempts in HKTC failed. Both of these strains proved to be indistinguishable from each other and from Chagres virus strain JW-10 by cross-CF test. A rise in antibody titer to this virus was demonstrated in both cases during convalescence by CF technique and reisolation attempts were successful. Chagres virus was first isolated from man by MARU in July 1960 as a new virus belonging in the Phlebotomus fever group. These two newly-isolated strains represent the second and third cases of this virus. No isolates were obtained from insect vectors or wild vertebrates.

Another untyped virus in the Phlebotomus fever group was isolated from a febrile adult male resident of Escobal in August 1964. It reacted with certain members of the Phlebotomus fever group in HI and CF tests. Rise in antibody titers were demonstrated when tested with Icoaraci virus, an agent in this group.

Besides the arboviruses mentioned above, other isolations from man in Panama, previously reported from our laboratory and from MARU, include St. Louis encephalitis, yellow fever, Madrid, Ossa, Wyeomyia and Changuinola viruses. Despite the low recovery rate of arboviruses from man in other areas where hundreds of virus strains have been detected from animal and insect sources, the data presented here indicates that human infections with arboviruses are not uncommon in Panama.

(Carl M. Johnson and Sunthorn Srihongse)

REPORT FROM THE MIDDLE AMERICA RESEARCH UNIT (NIAID), Panama

VEE Virus.

Continued sentinel surveillance at the Gamboa study site has

failed to disclose evidence of VEE activity so far this year. Thus in four consecutive years, it appears that the level of circulation of the agent has fallen below the threshold of detection using baby mice as indicator.

Further progress was made on the problems of antigenic variation among strains of VEE virus. Using the kinetic HI method of Casals and single injection sera prepared by injection of live virus into Proechimys rodents, several different sub-types were recognized. Strains from a single geographic area were homologous, often over several years and regardless of host source of virus. No differences were detected when one isolate was passaged 28 times in mice or 20 times in VERO cell cultures and new antisera prepared. Data showing relationships among representative isolates are given in Table 1. It will be seen that in most cases "distance" of relationship was reciprocal. There is also a suggestion that viruses from central Panama, Colombia and Venezuela may represent a "core" of antigens most closely resembling older prototype strains.

It was also of interest that reagents prepared with the old Trinidad donkey strain displayed the broadest cross-reactivity, particularly in the case of the antigen. This material represented 25 guinea pig passages and one in VERO cells. Passage in guinea pigs may alter antigenicity, or long storage of a virus might influence its antigenic configuration. Another possibility is that recently isolated viruses may all represent geographic "degradations" from an older parent. In any case, evidence for discrete foci of VEE-complex is accumulating.

Hemorrhagic Fever

A. Ecology

Preliminary study of the rodents collected during 1965 indicates that Calomys callosus is widely distributed in the tropical semideciduous forest regions of the broad basin separating the Planalto do Mato Grosso of Brasil and the Andean foothills. This rodent has now been collected from each of the four interfluves formed by the principal rivers draining the Llanos de Moxos. These are the Beni-Mamore interfluve (Santiago de Yacuma, San Ignacio de Moxos); Mamore-Machupo interfluve (San Joaquin, Trinidad, San Juan de Cercado); the Machupo-Itonama interfluve (San Ramon, Huerrasca, Magdelena); and the Itonama Blanco interfluve (Orobayaya, Baures, Huacaraje). Machupo virus activity in the form of human cases of hemorrhagic fever has been documented in three of the four interfluves listed above; no cases having been recorded from localities west of the Rio Mamore. The geographic distribution of this population of Calomys callosus appears to coincide with the distribution of grasslands (the

Llanos de Moxos) being limited to the north at about the 12th parallel by the appearance of tropical evergreen forest and to the south at about the 16th parallel by an arm of the Andean rain forest and, to the southeast, by the dense forests of the northern slopes of the Chiquitos highlands. The eastern and western limits of this population of Calomys callosus have not been accurately determined but their range probably extends continuously from the Rio Beni nearly, if not quite, to the Rio Itenez. Other populations of Calomys callosus were found in the Chiquitos highlands south of the 16th parallel (San Ignacio de Velasco), on the west bank of the upper Rio Paraguay (Urucum de Corumba and Caceres, Mato Grosso, Brasil), and the population which is widely distributed in the eastern foothills of the Andes between the 17th and 27th parallels, studied at Warnes, Santa Cruz, Bolivia. Calomys laucha, a small species of the genus believed to be an important reservoir of Junin virus in Argentina, was collected from house at Fortin Guachalla on the Rio Pilcomayo in southwestern Paraguay.

B. Laboratory Studies.

Studies begun in 1963 on isolation of Machupo virus from specimens obtained from serologically confirmed human HF cases are summarized in Table 2. Infant hamsters continue to be the most sensitive hosts for detection of virus. Of 44 persons studied, only 14 (32%) yielded the agent. Several isolates were usually made in positive subjects and the temporal pattern of virus recovery was suggestive of "peak" viremia with appearance of virus in the throat and oral cavity 7-12 days after onset of symptoms, precisely the interval during which the more severe manifestations of disease occurred. Several quantitative reisolation studies revealed that the amount of virus present rarely exceeded 10^2HLD50/ml.

Serological work has been furthered by the development of a plaque neutralization test in VERO cells, in which fixed virus dose and serial serum dilutions in a micro-system are employed. This test overcomes two major limitations of the CF procedure; lack of type-specificity and the problem of anti-complementary activity frequently encountered in animal sera. Selected convalescent sera from human cases of Bolivian HF with CF antibodies also showed clearly detectable N antibodies without cross reactivity with Junin or Tacaribe viruses. Comparison of CF and N titers in serial specimens from six subjects showed closely parallel curves. CF antibody appeared slightly earlier, but N antibody persisted longer. Continued observations are being made to determine whether N antibodies persist for sufficient years to afford a reliable cross-sectional survey tool.

Some 1,983 human sera collected during the 1965 field expedition were tested for CF antibodies to Machupo antigen. As shown in

Table 3, 1,678 were clearly negative. The remaining 305 reacted at dilutions of 2 to ≥ 8, but with varying AC activity. Seventy of the "best" candidates were tested for N antibodies to one or more of four HF viruses. No positives were encountered. To test the hypothesis that old infections might have been missed by CF, all males of 10 years or greater (576) are currently being tested for N antibodies to Junin and Machupo viruses. Although still in progress, it appears that no important focus of human infection will be discovered.

Preliminary titrations indicated that the Panamanian marmoset Saguinus geoffreyi was highly sensitive to Machupo virus, but not to prototype Tacaribe or XJ high passage Junin viruses. About $10^{5-5.5}$ HLD_{50} of each of the latter viruses was given IP to six animals. Some 70 days later five Junin and four Tacaribe infected animals with N antibody titers of 1:16-64 were available for challenge with a similar dose of Machupo virus. All Tacaribe "immune" and 4/5 Junin animals succumbed to Machupo infection after intervals not different from those observed for primary Machupo-inoculated animals. Although analysis of virus isolation data to determine if the primary infections were still active at the time of challenge is not complete, we conclude that neither heterologous antibody nor chornic infection if present was sufficient to protect marmosets against a fairly large dose of lethal Machupo virus.

(N.A. Young, L. Gauld, M.L. Kuns, P.A. Webb, and K.M. Johnson)

Table 1

Reciprocal Relationships among VEE Isolates Representing Different Geographical Regions as Measured by Kinetic HI Test

ANTIGENS			PROECHIMYS IMMUNE SERUM[+]							
Locality	Strain	Year	Fe3-7c	63A216	MENA-II	3880	V-209A	V-198	P676	Tr. Donkey #1
Florida	Fe3-7c	1963	1*	8	8	8	4-8	8	8-16	8
Mexico	63A216	1963	8-16	1	1	8	16	8-16	16	16
Panama (Almirante)	MENA-II	1962	8-16	1	1	4-8	8	8	16	16
Panama (Cañito)	3880	1961	8-16	8	8	1	1-2	4-8	8-16	4
Colombia (Santander)	V-209A	1960	8-16	4	4-8	1	1	4-8	8-16	2-4
Colombia (Guajira)	V-198	1962	8-16	4-8	4-8	2	2	1	1	2-4
Venezuela	P676	1963	16-32	4	4-8	2	2	1	1	2-4
Trinidad	Tr.Don. # 1	1943	>8	2	2-4	1-2	1	1	4	1

[+] All sera prepared by single IP injection and collection 18-21 after inoculation.

* Relationships shown in "fold" differences corrected to homologous system considered as unity.

Table 2

ISOLATION OF MACHUPO VIRUS FROM 44 PERSONS

WITH CONFIRMED BOLIVIAN HEMORRHAGIC FEVER, 1963, 1964

(38 positives from 14 patients)

SPECIMEN	DAY OF ILLNESS					
	1-3	4-6	7-9	10-12	12-18	Total
Throat Swab	0/9*	0/16	6/26	5/14	3/14	14/80
Saliva Swab	0/1	0/4	4/12	1/6	2/9	7/32
Blood +	0/33	3/29	8/36	6/18	0/14	7/130
Total	0/43	3/49	18/74	12/38	5/38	38/242
(%)	(0%)	(6%)	(24%)	(32%)	(13%)	(16%)

* Number positive / number specimens tested

\+ Includes - serum, plasma, buffy coat, heparinized blood, whole blood, sedimented wbc's and rbc's.

TABLE 3

COMPLEMENT FIXING ANTIBODIES TO MACHUPO ANTIGEN IN HUMAN SERA FROM RURAL BOLIVIA, PERU, PARAGUAY, AND BRAZIL, 1965

COUNTRY	LOCALITY	No. of Sera Tested	ANTIBODY TITER		
			< 2	≥ 2 but with varying degrees AC activity	
BOLIVIA	San Ignacio de Moxos	184	174	10	
	Riberalta	182	174	8	
	San Ignacio de Velasco	303	283	20	
	Warnes	195	181	14	
PERU	Shintuya	134	126	8	
	Pto. Maldonado	61	56	5	
PARAGUAY	Misiones	174	169	5	
	Gran Chaco	243	145	98	)
BRAZIL	Corumba	224	174	50	) 235*
	Caceres	283	196	87	)
	Totals:	1983	1678	305	

* These sera remained unfrozen for a considerable period of time prior to reaching the Panama Laboratory

REPORT FROM INSTITUTO NACIONAL DE VIROLOGIA MEXICO, D.F., MEXICO

Studies on the VEE Virus Strain Isolated in Mexico.

Routine serological surveys for arbovirus have shown a wider distribution of VEE in Mexico. Recently, in human beings of Chiapas State (located on the border with Guatemala) VEE HI antibodies have been detected. Also in Nayarit and Jalisco (Western Coast), serological evidence of VEE infections has been found in cattle and humans, respectively.

These findings and those previously obtained, pointed out a broad distribution of VEE in the Mexican Republic. However, epidemics or epizootics produced by VEE have not been so far recognized in the country (except the small outbreak in humans of Campeche in 1962, which was possibly due to VEE).

Multiple causes may account for such a situation. A natural attenuation of the local strain (63U2) of VEE, must be kept in mind. Therefore, comparative studies were carried on with the Mexican VEE strain and the FD (Trinidad) strain of VEE. Both were titrated in suckling and adult mice, which were inoculated by IC, SC and IP routes. Also, hamster kidney and chick embryo cell cultures were used to titrate both viruses. Plaque formation on chick embryo fibroblasts were also studied.

Incubation periods and average survival times in adult and suckling mice inoculated by the different routes used, showed no significant difference. Both titre and CPE in cell cultures employed were similar for the two strains, but in plaque formation studies, it was found that VEE 63U2 (local strain) produced larger and non-confluent plaques as compared with those formed by the FD strain. This difference can be explained considering that the Mexican strain has been passed 5 to 6 times in mice, whereas VEE FD has at least 80 passages. However, further studies are necessary to establish if plaque characteristics of VEE 63U2 mean a stable character which is related to a low virulence of the Mexican virus for humans and equines.

(Julio de Mucha-Macias and Carlos Campillo-Sainz)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF THE WEST INDIES, KINGSTON, JAMAICA

EEE Surveillance in Jamaica

As a follow up to the programme reported for the quarter ending

September 1965, the work of EEE surveillance has been extended to the parish of St. Elizabeth where bird netting is being carried out. Thus far, over 100 migrant birds have been netted and banded in this area. Second blood specimens were obtained from 12 of these birds, and they were tested against EEE in neutralization test. The results were negative.

Serological Survey - Sera from Peace Corps Volunteers.

A limited serological survey for arbovirus antibodies was carried out among U.S. Peace Corps Volunteers serving in Jamaica. Thirty-six sera were tested in HAI for EEE, VEE, SLE and dengue antibodies. All were negative except two sera which were positive (1:40) to SLE only. It is hoped that follow up studies can continue with these Volunteers while they are in Jamaica.

Ministry of Health Survey.

The Ministry of Health, Jamaica requested a serological survey of an area (Port Henderson - Gregory Park) which is adjacent to Caymanas where a previous survey was carried out. Twenty-two individuals were less than 15 years old. Two of these showed a low HAI titre to group B arbovirus; both were negative in CFT.

TABLE 1.

(a) HAI Results of Survey Sera - Port Henderson - Gregory Park area.

TOTAL SERA TESTED	Dengue		SLE		EEE		VEE	
BY HI	POS.	%	POS.	%	POS.	%	POS.	%
132	56	42.2	45	34.1	0	0	0	0
	(b)	CFT Results						
BY CF								
61	34	55.7	35	57.4	0	0	0	0

Table 1 (a) and (b) summarizes the HAI and CFT results respectively. HAI titre ranged from 1:20 to 1:320 and CFT titre from 1:4 to 1:32. The findings in this survey correlate with previous studies in adjacent areas.

Clinical Specimens.

Several paired sera from clinical cases were examined for antibodies to EEE, VEE, dengue and SLE. A significant rise in group B antibodies was demonstrated in one serum taken from a 40-year old female from St. Mary, one of the northern parishes. The diagnosis made by doctors at the University Hospital at that time was? meningo-encephalitis, ? encephalitis.

TABLE 2.

Laboratory Results of Clinical Specimens CV 95/66 from a case of ? encephalitis, ? meningo-encephalitis.

	Date Submitted	HAI Test titre				CFT titre	
		EEE	VEE	Deng.	SLE	Deng.	SLE
Serum 1	26.1.66	Neg.	Neg.	1:160	1:320	N.S.	1:8
Serum 2	15.2.66	Neg.	Neg.	1:640	1:1280	N.S.	1:64

N.S. = Non-specific

In neutralization test against SLE (Ja 7532), serum 2 showed more than two logs protection, whereas serum 1 did not protect. No neutralization test was done against dengue virus. In view of these results, this is regarded as a case of St. Louis infection.

REPORT FROM THE SOUTHWEST FOUNDATION
FOR RESEARCH AND EDUCATION
SAN ANTONIO, TEXAS

The Division of Microbiology and Infectious Diseases is most happy to have Dr. H.S. Hurlbut join its staff as Chief, Section

on Arboviruses. Dr. Hurlbut's presence will allow for expansion of activities to include greater coverage of the arboviruses as well as the various arthropod vectors associated with subhuman primates held in captivity as this facility and in the wild state in Africa.

A field trip to Africa has recently been completed (February-March, 1966). Approximately 125 baboons, at three major ecologic sites, were captured and sampled. The various specimens obtained (throat, nose, rectal, ear, eye, vaginal, skin swabs; hair and nail clippings; and serum) are now under study in Bacteriology, Mycology, and Virology. Parsitologic and pathologic examinations were completed in the field on about 85 animals. Special attention will be given to serologic indications of past infections with various arboviruses. A number of ectoparasites--Ambylomma sp., Rhipicephalus pulchellus, and Ixodes sp. were obtained from the baboons in the field (by Drs. R.E. Kuntz and B.J Myers).

A new research program on the transmission of arboviruses is being developed. This study will be oriented towards arthropod infection and the mechanism of transfer of virus to vertebrates. The virus content of the product of exit portals, particularly mosquito saliva, will be studied with reference to the dynamics of the arthropod infection.

(S.S. Kalter and H.S. Hurlbut)

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

In the last report of the Information Exchange, we reported two unidentified viruses. One was identified as Turlock virus.

At the present time (June 15) 3,062 mosquitoes from Wichita Falls, San Benito, Corpus Christi and Fort Worth have been processed. In addition, a special project is being conducted in conjunction with Fort Sam Houston entomology group on the Wildlife Refuge area near Sinton, Texas, which not only includes mosquitoes for virus isolation but also serology of sentinel chicken flocks and wild birds. So far, 9,079 mosquitoes from this area have been processed.

Three mosquito isolates from pools of Culex quinquefasciatus (one Hart Park isolate and two unidentified) from the Fort Worth area have been found. Seventy-four rattlesnake bloods collected during April 1966, from the Lubbock area were negative in HI tests

for WE, EE, and SLE. Nine of these blood clots were negative for virus isolation in suckling mice.

CF serological tests, employing 101 human convalescent sera from suspected cases of encephalitis and 106 horse sera have been tested with negative results against Hart Park, Turlock, and California encephalitis virus (strain LaX) antigens. Additional tests will be done employing the Texas strain - San Angelo Virus - of the California complex.

Since March 1966, five equine cases of WE encephalitis have been confirmed in four widely separated areas.

(J.V. Irons, Julian Feild, and Tom Guedea)

REPORT FROM THE ENCEPHALITIS RESEARCH CENTER STATE BOARD OF HEALTH, TAMPA, FLORIDA

New Viral Isolations and Identifications.

A new member of the California encephalitis virus complex has been isolated and identified by the ERC and the University of Pittsburgh Laboratories under the direction of Dr. W. McD. Hammon. The new virus, termed "Keystone" has been compared in the University of Pittsburgh Laboratory with other known members of the CEV Group from the U.S. and abroad and is significantly different from all so far available for study. The Keystone strain of virus was obtained from *Aedes atlanticus-tormentor* mosquitoes collected in August, 1964.

A new arbovirus from ticks (*Dermacentor variabilis* removed from a raccoon) has been isolated and identified by the Tampa and Pittsburgh Laboratories. This agent has been named "Sawgrass" virus and fixed complement only in the presence of the homologous system. It has been compared with 78 other arboviral hyper-immune sera in the YARU Laboratories including all the known tick agents. This work was done by Miss Gladys Sather of the University of Pittsburgh.

Other unidentified viral agents have been obtained from cotton rats (nine), house mice (three), and *Culex nigripalpus* mosquitoes (one). These agents have not been adequately studied as yet to identify them as new arboviruses. SDC sensitivity studies and RNA analyses are currently being carried out by the Tampa and Pittsburgh Laboratories. In the Pittsburgh and YARU Laboratories, the cotton rat and house mouse agents have been compared by CF against fifty representative antisera, including Modoc, LCM, GD7, and Reo 3 with negative results.

The unidentified viral agent from Culex nigripalpus is of special interest since it raises the problem of indigenous mouse viral agents in the ERC mouse colony. The new unidentified isolate from Culex nigripalpus mosquitoes was forwarded to the Pittsburgh Laboratory and identified there as mouse hepatitis virus. It was supposedly isolated from a pool of Culex nigripalpus mosquitoes in the Tampa Laboratory. At this time, it is uncertain whether the mouse hepatitis virus was picked up in passage from indigenous viral flora of the Tampa Laboratory mouse colony or whetner it indeed was present in the wild caught Culex nigripalpus mosquitoes, which are known from other studies to feed upon rodents. It is considered most likely that the virus came from the mouse colony. A subsequent check of fifty individual males, 12 to 14 weeks of age, from the Tampa mouse colony (test performed in CDC Laboratories, Atlanta, Georgia) indicated a prevalence of HI antibodies of titer of 1:20 or greater against the following agents: 66 per cent Reo 3, 38 per cent Sendai, 46 per cent GDVII, and 12 per cent mouse hepatitis virus. Negative results were obtained against polyoma, K virus, PVM and mouse adenovirus. These results emphasize the importance of including in the battery of antisera used for identifying new viral isolates available hyperimmune sera against indigenous murine viral agents.

California Encephalitis Virus Studies.

The ecologic studies for arboviruses in the Tampa Bay area of Florida have demonstrated the California Group of arboviruses to be the most commonly recovered viral agents from mosquitoes. Of 45,091 Aedes mosquitoes tested in 1,130 pools during the period January, 1963 through December 1965, 64 pools have been positive for California Group arbovirus. The trivittatus strain was recovered most frequently from Aedes infirmatus mosquitoes and a newly identified strain termed "Keystone" from Aedes atlanticus-tormentor.

Detected human disease related to California viruses by serologic test has been uncommon. Four cases have been identified in 984 tested patients with suspected viral infection of the central nervous system. However, human infection without disease is found commonly in 1 to 6 per cent of the general population. The importance of extracting human sera with kaolin rather than acetone to detect specific inhibitors to BFS-283 antigen is emphasized in our studies.

The vertebrate reservoir of California virus in Florida remains unknown. Extremely low rates of HI antibody have been found in mammals tested to date. Laboratory infection experiments have demonstrated domestic rabbits can be infected with the local trivittatus-like strain of CE virus. However, no detectable HI antibody was found at one and three weeks following infection,

using the BFS-283 HA antigen. The year-round, state-wide recovery of CEV from mosquitoes suggest its continual presence in one or more common hosts.

St. Louis Encephalitis Studies.

The major purpose of ERC is to study the epidemiology of SLE virus. This was frustrated again in 1965 by the virtual absence of SLE virus from the Tampa Bay environment. Serologic evidence indicated that cotton rats in Hillsborough County and backyard chickens in Hardee County may have been infected with SLE virus sometime during the spring of 1965. Despite intensive studies, however, no further evidence of SLE virus activity was detected. The volume of material collected for viral surveillance in 1965 exceeded any previous year. Specimens tested for SLE virus, either by serology or viral isolation, included over 175,000 mosquitoes, 2,832 wild birds, 747 sentinel chickens, 205 backyard chickens, 52 horses, 531 mammals, 419 human central nervous system (CNS) surveillance cases and 398 human serologic survey specimens.

Eastern and Western Encephalitis Studies.

Of particular interest have been the recoveries of Eastern encephalitis virus from mosquitoes in every season of the year in the Tampa Bay area. In 1964 and 1965 combined, EE viral isolations were made in each month of the year except January and December. A variety of mosquito species have been involved. predominantly Culiseta melanura and Culex nigripalpus with one isolation each from Aedes infirmatus, Culex salinarius, Anopheles crucians, Culex quinquefasciatus and Mansonia perturbans. Actual transmission of Eastern and Western virus to sentinel chickens was demonstrated in November and December, 1965. Two chickens converted by HI test against EE and one against WE. Both HI results were confirmed by SN test. During June and July, 1965, nineteen wild birds apparently developed EE-HI antibody and similarly in May and early June 1966, eleven wild birds developed EE-HI inhibitors. The failure to confirm a large number of these by the Sn test suggest the strong possibility that these are non-specific inhibitor related to ovulatory activity of the birds. Approximately 500 small mammals have been examined for EE-HI antibody and no detectable conversions have occurred. For the first time in ERC experience, persistent elevation in HI and neutralizing antibody titers for WE was detected in the sera of three patients with suspected viral disease. Their WE-CF titers were not elevated. In each instance there was a history of residence in western states but no record of encephalitis. Two of these patients also had elevated SLE-HI titers.

Other Special Studies or Activities.

Mosquito Attractant Studies.

The monthly average per trap night of mosquitoes collected by chick baited traps was compared with CDC miniature light traps in a special study area from May 1964 through October 1965. *Culex nigripalpus* responded better to light trap in the summer and slightly better to bait in the winter. The response by *Aedes infirmatus* was similar to both types of traps with the light slightly better in the summer. The monthly average for trap night collections for both *Culiseta melanura*, *Aedes atlanticus-tormentor* was greater for light trap than for the bait trap each month during the period.

A brief comparative study was carried out on twelve trap nights during July and August 1965, using a two to three week old chick, crushed dry ice alone, and a chick inside a trap with dry ice outside and above the trap. Results of this brief study indicate that of the three, the chick attracted the least number of mosquitoes, the chick with dry ice the most.

Migrant Bird Studies.

Special attention was given to the migrant bird population moving through Florida during the year. Tissues from more than one thousand migrants were screened by inoculation into one to two day old chicks in attempt to detect arbovirus activity. Serologic studies were carried out on 198 sera from heron and egret fledglings collected in the peninsular portion of the state. This was a portion of a larger group of 1,114 fledglings which were banded and color marked in an effort to trace movements following the nesting season. One banded and marked heron was recovered in Acapulco, Mexico. A special collection of 271 bloods for isolation was made in the Dry Tortugas in the spring of 1965 and 301 in the spring of 1966. Migrant birds were captured during periods of adverse weather which forces them to land on the small islands off the tip of Florida. No recoveries of virus have been made from these bloods.

Three infection experiments with California encephalitis viruses in domestic rabbits have been carried out. Both the local trivittatus-like strain and the prototype BFS-283 strain produced a detectable viremia in 50 per cent or more of the rabbits at the 48 hour period. However, only after injection with the BFS-283 virus did the rabbit produce detectable HI antibody at one and three weeks, using BFS-283 HA antigen. None of the local strains of CEV virus have yet been demonstrated to produce HA antigens.

REPORT FROM FLORIDA STATE BOARD OF HEALTH LABORATORY JACKSONVILLE, FLORIDA

The state-wide arbovirus surveillance program, which was instituted in 1962 following an epidemic of SLE in the Tampa Bay area of Florida, has continued. The combined efforts of the Bureaus of Preventable Diseases, Entomology and Laboratories of the State Board of Health have been extended to include an investigation of factors influencing the transmission of the arboviruses.

At the symposium on arbovirus research and surveillance, held in St. Petersburg, Fla., June 21-22, 1966, there was a report of the 643 arboviruses isolated in Florida within the period 1952-1965 (Table I). It is interesting that there were 23 isolations of VE by the USPHS from the Everglades area in 1963-1964. Table II shows the different species from which the arboviruses were isolated. With increased arthropod collections and improved laboratory technology, the SBH at Jacksonville isolated 75 arboviruses from 2,231 pools of mosquitoes in 1965.

EE was diagnosed in three patients in Florida during 1965: one case terminated fatally. Each was confirmed serologically. The EE virus was isolated from a covey of chukars and 46 clinical cases were reported in horses. State-wide study, surveillance and control of arbovirus infections, early recognition and promptly intensified control measures are directed toward elimination of future arbovirus epidemics.

TABLE I

ARBOVIRUS ISOLATIONS BY YEAR REPORTED, FLORIDA, 1952 - 1965

	Arbovirus Type							
Year	EE	WE	VE	SLE	CE	BG	HPL	Total
1952	1			1				2
1953								
1954	4							4
1955								
1956								
1957	8			1				9
1958	6							6
1959	2							2
1960	8	6				2		16
1961	9	1		2		3		15
1962	7			49	2	1		59
1963	14	3	6		39	77	1	140
1964	53	11	17	1	70	91	11	254
1965	27	4			55	38	12	136
TOTAL	139	25	23	54	166	212	24	643

WRH:ew

FSBH 6/66

TABLE II

ARBOVIRUS ISOLATIONS BY SOURCE OF SPECIMEN, FLORIDA, 1952 - 1965

	Arbovirus Type							
Source	EE	WE	VE	SLE	CE	BG	HPL	Total
Human	1			6				7
Equine	40	1						41
Bovine	1							1
Fox						1		1
Pheasant-Chukar	31	3						34
Pigeon				2				2
Other wild birds	10	4						14
Sentinal mouse		2						2
Sentinal chick	2	2						4
A. atlanticus	1	1	1		66	1		70
A. fulvus pallens					1			1
A. infirmatus	2	2			40	5		49
A. taeniorhynchus		1	1	1	39	53		95
Aedes ap.	2				8			10
An. crucians	1		2	1	2	137		143
An. punctipennis						1		1
An. quadrimaculatus						3		3
C. nigripalpus	14		1	41	3	3	6	68
C. (Melanoconion) sp.	2		18	1	4			25
C. quinquefasciatus	1							1
C. salinarius	1							1
Culex sp.	2			2				4
Cs. melanura	27	9			2	1	18	57
M. perturbans	1				1			2
P. confinnis						6		6
P. ferox						1		1
TOTAL	139	25	23	54	166	212	24	643

WRH:ew
FSBH 6/66

REPORT FROM THE ARBOVIRUS UNIT
THE COMMUNICABLE DISEASE CENTER
ATLANTA, GEORGIA

St. Louis Encephalitis in Dallas, Texas - 1966.

Several cases of human encephalitis were recognized during the last week of July 1966, by the house staff of Parkland Memorial Hospital in Dallas, Texas. Early laboratory results indicated haemagglutination inhibiting (HI) antibodies for St. Louis encephalitis (SLE). At the invitation of the Texas State Health Department, a CDC team arrived in Dallas on August 10, and in collaboration with the Dallas City Health Department, and Texas State Laboratories, Austin, commenced epidemiological, virological, entomological and ornithological investigations.

The date of onset of the first recognized human case was June 8. However, a significant number of cases did not appear until July, and the count did not assume epidemic proportions until the first week in August. At that time 26 human cases (laboratory confirmed or presumptive) were recognized. On September 27 the SLE case count is 61 confirmed and 108 presumptive.

To date, Dr. S. Edward Sulkin of Dallas has obtained three isolations of SLE virus from brain tissue. The patients from which these tissues were obtained expired on the fourth, sixth and seventh day following onset of illness. Histopathology of each supported the diagnosis of encephalitis. Attempts to isolate SLE virus from brain tissues of other expired patients are in progress.

The age distribution varied from infants a few months old to very elderly persons. Early calculations of attack rates, using 1960 census data, indicated the lowest rate (around 5 per 100,000 population) in the young, rising to the highest rate in the 70+ age group (70 per 100,000). No predominant sex or racial distribution was apparent.

Although early cases appeared in general to cluster near the river, there were cases found throughout the city. The greatest concentrations of cases were in the lower socioeconomic sections.

From past medical records, there was no indication that Dallas had previously experienced an epidemic of SLE. An obvious, unusual circumstance preceded the current epidemic. Heavy rainfall between April 22 and May 2, 1966, caused severe flooding of the Trinity River, which runs through Dallas. Dikes bounding the river were breached, garbage from sanitary fills dispersed widely, and sewage facilities were seriously impaired,

particularly in the lower-lying sections of the city. As a result, stagnant, organically enriched water catchments were left. During the drier period which followed cessation of the rains undoubtedly contributed significantly to an increase in breeding of Culex quinquefasciatus, the most commonly recognized urban vector of SLE in the south central states.

The entomologists collected mosquitoes from all parts of the city, establishing numerous collecting stations in culverts and outbuildings, primarily backyard chicken houses. Emphasis was given to neighborhoods where cases had occurred, but collections were not restricted to these areas. The purpose of the mosquito sampling was three-fold: to confirm the vector species involved, to determine mosquito infection rates in various parts of the city, and to provide a basis for evaluation of mosquito control measures.

Upon recognition of an epidemic condition, local mosquito control measures were emphasized. These consisted primarily of evening dusting of infected districts with 3% benzine hexachloride by means of five truck-mounted Buffalo turbines. In addition, malathion fogging with three truck-mounted units was commenced, as well as selective larviciding of known mosquito breeding sites. It soon became apparent that these measures were probably inadequate for the degree of mosquito reduction required.

Through arrangements with the United States Air Force, the services of specially equipped C-123 spray plans and crews were obtained for low-level spraying of the city of Dallas and other communities in Dallas County. The insecticide used was undiluted malathion, applied as a fine mist at the rate of three ounces per acre. The time of spraying was from dawn to about 7:30 a.m. The city and county were treated in sections, commencing on August 19 and ending on August 27.

Judging from reduction of mosquito counts in the various sampling stations, the airplane spraying probably reduced the adult Culex quinquefasciatus population by as much as 90 to 95 percent in most areas. The counts remained very low for 7 to 14 days; thereafter original counts were again obtained in a number of the sites, indicating renewed breeding. However, in some areas the counts have remained at low levels.

The virus isolation rates from C. quinquefasciatus, before and after the airplane spraying, attest to the effectiveness of the aerial treatment in reducing the populations of infected mosquitoes. Although all of the mosquito isolates have not yet been confirmed by serological tests, it is apparent that about 60 were made from approximately 10,000 mosquitoes collected before, during and on the first day after spraying, but only a single isolation

or possibly none, from 12,000 mosquitoes collected two to three weeks thereafter. Several thousand additional mosquitoes collected later are currently on test.

The results from the Dallas birds are not yet complete. During the first week, about 90% of the birds collected and tested were house sparrows. A tentative identification of SLE virus has been made on an isolation in DETC from the blood of a house sparrow caught during the first week of investigation. Serological studies on other bird sera are in progress. A census of the birds of Dallas shows the house sparrow to be the most abundant species (about 64%), followed by the blue jay (12%) and the feral pigeon (10%).

Following the spraying, human cases continued at the same rate for approximately two weeks. At that time there was a marked decrease in cases and, as of now, the epidemic appears to be ended. These results indicate that the aerial spray probably had significant effect in reducing virus transmission to the human inhabitants of Dallas.

Isolation and Identification of Arbovirus Group C - Isolates from the Florida Everglades.

Continuing investigations of the ecology of VEE and other arboviruses in the Florida Everglades have isolated a number of arboviruses. Isolations of California, VEE, Bunyamwera and Guama group and other arbovirus strains have been described in previous issues of the Information Exchange. Some of these other isolates have now been characterized as arbovirus Group C strains.

Forty-five strains isolated from the Everglades during the period 1963-1965 have been studied. They fall into three types which appear to be new Group C viruses. Mosquito pools yielded 42, mammalian sera, three. Virus strains related to each prototype were isolated during each year of collection. All but two of the mosquito isolates were from pools of Culex (Melanoconion).

After serial passage usable hemagglutinins were produced with each of the prototype strains, Gumbo Limbo, Shark River, and Pahayokee which are the tentative names given to these agents. Neutralization tests were performed by plaque reduction technique in BHK-21 tissue culture.

In tests performed to date, it is apparent that strain, Gumbo Limbo virus shows an antigenic relationship to all known Group C strains except Patois and Zegla. Gumbo Limbo is not related by HI, CF or NT to Pahayokee and Shark River viruses which do show antigenic relationship to Patois and Zegla.

REPORT FROM THE WALTER REED ARMY INSTITUTE OF RESEARCH THE DEPARTMENTS OF VIRUS DISEASES AND ENTOMOLOGY AND THE VETERINARY SCIENCE DEPARTMENT UNIVERSITY OF MARYLAND

Arbovirus Studies on Maryland's Eastern Shore.

Both mosquitoes and vertebrates were collected in the Pocomoke Cypress Swamp, Maryland, and vicinity during 1965-66. As was observed in areas along the East Coast during the summer of 1965 by others, Eastern (EEE) and Western (WEE) equine encephalitis viruses and Flanders virus were recovered on frequent occasions (Table 1). Flanders virus was recovered almost as soon as the first *Culiseta melanura* were collected in the spring, continued to be recovered frequently in June and was less frequently encountered when EEE and WEE viruses were first detected in *Culiseta melanura*. Flanders virus had entirely disappeared from *Culiseta melanura* by late summer - early fall, a time when EEE and WEE viruses were recovered with regularity.

Virus isolation attempts from vertebrates sampled on a year-round basis have failed to yield any virus, despite numerous virus recoveries from *Culiseta melanura* in the same area, Serological surveillance of mammals and reptiles shows that neutralizing substances to EEE virus occur in sera of medium-sized mammals (i.e. raccoons, opossums, skunks) fairly commonly, whereas serum from small mammals (i.e. wood mice, shrews) rarely neutralized EEE virus (Table 2). The acetone-extracted sera of turtles bled during the summer of 1965 commonly inhibited hemagglutination by EEE virus. Electrophoresis of field-collected mud turtle serum and serum from a mud turtle inoculated with EEE virus showed that hemagglutination-inhibiting properties of the wild turtle sera were not associated with immune globulins (Table 3). These sera failed to neutralize EEE virus in tissue culture or mouse systems, hence, were interpreted as non-specific reactions. Neutralization tests showed comparatively few reptiles to have EEE virus antibody (Table 4).

Since monitoring of many sedentary vertebrates has failed to suggest the source of *Culiseta melanura* EEE virus infection, the current season's efforts are directed toward the natural history of *Culiseta melanura* mosquitoes in the Pocomoke Cypress Swamp.

(E.L. Buescher, T.M. Yuill, M.J. Collins, Jr. and J. Scanlon).

Table 1 Arbovirus Infection of C. melanura, Pocomoke Cypress Swamp, 1965

Week of	No. Female C. melanura Captured	Blooded	Virus Infection Rates/Thousand Mosq. EEE	WEE	Flanders
Apr 18-24	1	0	0	0	0
25-1	5	0	0	0	0
May 2-8	183	0	0	0	5.5
9-15	259	0	0	0	9.7
16-22	1856	3	0	0	0.5
23-29	2420	64	0	0	0.7
30-5	1800	16	0	0	0.9
Jun 6-12	1525	0	0	0	2.3
13-19	1200	29	0	0	1.4
20-26	4400	298	0	0	0.2
27-3	2360	28	0.4	0.1	1.7
Jul 4-10	2200	181	0	0.4	1.6
11-17	1900	23	0	3.4	0.5
18-24	700	44	4.3	1.5	1.2
25-31	1900	39	3.2	1.7	0
Aug 1-7	1425	6	7.8	2.8	0.9
8-14	1100	0	2.1	2.1	0.9
15-21	3348	1	4.3	2.2	0
22-28	3718	1	4.7	1.5	0.3
29-4	1120	0	11.3	0	0
Sep 5-11	1648	0	4.7	3.2	0
12-18	2468	34	1.1	2.2	0
19-25	1590	10	0.9	0	0
26-2	352	0	0	0	0
Oct 3-9	170	0	0	7.7	0
10-16	68	0	0	0	0
17-23	1380	1	2.8	0	0
24-31	2		0	0	0
Nov 1-6	0		0	0	0

Table 2 Prevalence of Neutralizing Antibody to EEE Virus in Mammals From the Pocomoke Cypress Swamp, 1964-1966

Mammal	Serology: 1 Oct 64-31 Mar 65	1 Apr 65-31 Mar 66	1 Apr 66-6 Jun 66	Total
Wood Mouse (Peromyscus leucopis)	0/270	4/411	0/76	4/757
House Mouse (Mus musculus)	0/45	0/5		0/50
Meadow Vole (Microtus pennsylvanicus)	0/11			0/11
Eastern Jumping Mouse (Zapus hudsonicus)	0/3			0/3
Pine Vole (Pitymys pinetorum)		0/1		0/1
Southern Flying Squirrel (Glaucomys volans)			0/1	0/1
Gray Squirrel (Sciurus carolinensis)	0/2	1/4		1/6*
Muskrat (Odontra zebithica)		0/80		0/80
Short-tailed Shrew (Blarina brevicauda)	0/3	0/5	0/4	0/12
Least Shrew (Cryptotis parva)	0/4			0/4
Masked Shrew (Sorex cinereus)	0/4	0/2	0/6	0/12
Eastern Cottontail Rabbit (Sylvilagus floridanus)		0/3		0/3
Striped Skunk (Mephitis mephitis)	0/2	2/7		2/9
Raccoon (Procyon lotor)	0/6	3/14	1/11	4/31
Opossum (Didelphis virginiana)	0/21	3/14	1/3	4/38
Gray Fox (Urocyon cineroargentius)		1/1		1/1
White-tailed Deer (Odocoilous virginiana)			0/1	0/1

* Squirrel shot and bled. Neutralization may be nonspecific.

Table 3 Comparison of Location of HI-Reactive Fractions (Reciprocal Titer) in Turtle Sera from the Field and a Turtle Serum 2 Months After a Laboratory EEE Virus Infection

Fraction #		Field Sera		EEE - Convalescent Serum	
		Protein Com.	HI Titer	Protein Com.	HI Activity
10	IGG	0.04	0	0.03	0
12	IGG	0.18	0	0.09	0
14	IGG	0.36	0	0.40	2
16	IGG	0.73	0	0.58	2
18	IGG	1.10	0	0.67	4
20	IGG	1.30	0	0.44	4
22		1.10	0	0.30	0
24		0.97	2	0.26	0
26		1.05	4	0.28	0
28		1.35	8	0.41	0
30		1.40	2	0.56	0
32		1.1	0	0.40	0
34		0.72	0	0.06	0
36		0.38	0	0.01	0

Table 4 Prevalence of Neutralizing Antibody to EEE Virus and Isolation Attempts from Clots in Reptiles from the Pocomoke Cypress Swamp, 1964-1966

Reptile	1 Oct 64- 31 Mar 65	1 Apr 65- 31 Mar 66	1 Apr 66- 7 Jun 66	Totals	Isolation Attempts From Clots
Painted Turtle (*Chrysemys picta*)	0/1	5/130	0/97	5/228	0/159
Spotted Turtle (*Clemmys guttata*)	0/1	2/49	0/54	2/104	0/94
Mud Turtle (*Kinosternon subrubrum*)		3/64	2/105	5/169	0/115
Snapping Turtle (*Chelydra serpentina*)		1/34	0/11	1/45	0/14
Eastern Box Turtle (*Terrapene carolina*)	0/2	0/30	0/3	0/35	0/6
Northern Rat Snake (*Elaphne obsoleta*)	0/2	1/7	0/4	1/13	0/6
Black Racer (*Coluber constrictor*)	0/1	0/11		0/12	0/6
Brown Snake (*Storeria decayi*)	0/1			0/1	
Common Kingsnake (*Lampropeltus getulus*)	0/1	0/3	0/1	0/5	0/2
Common Watersnake (*Natrix sipedon*)		0/5	0/2	0/7	0/3
Rough Green Snake (*Opheodrys aestivus*)		0/1		0/1	
Eastern Fence Lizard (*Sceloporus undulatus*)		0/4	0/6	0/11	0/5

REPORT FROM THE LABORATORY OF VIROLOGY & RICKETTSIOLOGY
DIVISION OF BIOLOGICS STANDARDS, NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

Until recently investigators in the United States have been unable to confirm the observations of Argentinian workers on Junin virus pathogenicity for the adult guinea pig (GP). There has been much speculation about the possible differences in the GP's found in the two countries, but, as yet, there is little evidence that this could explain the conflicting results. We feel that our recent experience might shed some light in this area.

In early 1964 we were able to infect adult GP's with a strain of Junin virus which was passaged twice in guinea pigs, 13 times in newborn mice and 21 times more in guinea pigs (GP/2, SM/13, GP/21). This particular GP-adapted strain was in the 19th GP passage when it was brought to the Rockefeller Virus Laboratory in New York City by Dr. N. Mettler who passaged it one additional time in GP's (GP/20). Dr. Jordi Casals provided an aliquot of this GP/20 pool to the Laboratory of Tropical Virology (LTV) of NIAID. At LTV this virus was again passaged in GP's (GP/21). Although several animals became sick and died, no definite hemorrhagic lesions were noted. In our studies at LVR, DBS we have been using virus pools prepared from animals inoculated with the harvest of these animals.

This GP-adapted virus is highly pathogenic for baby and adult GP's (Hartley and NIH strains and inbred strains 2 and 13). At autopsy, many, not all, animals have hemorrhagic lesions, such as gross hemorrhage in the lung, kidney, stomach and intestines. All GP's which become clinically ill die. Animals which survive inoculation with high dilutions of the virus do not develop complement-fixing (CF) or plaque neutralizing (PN) antibody. Animals given Tacaribe virus develop CF and PN antibody to the homologous Tacaribe virus but develop only CF antibodies to the heterologous Junin virus. Tacaribe-immunized animals inoculated with the GP-adapted Junin virus not only survive but also develop high levels of CF and PN antibodies to both Tacaribe and Junin viruses. This GP-adapted Junin virus causes disease in suckling mice only when high doses (4-5 Log_{10} guinea-pig LD_{50}) are inoculated intracerebrally.

However, experience with a mouse-adapted Junin virus (GP/2, SM/17) differs from what we observe when the GP-adapted virus is used. The mouse-adapted virus causes disease in suckling GP's only after intracerebral inoculation of at least 4-5 Log_{10} suckling mouse LD_{50} of virus. It does not cause overt disease in adult GP's at any dose. The CF antibody response of these adult animals is highly irregular varying from <4 to 32-64.

We feel that Junin virus is capable of infecting both suckling mice and suckling and adult GP's but that it grows better in that animal to which it has been adapted. For those interested in performing experiments with Junin virus in adult GP's, it would be advantageous to select a suitably adapted strain.

(Nicola M. Tauraso and Alexis Shelokov)

REPORT FROM THE LABORATORY OF TROPICAL VIROLOGY NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, NIH BETHESDA, MARYLAND

Recovery of Machupo Virus from Experimentally Inoculated Ticks (*O. savignyi*).

Collaborative studies begun with Dr. H. Hurlbut last year failed to demonstrate recovery of virus from mosquitoes (*C. pipiens*), ticks (*O. savignyi*), bedbugs (*C. lectularius*), and carpet beetles (*A. piceus*) after experimental infection with Tacaribe, Junin (XJ) and Amapari viruses. Results of similar studies with the Carvallo strain of Machupo virus suggested positive results from more than one arthropod species, based on characteristic illness in suckling hamsters used to isolate virus from test arthropods.

For reasons of expediency, continued studies were restricted to the virus recovered in suckling hamsters from ticks inoculated 7 days prior to test.

The recovered virus produces a characteristic CF antigen and antibody in hamsters and plaques in both MA-111 and Vero cell lines. Neutralization tests are in progress.

This data only indicates recovery of Machupo virus from experimentally infected ticks. Studies will be continued to determine whether or not transmission is possible through 5 serial passages in ticks.

On July 1, 1966, Dr. Wiebenga leaves Bethesda for an assignment by NIAID at the 406th Medical Laboratory, U.S. Army Medical Command, Japan (APO San Francisco 96343). Mr. John I. Thomas is expected to follow about October 1, 1966. Continued study of hemorrhagic fevers is the motive for this move.

Dr. William D. Hann will be responsible for the continuing interests of LTV in both hemorrhagic fever and arbovirus.

REPORT FROM EPIDEMIOLOGY BRANCH AND LABORATORY OF SLOW, LATENT, AND TEMPERATE VIRUS INFECTIONS NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS NIH, BETHESDA, MARYLAND

The National Institute of Neurological Diseases and Blindness, through its Epidemiology Branch in Bethesda, its Research Center in Guam, its Study Group on Child Growth and Development of Disease Patterns in Primitive Cultures, and its Laboratory of Slow, Latent, and Temperate Virus Infections, is conducting studies of Japanese B Encephalitis (JBE) on Guam. The long range purpose of these studies is to determine if there are sequelae to arbovirus infections particularly among infants who may have had inapparent infections during the perinatal period. Sequelae have been observed following clinically apparent Western Encephalitis in California during the perinatal period by Dr. Knox Finley. The situation on Guam offers a unique possibility to conduct this type of investigation. The NINDB has a permanent staff of neurologists investigating the unusually high incidence of amyotrophic lateral sclerosis and other degenerative diseases among Guamanians. An epidemic of Japanese B encephalitis occurred on Guam in 1948. This event was well documented by Drs. Hammon, Reeves, and others. Their evidence indicated that the virus completely disappeared from the population following the epidemic.

Our efforts on Guam are proceeding in three phases: (1) to document the impression that JBE virus disappeared from the island shortly after the 1948 epidemic. (2) To study the serological responses of individuals who were exposed to JBE at one time in 1948 and possibly never since. (3) To investigate the neurological and psychological sequelae of apparent and inapparent infections particularly in the perinatal period with JBE virus.

After we embarked upon this study, we learned through the Medical Officer, CommNavMarianas and members of NAMRU 6 that a recent entomologic survey of Guam revealed an increasing prevalence of the *Culex tritaeneorhincus* mosquito. Although the most important vector of JBE in other areas, this mosquito was reported not to be involved in the Guam epidemic of 1948.

We have screened 103 children born since 1950 by routine HI tests for JBE, RSSE, Dengue 1, and 41 sera have been tested for SLE. Of the 103 children, six had titers against JBE (5 with titers of 1:40 or greater; 1 with a titer of 1:20); and two had titers of 1:20 against Dengue 1. There were no titers of 1:20 or greater against RSSE. Of the 41 sera tested against SLE, three had titers of 1:20 and three had titers of 1:40 or greater. Positive reactors are being tested now in mouse neutralization tests.

To date we have screened 123 pigs and five dogs by HI against JBE only. Bloods were collected on filter paper discs using a technique which we have found satisfactory in other studies for Group B arboviruses. One dog and one pig had titers of 1:20 to JBE. Following the epidemic in 1948, 100% of the pigs tested and most of the dogs had antibody to JBE.

Our impression at this time is that minimal activity by a Group B virus occurs on Guam. There may be a small focus of JBE virus but the greater likelihood is that another virus is involved because the rates of positivity to SLE are somewhat higher than to JBE, and the pigs, usually a good sentinel for JBE, had virtually no antibody to this virus. In view of the potentially explosive situation with the *Culex tritaeneorhincus* now abundant on the island, these studies will continue until more complete documentation is available. In the meantime we will start to screen individuals born between 1946 and 1950 in order to develop a study population of individuals who have evidence of contact with JBE virus and individuals who do not. Subsequently, neurologists will examine positives and controls with no knowledge of their serological history in order to determine whether the infections gave rise to any detectable neurological or psychological deficits.

We would like to appeal to other recipients of the Arthropod-borne Virus Information Exchange to review their records in order to see if they know of situations analogous to that in Guam, where an arbovirus invaded a community and then disappeared. As we work out our protocols for neurological and psychiatric testing, it would be appropriate to conduct investigations of this nature on a number of selected arboviruses.

(Jacob A. Brody and C. Joseph Gibbs, Jr.)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE BALTIMORE, MARYLAND

Studies on Accessory Factor-Enhanced West Nile Virus Neutralization.

In previous reports we have described the enhancement of neutralization of West Nile virus (WNV) by accessory factor (AF) *in vitro*, AF activity of sera from different animal species, the influence of various levels of human AF on WNV neutralization and preliminary data on the nature of AF. This report is concerned with the sequence with which WNV, hyperimmune antibody and AF react.

Attempts were made to determine the sequence with which AF interacted with WNV and immune serum. In order to determine whether or not AF acted after virus antibody interaction, high titered WNV was reacted with a 1:500 dilution of hyperimmune serum at pH 7.0, at 37 C in the absence of AF. After 150 minutes neutralization two aliquots of the reaction mixture were removed and diluted 1:100 in cold buffer solution containing, 2) 25% accessory factor, or b) accessory factor heat inactivated at 56 C for 30 minutes. Samples of each of the two reaction mixtures were assayed at various time intervals for virus content. As a control the original virus antibody mixtures was allowed to incubate and the contents were assayed at predetermined time intervals. The neutralization process advanced at a fairly slow rate in the absence of any form of AF and a large persistent fraction was noted. However, when antibody activity was removed by dilution and, simulatenously, AF was added, the neutralization process accelerated markedly. When the virus-antibody mixture was diluted in heat inactivated AF, the rate of neutralization did not change and the resultant neutralization curve paralleled the thermal inactivation curve and the control curve. Similar results were obtained when the antibody used for initial neutralization of WNV was reduced to 1:16,000, although the rate of neutralization in the presence of AF was reduced over that observed when 1:500 antibody was employed. When the antibody concentration in the initial portion of the experiment was reduced to 1:180,000, no AF potentiated neutralization was observed. The results of these experiments suggest that AF acts upon preformed virus-antibody complexes and not necessarily in the initial interaction of antibody with virus.

Experiments were designed to determine the influence of AF upon, a) the neutralization of virus preadsorbed to susceptible cells but prior to penetration, and b) penetration of virus-antibody complexes into susceptible cells.

Virus was pulse-adsorbed to chick embryo cultures for two minutes. The concentration of virus selected was such that approximately 100 plaque forming units remained attached even after three wash cycles with Hanks' BSS. To one set of cultures was added a mixture containing 1:500 antibody and 25% AF and to the second set of cultures 1:500 antibody plus 25% heat inactivated AF. At intervals replicate cultures were repeatedly washed with BSS and overlayed with agar medium. The results of these experiments show that antibody plus AF neutralized adsorbed virus more rapidly and to a greater extent than antibody and heated AF. These results suggest that AF does enhance neutralization of preadsorbed virus but does not necessarily render all virus particles incapable of penetration.

To test the influence of AF upon neutralization of virus during the antibody sensitive penetration period, cultures were pulse

infected, washed, and the virus allowed to penetrate under BSS. At intervals the BSS was removed from replicate cultures, and antibody plus AF or antibody plus heat inactivated AF was added for a two minute period. The dilution of antibody selected was such that 99% virus neutralization occurred in two minutes. The antibody was then removed by repeated washings and the cultures overlayed. A total plaque count control, i.e., normal serum, was included in each time interval. Antibody plus AF neutralized 40-60% of the adsorbed virus during the first 40-50 minutes. Then the per cent virus neutralized decreased rapidly over the next 10-15 minutes. Virus was no longer antibody-sensitive after 60 minutes adsorption to cells. In the presence of heated AF, 20-40 percent of the adsorbed virus was neutralized for the first 40-60 minutes. Penetration however, occurred at about the same time as when potent AF was used and was complete within 60-75 minutes. These data suggest that AF does not exert its influence by altering penetration of antibody sensitized adsorbed virus.

The Cultivation of Dengue Viruses In Vitro

Continued efforts have been directed towards the development of a satisfactory tissue culture assay system for the dengue viruses, especially type 1 dengue virus. Although previous work had indicated that several strains of type 1 dengue virus would form plaques in chick embryo cell cultures (CEC) and in HeLa cell cultures, plaque size rarely exceeded 2mm in diameter in CEC, and plaques were often diffuse and difficult to count. Manipulation of medium components and/or extended incubation failed to significantly alter plaque size or morphology.

Another cell culture system, the BS-C-1 line of African grivet monkey cells, was selected for study with the hope that a more satisfactory plaque assay system could be developed for type 1 dengue virus. This line of cells was obtained from Mrs. Hope Hopps at the Division of Biologic Standards at the National Institutes of Health and was in the 344th passage when received. The growth medium consisted of 10% bovine serum, double strength Eagles BME vitamins and amino acids with glutamine, in Hanks' BSS. In all cases the pH of the medium was adjusted to pH 7.4-7.6 with sodium bicarbonate and incubation was carried out in a humidified atmosphere of 5% carbon dioxide, 95% air.

The virus diluent was 1/15 M phosphate buffer supplemented with 5% heat inactivated rabbit serum at pH 7.0. Adsorption of virus was carried out at 26 C for 120 minutes and the infected monolayer cultures were overlayed with a medium consisting of 10% bovine serum, 2X BME vitamins, 2X BME amino acids with glutamine, 15 mM bicarbonate, in Hanks' BSS with 2% methyl cellulose as solidifying agent. Sterile NaOH was added to adjust the medium to pH 7.2. Incubation was at 37 C in 5% CO_2-95% air. After

six days incubation a feeder layer was added. This medium was similar to the original overlay medium except that 5% bovine serum was utilized and 1% washed Noble agar was employed as a solidifying agent. Plaques were stained by the addition of 2 ml of 2% agar containing 1:12,500 neutral red stain. Bottles were reincubated in the dark and plaques observed on the following day.

Type 1 dengue virus, Hawaiian strain, produced plaques in about 11 days with the plaque diameter increasing to about 2 mm after 15 days incubation. The New Guinea B strain of type 2 dengue virus first produced plaques on day 7 with a slight increase in plaque size to 2 mm by the 15th day. Type 3 dengue (H-87) also produced plaques on day 7 with a maximum diameter of 2-3 mm on the 14th day. Dengue type 4, (H-241), produced plaques by the 7th day with an increase in size to 4 mm by the 13th day.

During the course of these experiments, it was noted that the BS-C-1 cell monolayers, unlike those of KB, HeLa and CEC, remained firmly attached to the glass even after extended incubation. The above experiments were repeated and after plaques were counted by the neutral red staining technique, the methyl cellulose-agar overlays were washed off of the cell sheets and the monolayers stained with crystal violet stain. The results of these experiments were similar to those obtained with neutral red staining alone except that the plaque diameter was uniformly larger after the crystal violet stain.

This technique was modified in an attempt to increase plaque diameter. Instead of the usual 2% methyl cellulose concentration, the final overlay medium employed only 1% methyl cellulose. Since the medium was rather fluid, the second agar feeding overlay was deleted. Monolayers survived for 15-17 days without additional feeding provided the initial volume was increased to supply nutrients over the extended incubation period. In most instances plaque diameter was significantly increased, this being especially true in the tests involving various strains of type 1 dengue virus.

Work is continuing on the development of this assay system in the hope that it will result in a uniformly sensitive system where large plaques may be readily counted.

(Eylar and Chandler).

REPORT FROM THE DEPARTMENT OF ZOOLOGY PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA

A grant to train students in the study of birds as reservoirs of disease has been in operation at Pennsylvania State University

for several years. The primary emphasis has been on work with wild bird populations on a 132-acre tract of mixed forest habitat near the university. Mist-nets are arranged in a square grid of 64 nets. The birds are banded and blood samples taken from about half of them. Although serological tests for the activity of viruses are not yet completed, the blood smears have been examined for protozoan parasites. Mosquito populations in the area have also been surveyed.

During 1965, 1,135 blood smears and 882 samples of serum were obtained from a total capture of 2,089 birds of 73 species.

An estimate of the population levels of various species (Table 1) was made by the Schnabel method using the release and subsequent recapture of resident birds. The length and season of the estimation period was chosen to minimize error due to migrality and fledging; the number of birds captured and recaptured apply only to the period of estimation. The estimates indicate a fairly stable population level with some fluctuation in species composition.

A preference shown by any bird species for a certain vegetation type could be determined, since the mist nets were located in a variety of habitats. Table 2 lists the vegetation preferences of 16 of the most commonly captured species. The preferences were determined by use of the Chi-square test comparing the actual captures in each type of vegetation with the captures expected if the birds had been randomly distributed.

The blood smears obtained from the birds were stained with Giemsa stain and searched for parasites for ten minutes. The prevalences of the three common genera in the period 1963-1965 are shown in Table 3. In addition to the common genera of Plasmodium, Leucocytozoon, and Haemoproteus, the genera Trypanosoma, Hemogregarina, Toxoplasma, Microfilaria, and Hepatocystis also occurred.

Among the bird species screened, Robins demonstrated the highest prevalence of Plasmodium (43% positive), Wood Thrushes the highest of Leucocytozoon (33% positive), and the Scarlet Tanager, Red-eyed Vireo, and White-throated Sparrow were high for Haemoproteus (49%, 46%, and 44% positive respectively). Forty-four birds had multiple infections with two parasites, eleven with three, and two (an Ovenbird and a Scarlet Tanager) had four.

The mosquitoes in the same area wre surveyed briefly. Six species of adult Aedes were attracted to human bait between May and September, 1965: A. punctor, A. trivittatus, A. vexans, A. triseriatus, A. canadensis, and A. stimulans, the most abundant species in August and September, and hence might be an important

vector species if an arbovirus epidemic were to occur in the area. Mosquito larvae were collected from a permanent pond, from treeholes, and from black plastic quart containers fastened near the bases of trees to simulate treeholes. These plastic containers were stocked with distilled water and some forest floor litter. A. triseriatus bred in the treeholes and containers in deciduous woods in late summer, with the greatest numbers being in the natural treeholes. The pond produced other Aedes larvae, and, late in the summer, Anopheles punctipennis.

(David E. Davis and Edwin Franks).

Table 1

Estimates of the population of selected bird species on the forestry tract (132 years)

Species	Year	Population estimate	95% confidence limits lower	95% confidence limits upper	Estimation period from - to	Number of individuals captured	Number of individuals recaptured
Wood Thrush	1963	86	38	118	April-July	59	37
(Hylocichla	1964	106	51	192	April-July	43	11
mustelina)	1965	90	narrow		April-July	75	65
Robin	1964	36	12	93	April-July	16	5
(Turdus	1965	74	50	105	April-July	50	31
migratorius)							
Tufted Titmouse	1963	18	7	38	April-Dec.	10	7
(Parus bicolor)	1964	58	45	113	April-Dec.	29	9
	1965	46	27	75	April-Dec.	26	17
Ovenbird	1963	67	40	105	April-July	44	19
(Seiurus	1964	-	-	-	April-July	14	-
aurocapillus)	1965	25	35	105	April-July	41	25
Catbird	1963	134	70	231	April-July	52	13
(Dumetella	1964	114	31	335	April-July	28	4
carolinensis)	1965	94	57	145	April-July	52	21
Field Sparrow	1963	64	28	124	April-Aug.	30	9
(Spizella	1964	-	-	-	April-Aug.	20	-
pusilla)	1965	35	93	188	April-Aug.	79	35
Summary of all species							
	1963	1227	900	1618	June-July	438	49
	1964	1144	576	1758	June-July	498	12
	1965	1063	narrow		June-July	437	82

Table 2

Vegetation preferences of 16 species of birds

Species	Total captures (3 years)	Vegetation preference
Blue Jay (*Cyanocitta cristata*)	64	randomly captured
Cowbird (*Molothrus ater*)	74	randomly captured
Swainson's Thrush (*Hylocichla ustulata*)	89	randomly captured
Scarlet Tanager (*Piranga olivacea*)	103	randomly captured
Ovenbird (*Seiurus aurocapillus*)	268	edge[1]
Wood Thrush (*Hylocichla mustelina*)	389	edge
Black-capped Chickadee (*Parus atricapillus*)	247	field, scrub and edge
Catbird (*Dumetella carolinensis*)	270	field, scrub and edge
White-throated Sparrow (*Zonotrichia albicollis*)	78	field and scrub
Field Sparrow (*Spizella pusilla*)	205	field and scrub
Goldfinch (*Spinus tristis*)	82	field, scrub and aspen
Robin (*Turdus migratorius*)	293	field, scrub and conifers
Red-eyed Vireo (*Vireo olivaceus*)	142	oak-hardwood and aspen
Rufous-sided Towhee (*Pipilo erythrophthalmus*)	178	locust, aspen and scrub
Downy Woodpecker (*Dendrocopus pubescens*)	73	large deciduous and edge
Tufted Titmouse (*Parus bicolor*)	110	large deciduous

[1]Edge denotes capture in a net which extends from one vegetation type into another.

Table 3

Prevalence of blood protozoans in captured birds

Parasite	Prevalence in		
	1963 (296 birds)	1964 (482 birds)	1965 (1043 birds)
Plasmodium sp.	9.5%	11.0%	10.5%
Leucocytozoon sp.	3.7%	4.4%	9.6%
Haemoproteus sp.	11.8%	9.5%	7.9%

REPORT FROM DEPARTMENT OF EPIDEMIOLOGY AND MICROBIOLOGY UNIVERSITY OF PITTSBURGH GRADUATE SCHOOL OF PUBLIC HEALTH

I. Typing of Viruses in California Encephalitis Virus Group

From isolates submitted from many persons in the United States and Canada, Miss Gladys Sather has distinguished eight types. These, plus the three already known exotic types (Melao, Tahyna, Lumbo), make a total of 11. The domestic prototypes now carry the following names: California encephalitis (BFS-283), group prototype, trivittatus, San Angelo, snowshoe hare, LaCrosse, Jamestown Canyon, Jerry Slough and Keystone.

II. Characteristics of Another Strain of Attenuated Japanese B Encephalitis (JBE) Virus, T-11.

A two-year study of the characteristics of this new, highly attenuated strain was made by Captain George French as a Ph.D. thesis research project. This strain is more highly infectious to many laboratory animals by the peripheral route than the attenuated strain obtained by passage at low temperature and used for the above inactivated vaccine. T-11 strain has a number of distinctive markers; plaque size was particularly studied to determine causes and significance. This involved studies of complex polysaccharide inhibitors, polyanions, pH, temperature, bicarbonate effect, growth cycles, interferon production and sensitivity, effect of actinomycin D, etc.

III. Typing of Dengue Viruses

Gel precipitin tests have been developed by Dr. Adly Ibrahim, using rabbit immune sera, which demonstrate several antigens in each dengue virus and are capable of demonstrating lines of non-identity between D-1 and TH-Sman (?D-6) and between D-2 and TH-36 (?D-5). Cross plaque neutralization tests also show consistent but small differences. Whether or not these differences between members of the two closely related pairs are significant in regard to disease potential (classical dengue *vs* hemorrhagic fever) is unknown, or whether many dengue strains closely related by more conventional serological tests show distinct antigenic heterogeniety by properly designed gel diffusion tests is not known.

IV. Formalin Inactivated Japanese B Encephalitis Tissue Culture Vaccine in Human Volunteers

Dr. Darwish and others have completed serological tests on 41 volunteers given the inactivated, attenuated (OCT-541 24°C) strain grown in hamster kidney cells. Group B virus virgins responded to three inoculations by 92% gaining neutralization

indices of 3.0 to > 6.0 logs. Titers in most persisted well for eight months and a booster at this time gave 100% response, all at higher titers than ever before. CF and HI antibody responses seldom developed in virgins but developed promptly to many group B viruses in those with previous group B infections other than that of 17-D Y.F. vaccine. Local reactions were negligible and there were no systemic reactions. This vaccine is considered suitable for commercial scale production and large scale field trial.

V. Formalin Inactivated JBE Virus Vaccine From Wild, Virulent OCT-541 Strain

Several lots of vaccine prepared in the same way in hamster kidney cells from the virulent, early passage virus, though of similar infectious titer to that of the attenuated before inactivation produced a product which by the mouse potency test was two to three times that from the attenuated strain. An equivalent potency was then obtained with the avirulent strain by simply reducing the amount of tissue culture fluid present at the time of harvest. The Nakayama strain failed to produce suitable potency.

VI. Formalin Inactivated St. Louis and West Nile Encephalitis Vaccines From Hamster Kidney Cells

Using a stable, large plaque derivative of the Florida P-15 SLE virus an inactivated vaccine was prepared exactly as for the JBE viruses. The potency of several lots as tested in mice was consistently higher (MID < 0.0015 ml) than those for JBE viruses. Preinactivation titers were $10^{9.7}$ per 1.0 ml with no reduction of amount of culture fluid.

One strain of West Nile virus was tested, and it made an entirely unacceptable product.

VII. Identification of New Tick Arboviruses From Florida

Two similar viruses submitted for identification by Dr. Bond from the Tampa Bay Area were tested by Miss Sather against the tick viruses recognized in North America and then with antigens and antisera from the Florida isolates. She was afforded an opportunity by Dr. Casals at YARU to check them against all other tick-borne virus sera available there and representatives of all other grouped and non-grouped arboviruses available there. The Florida agent, now named Sawgrass virus, proved unrelated to all.

VIII. Cotton Rat and House Mouse Viruses From Tampa Bay Area

These two agents submitted by Dr. Bond for identification were not related. Miss Sather checked these against a number of

possible, known murine agents, then against arbovirus groups available in our laboratory, then against the essentially complete battery of grouped and non-grouped arboviruses made available at YARU, without finding any antigenic relations.

IX. Mouse Hepatitis Epizootic in Laboratory

While Capt. Thomas Keefe was working on the epidemiology of the low grade, occasional spontaneous transmission of MHV in our building (which on occasion has developed into a severe epizootic) a severe epizootic occurred! The source was an agent submitted from the Tampa Bay Laboratory for identification, supposedly from *Culex nigripalpus*. This agent, subsequently identified as MHV, promptly gave rise to a massive epizootic among suckling mice and spread to several different rooms. It was fed by supplying susceptibles under a variety of conditions for four months. Deaths and serological conversions under a great variety of environmental conditions were studied, and results are being analyzed as a doctoral thesis in epidemiology.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
RUTGERS MEDICAL SCHOOL, NEW BRUNSWICK, NEW JERSEY

Further studies have been carried out on the physical-chemical characterization of dengue-2 virus and its RNA. In the work previously reported, virus harvested from KB cell cultures was subjected to estraction with fluorocarbon and CsCl equilibrium density gradient centrifugation. The latter yielded two major classes of hemagglutinating (HA) particles of which the denser one (mean density 1.24 gm/ml) contained 90-99% of the residual plaque-forming activity, while the other one (mean 1.19 gm/ml) consisted largely of non-infectious HA. Because CsCl reduced infectivity by 90-99.5% and also in order to avoid possible deleterious effects of fluorocarbon, further biochemical analyses were done avoiding these two steps. Freshly harvested, P^{32}- or H^3-uridine labeled virus was pelleted at 78,000 g for two hours. The pellet was resuspended in 1/50 to 1/100 volume of borate saline pH 9 containing 0.2% bovine serum albumin (BABS9). Two ml of the concentrate were layered on 27 ml of a linear sucrose gradient (5-25% sucrose in BABS9) and centrifuged for three hours in the Spinco SW25.1 rotor at 25,000 rpm. This procedure preserved undiminished HA and 30-100% of the plaque-forming activity.

The virus again separated into two major HA components of which the faster settling one contained most of the infectivity. The 3-4 fractions constituting this peak were combined as "Pool I" and those from the slow-moving component made up "Pool II."

The RNA was extracted from each pool with 80% phenol at room temperature. The extracted RNA was subjected to sucrose gradient centrifugation with rat liver RNA added as a reference marker.

Virus from Pool I yielded a single homogeneous species of RNA which had a calculated sedimentation coefficient of 45S and an estimated M.W. of 3.3×10^6. Pool II also contained a small amount of 45S RNA and, in addition, a larger peak of RNA of sedimentation coefficient 6-8S.

Base analyses on these three kinds of RNA are summarized in Table 1 which shows that the 45S components from Pools I and II were probably identical and very similar in composition to the Sindbis virus RNA previously analyzed by Pfefferkorn and Hunter. The 6-8S species is obviously different. Its origin and significance are unknown.

The 45S RNA is apparently the basic infectious unit of dengue-2 virus. The amount of P^{32} incorporated in it is, in any given experiment, in a constant ratio to the PFU titer regardless of whether the RNA is derived from Pool I or II.

Inhibition experiments were carried out with actinomycin D, 5-fluorouridine, and 6-azauridine. Actinomycin D, present throughout the experiments, failed to inhibit virus synthesis and, at concentrations of 0.01 - 0.03 ug/ml, induced a slightly increased yield. FUR and AUR inhibited viral growth both in terms of PFU and HA. The time course of viral RNA synthesis was studied with the aid of AUR inhibition, with or without reversal with uridine. These experiments showed that RNA synthesis essential for virus growth began about six hours after infection and preceded viral maturation by six to seven hours.

(V. Stollar, T.M. Stevens, and R.W. Schlesinger)

TABLE 1

BASE COMPOSITION OF VIRAL RNA

Virus	Sample		Nucleotides per 100 nucleotides			
			U	G	C	A
Dengue-2	Pool I	45S	21.6±1.6	26.4±0.9	21.3±0.6	30.6±1.7
Dengue-2	Pool II	45S	21.8	25.2	21.8	31.3
Dengue-2	Pool II	6-8S	24.4	29.1	22.1	24.4
Sindbis[a]			19.7±0.3	25.8±1.1	24.9±0.9	29.6±1.0

[a] Pfefferkorn, E.R., and Hunter, H.S. (1963). Purification and partial chemical analysis of Sindbis virus. Virology 20, 433-445.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
CORNELL UNIVERSITY MEDICAL COLLEGE

I. Investigations of Arthropod-Borne Viruses Along the Eastern Coastal Tropics of Mexico.

Investigations of arthropod-borne viruses along the eastern coastal tropics of Mexico were continued during 1965. Habitats near Sontecomapan and Minatitlan, Veracruz have yielded numerous strains of Venezuelan encephalitis virus from mosquitoes, sentinel hamsters, sentinel mice, a Philander opossum and an Artibeus bat. A group C arbovirus has been recovered repeatedly from sentinel hamsters and sentinel mice and an unidentified virus, 63A49, from mosquitoes, sentinel suckling mice and sentinel hamsters. A preliminary HI antibody survey indicated that since 1963, VE virus has been infecting humans, domestic animals, particularly pigs, wild terrestrial mammals, young herons and perhaps resident wild birds along the southeastern tropical coast of Mexico. Exposure of three horses and three burros at Sontecomapan during summer 1965 showed that the strain or strains of VE virus in nature at that time infected but did not produce overt disease in these animals. The quantities of VE virus in hamster organs at illness or death ranged from $10^{5.5}$ - $10^{6.3}$/0.1 ml for brain, heart, skeletal muscle, lung, kidney, liver and spleen. Thus, almost any visceral organ or brain or skeletal muscle could be employed as a source of VE virus from sentinel hamsters dying or found ill in the field. Contact, intra-cage infection of hamsters occurred with VE virus, and an overt VE laboratory infection occurred in a person vaccinated 612 days prior to exposure with Ft. Detrick vaccine despite the presence of neutralizing antibody to the infecting strain at the time of exposure.

The group C arbovirus (prototype 63U11) apparently is not Caraparu, Marituba, Murutucu, Itaqui, Oriboca, or Apeu by various serologic tests. It grew and produced cytopathic effects in HeLa and L cell cultures. Infection of two humans in the field in Mexico occurred during 1964 with development of CF antibody but without clear-cut relationship to disease. The virus was inactivated by sodium deoxycholate in the presence of low-protein concentrations but not in the presence of human serum. Potent, complement-fixing antigens were obtained from liver extracted with saline, and low-titer goose erythrocyte agglutinins were made by acetone extraction of serum or liver.

63A49 virus also produced cytopathic effects in HeLa cell cultures, but only grew without CPE in L cell culture. Goose erythrocyte agglutinins were obtained from infected liver, the tissue which also yielded potent saline extracted CF antigens.

The Bunyamwera group virus from Tlacotalpan, Veracruz, (61D240) a river delta, savannah habitat studied during 1961-63 and located 75 miles west of Sontecomapan, still has not been identified with certainty within the Bunyamwera group. From previous studies it is known to be related to Cache Valley, Maguari and Tensaw viruses. During 1965 it was found to be chloroform sensitive and to produce cytopathic effects in HeLa but not in L cell cultures on first passage, although virus apparently grew in L cell cultures, and with passage, produced cytopathology.

To test the possibility that Nodamura virus from Japanese *Culex tritaeniorhynchus* might be a mixture of an arbovirus and a non-arbovirus, substrains of virus obtained after propagation in and transmission by *Aedes aegypti*, and after propagation by needle inoculation through five passages in *Culex tarsalis* or *Ornithodoros* ticks (by Dr. H. Hurlbut several years ago) were studied for sensitivity to ether, chloroform and sodium deoxycholate. All substrains were resistant to ether and to chloroform. Investigations of these substrains are currently underway with sodium deoxycholate since during the past year, irregular results have been obtained with sodium deoxycholate such that sometimes the virus was inactivated by SDC and at other times, it was not.

II. Maintenance of Sarcoma 180 T/G Cells by Freezing with Glycerin on Dry Ice Rather Than by Serial Transfer in Mice.

Using the principles originally developed to preserve human HeLa and mouse L cells in a frozen state on dry ice (Proc. Soc. Exp. Biol. & Med. 87:480, 1954), Sarcoma 180 T/G cells have been similarly preserved for as long as 227 days.

Upon receipt from YARU in October 1965, ascitic fluid removed from a mouse inoculated with sarcoma cells 20 days previously was diluted to contain 16-20 million cells per ml and final concentrations of a) glycerin (reagent grade) 10%, ascitic fluid, 90%, or b) glycerin 10%, ascitic fluid, 10% and Hanks' solution, 80%. One ml of each of these cell suspensions was placed in sealed glass ampoules, which in turn were put in the bottom of a dry ice box to freeze slowly. For recovery of viable cells an ampoule was removed, thawed rapidly by immediate transfer to a 37°C water bath, and the cell suspension (usually 0.2 ml) inoculated intraperitoneally into each of two to five adult mice. One hundred and twenty-six days after freezing each of the two cell suspensions produced ascites in five of five mice, 8-12 days after inoculation. Other ascitic fluid suspensions of Sarcoma 180 T/G cells were prepared similarly in October 1965 or in November 1965 and March 1966, merely by addition of glycerin to the ascitic fluid to achieve a final concentration of 10% glycerin. To date these cell suspensions, 29-227 days

after freezing, have also produced ascites and sarcomas in each of the two-five mice within one to two weeks of inoculation.

Thus, it appears that Sarcoma 180 T/G cells can be frozen with equal facility to the numerous continuous cell lines which are now in the American Type Culture Collection repository (Science 146: 241, 1964).

Judging from experience with continuous cell lines and cells from solid tumors, the lower temperatures of liquid nitrogen or its vapor would probably be superior for long-term preservation to the temperature at the bottom of a dry ice box. Nevertheless, preservation by freezing avoids the risks of genetic change of cells, contamination with passenger viruses or other microorganisms and the inconvenience inherent in serial transfer of cells in animals.

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

Antigenic Comparison of Virus Strains in the Hart Park Group

We have to report at this time a comparative study of antigenic properties of a virus isolated by Dr. LaMotte of Greeley, Colorado, from *Culex tarsalis* collected in June, 1965, in Texas. We compared his agent with Flanders virus, strain 61-7484 and Hart Park virus using two serologic technics, complement fixation and neutralization. The results are shown in the two tables attached.

The new agent, 66-7008, from Dr. LaMotte is more closely related to Flanders virus than Hart Park virus as is demonstrated by both technics. All three viruses show group relationship.
We had received previously from Dr. Irons, in 1962, two immune sera prepared with strains isolated from Texas mosquitoes.
When these sera were tested in complement fixation with Flanders virus antigen, the titers of the test sera and the homologous serum were the same. (Elinor Whitney)

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

Table 1

Antigenic Comparison by Complement Fixation of 3 Strains of the Hart Park Group of Viruses Isolated from 3 Different Areas of the United States

Antigens		Mouse Sera					
		Strain : 61-7484	64-7030		66-7008		Normal
Virus	Date	No. inj. : 5	2	5	2	5	
Strain	Prepared	Date bled: 4/14/64	5/18/64	6/10/64	4/19/66	5/11/66	9/23/65
61-7484 Flanders	1/22/63	16/64*	< 4	< 4	8/32	32/64	< 4
64-7030 Hart Park	5/28/64	4/64	< 4	16/32	< 4	8/32	< 4
66-7008 Greeley	4/11/66	32/32	< 4	< 4	8/32	32/64	< 4
Normal	2/16/66	< 4	< 4	< 4	< 4	< 4	< 4

*Reciprocal of the serum dilution which gave 50 per cent hemolysis/Reciprocal of the antigen dilution which gave 50 per cent hemolysis.

Table 2

Antigenic Comparison by Animal Neutralization Tests of 3 Strains of the Hart Park Group of Viruses Isolated from 3 Different Areas of the United States

			Mouse Sera				
			Strain : 61-7484	64-7030		66-7008	
Virus	Date	LD_{50}	No. inj. : 5	2	5	2	5
Strain	Tested	Used	Date bled: 4/14/64	5/18/64	6/10/64	4/19/66	5/11/66
61-7484 Flanders	5/31/66	100	215.2*	< 4	10.2	4	107.6
64-7030 Hart Park	5/23/66	25	205	5.8	>256	Approx.4	34.6
66-7008 Greeley	5/16/66	100	> 256	< 4	35.5	20.3	>256

*Reciprocal of the serum dilution which protects 50 per cent of the animals as calculated by moving averages.

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT
YALE UNIVERSITY SCHOOL OF MEDICINE, NEW HAVEN, CONNECTICUT

Powassan studies on mammal sera submitted by Dr. Robert Tonn of Encephalitis Field Station, Lakeville, Massachusetts, and Dr. David E. Davis, Pennsylvania State University.

Testing with Powassan virus and intraperitoneal inoculation of infant mice, test dosages in several tests being 317, 400, 400, 500 and 2300 LD_{50}.

Animal	Positives/Total No. Examined
Cottontail rabbits	0/24
Gray squirrel	0/35
Red squirrel	0/4
Chipmunk	0/13
Short tailed shrew	0/3
Peromyscus	1/44
Microtus	0/27
Mus musculus	0/1
Rattus norvegicus	0/13
Clethrionomys	0/13
Napaeozapus	0/6
Red fox	0/1
Raccoon	1/3
Woodchuck	2/4
Skunk	10/12

Woodchuck sera submitted by Dr. David E. Davis showed in one series 16/27 positives and in a later series 14/22 positives.

Studies are continuing on specimens from Connecticut and other northeastern states.

REPORT FROM THE ENCEPHALITIS FIELD STATION
LAKEVILLE HOSPITAL, MIDDLEBORO, MASSACHUSETTS

During the 1966 spring migration period (April 15 - June 4), a total of 348 birds belonging to 39 species were captured at Site I, on the dike, in Raynham, Massachusetts. Permanent resident birds were included in the survey total. Three hundred and seventeen blood speciments were taken and tested for EE and WE virus in chick embroy tissue culture. No virus was isolated.

In 1965, an antibody study was initiated. Twenty blood specimens from 20 species of birds common to the study sites were selected to be tested for EE and WE antibody. To date test results are complete for the following species.

Bird	EE Ab.	WE Ab.
Blackpoll Warbler	0/20	0/20
Song Sparrow	2/20	1/20
House Sparrow	1/20	1/20
Ovenbird	2/20	1/20

The complete report, including the age and sex of those selected birds tested, will be available in the next report.

The Yale Arbovirus Unit has completed preliminary testing of some selected mammal bloods collected by this Station. There is an indication that Powassan antibody is present in skunks, woodchucks, raccoons, and white-footed mice. More mammals are being collected for subsequent testing. In addition, sentinel rabbits and white-footed mice have been placed at several different sites in Southeastern Massachusetts.

Infant mice are now being used to screen arthropods for the presence of virus. To date, pools of *Ixodes cookei*, *Ixodes sp.*, *Dermacentor variabilis*, *Aedes abserratus* and *Culex restuans* have been negative. This is the first use of the Virus Laboratory at the Field Station. (R. J. Tonn)

REPORT FROM THE MASSACHUSETTS STATE VIRUS LABORATORY, BOSTON, MASSACHUSETTS

St. Louis Virus Studies

1. Isolation from arthropods

Capability of isolation of SLE virus from arthropods without recourse to infant mice was sought. The simple techniques used for EE and WE were not successful with stock strains of SLE but very particular conditions for chick embryo tissue cultures had been successful with stock strains, and the neutralization test had been worked out with these findings.

Wild SLE virus in arthropods might be expected to have a different host range than even a first passage laboratory strain where selective pressure (in the form of the laboratory host) has already been applied.

In order that we might test our tissue culture system, Dr. Telford Work and Dr. Roy Chamberlain kindly supplied us with 22 coded arthropod pools.

Code Letter	No. SM Died / No. SM Inoc.	PFU/ml	Code Letter	No. SM Died / No. SM Inoc.	PFU/ml
A	6/6	70×10^4	B	0/6	None Rep.
D	6/6	2×10^3	C	0/6	None Rep.
F	6/6	18×10^3	E	0/6	None Rep.
G	6/6	23×10^3	H	0/6	None Rep.
I	6/6	7×10^3	J	0/6	None Rep.
L	6/6	1×10^3	K	0/6	None Rep.
M	5/6	Missed	N	0/6	None Rep.
P	2/6	Missed	O	0/6	None Rep.
R	5/6	7×10^3	Q	0/6	None Rep.
T	6/6	17×10^3	S	0/6	None Rep.
U	6/6	24×10^3	V	0/6	None Rep.

When pools were tested in fluid fed tissue cultures, some of the positives produced virus, as evidenced by subinoculation on plaque plates (there is little or no cytopathic effect) while others were detected by their production of interferon, as evidenced by failure of EE challenge virus to grow. When these pools were inoculated directly onto monolayer plates, they all produced plaques. The agar overlay perhaps prevents diffusion of interferon, permitting the virus to grow. This may explain why some non-cytopathogenic arboviruses form plaques as first reported in an earlier Information Exchange by J. R. Henderson of Yale.

Nine out of 11 positives were detected by direct plaquing of a 1/100 dilution on chick embryo monolayers. The two pools missed by us did not kill all mice inoculated at CDC. The small amount of virus present was perhaps lost during several thawings, for when tested recently in newborn mice, these did not sicken nor did they have neutralizing antibody when bled out at three weeks of age.

The 1965 arthropod pools (435) collected by Dr. Robert Tonn have been re-examined using the conditions favorable to SLE isolation. One WE was reisolated, six pools are suspicious, i.e., require further study, and 90 remain to be tested.

2. Antibody

The plaque reduction neutralization test was applied to 1964 and 1965 human sera (all unsolved cases of encephalitis from which sera were received) and to 78 urban pigeons which were collected in 1964 and 1965 for the Virus Laboratory through the courtesy of Mr. Hickling of the U. S. Department of Agriculture and Mr. John Peterson of the Fish and Wild Life Service, University of Massachusetts. This bird was chosen because it had high antibody rates in Florida SLE outbreaks. House sparrows collected by the Field Station are being tested for SLE antibody.

Two other viruses of particular interest to this region, California complex (BFS strain courtesy of Gladys Sather) and Powassan (courtesy of Dr. Donald McLean), plaque well on chick embryo monolayers. Studies have begun on human and small mammal sera.

REPORT FROM THE HOSPITAL FOR SICK CHILDREN TORONTO 2, ONTARIO, CANADA

Between 5 May and 3 June 1966, neutralizing antibody to Powassan virus was found in sera from 51 of 116 forest rodents collected in the Powassan-North Bay region of northern Ontario, Canada. Hemagglutination inhibition activity against Powassan antigen was detected in 24 of 108 acetone-treated sera. In the three Townships of Bonfield, Chisholm and East Ferris, neutralizing antibody was found in 40 of 65 adult groundhogs (Marmota monax), four of 20 juveniles and none of four animals born during 1966. A presumptive isolation of Powassan virus has been achieved from one of 21 pools of nymphal or adult _Ixodes cookei_ ticks (removed from groundhogs) which have been examined so far. Results of neutralization tests using monolayers of primary swine kidney have shown satisfactory agreement with those obtained by intracerebral injection of weaned mice. (Donald M. McLean, M.D.)

REPORT FROM THE WESTERN COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF SASKATCHEWAN, SASKATOON; THE CANADA AGRICULTURE RESEARCH STATION, SASKATOON; THE PROVINCIAL LABORATORY, DEPARTMENT OF PUBLIC HEALTH, REGINA; AND THE DEPARTMENT OF BIOLOGY, UNIVERSITY OF SASKATCHEWAN, SASKATOON, SASKATCHEWAN

Western Encephalitis in Saskatchewan in 1965

Epidemics of WE occurred in man and horses in Saskatchewan during the summer of 1965. All the evidence associated with these epidemics is not yet available, but the following can be reported at this time.

In man, 77 cases were confirmed by serology or virus isolation on 306 notified cases hospitalized with suspected viral encephalitis. A variety of other viruses were also present, but WE virus was recovered from the brains of seven fatal cases, from the cerebrospinal fluid of six additional cases and from the throat washings of one infant.

In horses, 106 suspected cases of WE were reported of which 22 were confirmed serologically, and seven deaths were attributed to WE.

The 1965 mosquito populations were the largest that we have encountered in the four years of our study, being two to three times greater than in the previous epidemic year of 1963. *Aedes vexans* was the most abundant species in the agricultural area of the Province; it was followed closely by *Culiseta inornata* and together these two species made up about 70% of the total mosquito population from June 1 to September 30. *Culex tarsalis* varied in relative abundance from second to fourth depending on location, but at Delisle and Swift Current it outnumbered *A. vexans*. Examination for virus of the 1,323 species pools preserved on dry ice has not yet been completed, but at least three isolations of WE virus (two from *C. tarsalis* and one from *C. inornata*) have been obtained for the first time in four years from Delisle, about 25 miles southwest of Saskatoon.

Results from our six sentinel chicken flocks indicated intense virus activity in nature. All the birds in the most southerly flock at Estevan (Lat. 49° 08' N) had acquired WE infections by August 13, all the birds in the Kindersley flock by August 31, and all the birds in the Saskatoon flock by September 14. At Outlook 75% were infected by September 27 and at Aberdeen 41.7%, by September 10. At St. Walburg, the most northerly flock (Lat. 53° 38' N) two out of the 24 birds became infected.

The epidemics in man and horses ran similar courses to those that occurred in 1963. Horse cases were reported first (week

ending July 12); the peak in horse cases occurred during the week ending August 16, and the last cases were reported in the week ending September 20. The first human case was reported on August 2, this epidemic reached its peak in the week ending August 30, and the last case was reported on September 22. Both epidemics reached their peaks during a period of above-normal temperatures that persisted from the week ending August 2 to the week ending August 30. For the remainder of the season temperatures were well below normal with heavy rains, and the early end of the epidemics was probably caused by this inclement weather that reduced mosquito activity to a low level. During the month of September, our six traps took only 863 female mosquitoes of which 580 were C. inornata; during the week ending July 26, the same six traps had taken 89,965 female mosquitoes and 13 species.

The infections in the sentinel chickens and horses ran almost parallel courses. The first chicken acquired its infection between June 21 and July 7 (flocks are bled twice-a-month), the peak in chicken infections occurred during the week ending August 16, and the last chicken infections were acquired between August 30 and September 13 (St. Walburg). As in 1963, the mosquito population was well past its peak by the time the chicken, horse and human infections reached their peaks.

Vectors of WE in Saskatchewan

Up to the end of 1964, we had obtained 35 isolations of WE virus from seven species of mosquitoes. These are summarized in Table 1 with minimum infection rates over the four summer months (June-September) in each year, and the dates on which the infected mosquitoes were collected. Over the three years, 17 of the isolations have been from Culex tarsalis. In the epidemic year of 1963, 13 of the 18 isolations in that year were from C. tarsalis and its infection rate was higher than in 1962 and 1964. In the non-epidemic year of 1964, only one out of 11 isolations was from C. tarsalis and its infection rate was lower than in the other two years.

The large proportion of isolations of WE virus from species other than C. tarsalis suggests that these species might play a more important role in dissemination of the virus at Saskatchewan latitudes than elsewhere.

In 1964, although there was no horse or human epidemic, there was evidence of considerable virus activity in nature in addition to the isolations of virus from the mosquitoes. WE virus was isolated from 11 out of 863 bird (the majority nestlings) bloods tested; 31% of 212 breeder flock chickens and 12% of 104 turkey poults acquired WE infections during the summer. In the six sentinel chicken flocks the infection rates were not as high as in 1963 and 1965, but in the Estevan flock 29% of the birds

acquired infections and 21% in the Kindersley flock; all infections in the sentinel flocks were acquired in the two-week period from August 12 - 26. The bloods of 77 pigs out of 260, from the southern part of the Province and born in January and February of 1964, reacted to the SN test for WE antibodies.

A number of isolations of WE virus have been obtained from naturally infected frogs (*Rana pipiens*) and garter snakes (*Thamnophis spp.*) in 1964, 1965 and 1966. (A. N. Burton, J. McLintock, H. E. Robertson and J. G. Rempel)

Table 1. Known Mosquito Vectors of WE Virus in Saskatchewan

Species	Number Tested	Number of Pools	Number of Isolations	Infections /1000*	Dates
			1962		
Culex tarsalis	1348	80	3	2.2	July 31, Aug. 9, 25, Sept. 8
Culiseta inornata	5145	202	1	0.2	July 1
Aedes dorsalis	1262	97	1	0.8	Aug. 10
Aedes flavescens	63	9	1	15.8	June 25
Aedes campestris	335	22	0	0	
Aedes vexans	3309	94	0	0	
Aedes spencerii	124	5	0	0	
			1963		
Culex tarsalis	4118	189	13	3.2	June 22, July 31-Aug. 21
Culiseta inornata	19984	673	4	0.2	July 27, Aug. 6, 10
Aedes dorsalis	10573	351	1	0.09	Aug. 5 - 6
Aedes flavescens	634	69	0	0	
Aedes campestris	4087	177	0	0	
Aedes vexans	1064	75	0	0	
Aedes spencerii	1759	78	0	0	
			1964		
Culex tarsalis	521	67	1	1.9	Aug. 15
Culiseta inornata	4631	228	3	0.6	July 16 - 26
Aedes dorsalis	1059	76	1	0.9	July 21 - 25
Aedes flavescens	1148	84	1	0.9	June 23
Aedes campestris	976	83	2	2.1	July 4 - 9, 19 - 26
Aedes vexans	2281	117	2	0.9	July 5, 23 - 26
Aedes spencerii	104	21	1	9.6	July 14

* Minimum Infection Rate

REPORT FROM DEPARTMENT OF MEDICAL BACTERIOLOGY UNIVERSITY OF ALBERTA, EDMONTON AND CANADA AGRICULTURE RESEARCH STATION, LETHBRIDGE, ALBERTA

RESULTS FROM SPECIMENS COLLECTED IN 1965.

In summer and early fall of 1965 an epizootic of equine encephalomyelitis occurred in Alberta, with a peak from the third week of August until the third week of September (Table 1). At that time the Province experienced unusual freezing temperatures and an abundant snowfall that remained on the ground for several days. The appearance of cold weather and its persistence for several weeks in early fall may have contributed greatly to the almost abrupt ending of the epizootic. Since the Department of Medical Bacteriology of the University of Alberta contains the only well-established arbovirus unit of the Province, it was called on by the laboratory branch of the Provincial Veterinary Services to carry out examinations of sera from sick horses and of brains from animals suspected to have died of WE virus encephalomyelitis.

Horse sera

Paired sera, collected in the acute and convalescent stage of the disease, were received from 92 animals. Single sera were received from 133 animals. All were tested for the presence of complement fixing antibodies to WE and EE virus, but only the 92 paired sera for St. Louis Encephalitis Virus (Table). Sixty-five horses of the 92 from which paired sera were received showed a diagnostic rise of complement fixing antibodies to WE virus.

Horse brains

The brains of 35 animals were received. Isolation of viurs was attempted by intracerebral and intraperitoneal inoculation of suckling mice, 1-2 days old and of embryonated hens' eggs. From three brains WE Virus was isolated. One horse was a race horse, the second one came from the southeastern regions of the Province, but the third horse came from Edmonton. It was a draft horse (10 years old) - the only horse on the farm and it had never moved from Edmonton.

Human sera

Coincidental with an epizootic of WE infection in horses, a clinical suspicion of a considerable increase of such infection in humans was postulated. This was not confirmed. Only seven cases (4 children, 2 adults, 1 age unknown) have been proved serologically to be caused by WE virus.

Chicken sera from the irrigated areas of Lethbridge.

Four sentinel flocks, each of 15 roosters and 15 capons, hatched at the beginning of May, were set out in four locations of the irrigated areas of Lethbridge at the end of May and beginning of June. Four additional flocks, each of 10-12 roosters, hatched at the beginning of August, were set out on August 23, 1965. The birds were tagged, bled before exposure in the field and bled at two week intervals thereafter. From May 31 until November 9, the sera of 1,546 chickens were collected aseptically by venepuncture and tested by hemagglutination-inhibition (HAI) test for evidence of infection with WE, EE and SLE virus. The method used was a modification of Clarke and Casals' acetone extraction method as described by Holden and LaMotte (SCIENCE, 147: 169, 1965): All sera, after acetone extraction, were filtered through a SEITZ EK pad to remove non-specific inhibitors and thereafter were tested for HAI antibodies by the micro-technique of SEVER (J. IMMUN. 88: 320, 1962). The HA antigens were prepared in our own laboratory according to the method described by Clarke and Casals (AMER. J. TROP. MED. HYG. 7: 561, 1958). The virus strains used were: WEE-848, EEE ARTH - 167, SLE TBH-28. The results obtained are summarised in the Tables 3, 4 and 5.

Mosquitoes.

Four hundred and thirteen pools of 30-50 mosquitoes have been collected in the irrigated areas and other parts of the Province from June 3 until September 15, 1965 (Table 6). At that time the sudden appearance of abundant snow and of cold weather, which persisted for several weeks in early fall, rendered impossible further collections of mosquitoes. One hundred and eighty three of the 413 pools consisted of three species of females - *C. inornata*, *C. tarsalis* and *A. earlei*, collected in mammalian burrows in the same manner as reported by Shemanckuk (Mosquito News, 25: 456, 1965). The adults of these species enter the burrows during late summer and fall, to hibernate. Of the three species collected, *C. inornata* and *C. tarsalis* were found to contain blood in their gut (Table 7).

Mosquitoes were identified and examined for blood contents on the same day of collection, while they were still alive. Thereafter, they were grouped into pools of 30-50 mosquitoes, placed in bijou bottles, tightly screwed, sealed with adhesive tape, quickly frozen on dry ice and shipped in the frozen state to the virus laboratory, where they were kept in the freezer at -70° C, until they were processed for virus isolation.

The basic procedures employed in the isolation of arboviruses were those described by Chamberlain et al. (Amer. J. Trop. Med. Hyg. 13: 456, 1964). Mosquito suspensions were inoculated intra-

cerebrally in suckling mice, 1-2 days old. The inoculated mice were observed daily for 14 days, at the end of which period they were discarded if they remained healthy. No "blind" passages were made. Only sick mice were selected for passage. Dead mice were discarded. Reisolation of the strains from the original mosquito suspension was attempted in all instances. Mosquito suspensions from which the virus was not reisolated were regarded as negative. WE virus was isolated from seven of the mosquito pools collected in mammalian burrows (Table 8) and from two pools of mosquitoes collected elsewhere (Table 9). California virus was isolated from one pool of *C. inornata* collected in the mammalian burrows, result confirmed by the Arbovirus Reference Laboratory, CDC, Atlanta, Ga. Another pool of *C. inornata* yielded a virus not yet identified.

SUMMARY

1. An epizootic of WE equine encephalomyelitis occurred in Alberta in summer and early fall of 1965, with a peak from the third week of August until the third week of September. Extensive work has been done in connection with this, but the final results are not yet completed. A high percentage of suspected horses have been proved serologically to be suffering from WE virus infection. WE virus was isolated from three horse brains. The isolation this year, for the first time, of WE virus from a horse in the Edmonton area demonstrates that WE virus activity is approaching centres of large human population. Some indication has been obtained of a geographical distribution of the virus in the Province, wider than previously suspected.

2. The comparatively low incidenc of WE virus in humans in Alberta should not be interpreted to mean that a potentially serious situation does not exist. The difficulty in obtaining proper human specimens is a too well recognized problem in virus laboratories to deserve any comment.

3. One thousand five hundred and forty six chickens were collected from four sentinel flocks, placed in the field in the irrigated southeastern areas of the Province, were tested for the presence of HAI antibodies to WE, EE and SLE virus. All sera were negative to EE and SLE virus. Conversion from negativity to WE virus antibodies was observed in some of the chicken sera during the second week of August. It was almost complete in the third week of September, with no further changes occurring until the end of the exposure time, in the sera of the chiceksn placed in the field at the beginning of May. The results obtained on the chickens placed in the field on August 23, are difficult to interpret because of the abrupt occurrence of freezing temperatures on September 15 - temperatures that lasted for several weeks.

4. Four hundred and thirteen pools of 30-50 mosquitoes were collected in the irrigated areas of Lethbridge and other parts of the Province from June 9 until September 15, 1965. Of the 413 pools, 183 were constituted by three species of females, C. inornata, C. tarsalis and A. earlei, collected in mammalian burrows. WE virus was isolated from four pools of C. tarsalis and three of C. inornata, collected in the mammalian burrows. It was also isolated from one pool of C. inornata and one pool of A. vexans, collected in the field. We record the isolation of California virus from one pool of C. inornata collected in one mammalian burrow.

(Dr. Odosca Morgante and J.A. Shemanchuk).

TABLE 1.

Number of clinical cases of horses with encephalitis, reported weekly to the laboratory branch of the Provincial Veterinary Services of Alberta, in 1965.

	1st week	2nd week	3rd week	4th week	5th week
JUNE			1		2
JULY					1
AUGUST	7	21	84	109	22
SEPTEMBER	21	30	8	6	1
OCTOBER		6	2	1	

TABLE 2.

Complement Fixing antibodies to WE virus in paired sera of horses suspected to be clinically ill with encephalitis.

		No. paired sera received	No. positive	No. with No Diagnostic rise.	No. negative
AUGUST	1st week	3	2	0	1
	2nd week	9	7	1	1
	3rd week	19	16	1	2
	4th week	26	16	8	2
	5th week	13	9	2	2
SEPTEMBER					
	1st week	6	5	0	1
	2nd week	13	8	4	1
	3rd week	1	1	0	0
	4th week	1	0	1	0
	5th week	0			
OCTOBER	1st week	0			
	2nd week	1	1		
	3rd week - 5th week	0			

0 = NONE.

TABLE 2A.

Complement Fixing antibodies to WE virus in single sera of horses suspected to be clinically ill with encephalitis.

		CF. antibody serum titers 1:							
		< 8	8	16	32	64	128	256	512
AUGUST	1st week	1							
	2nd week	6	2	0	1	0	0	0	0
	3rd week	9	2	3	11	10	7	1	0
	4th week	10	3	8	14	4	4	0	0
	5th week	1	2	0	0	3	0	1	1
SEPTEMBER	1st week	4	2	3	3	2	2	0	1
	2nd week	1	0	0	2	3	0	0	0
	3rd week	1	0	0	0	0	1	0	0
	4th week	0	0	0	0	1	0	1	0
	5th week	No sera received							

JUNE One blood received during the 3rd week : negative

JULY One blood received during the 5th week : negative

OCTOBER No blood received.

0 = NONE

CHICKEN 1965.

TABLE 3.

HAI antibodies to WE virus in chicken sera from sentinel flocks of roosters located in four areas of Lethbridge.

	LUCO	McADOO	NILSSON	8 MILE LAKE
MAY 31	0/15	0/15	0/15	0/15
JUNE 15	0/15	0/15	0/15	0/15
JUNE 28	0/15	0/15	0/15	0/15
JULY 13	0/15	0/15	0/15	0/15
JULY 27	0/15	0/15	0/12	0/15
AUGUST 10	4/15	1/15	0/12	1/14
AUGUST 24	11/15	7/13	3/12	6/14
SEPTEMBER 7	13/15	11/12	1/12	13/13
SEPTEMBER 23	13/15	10/11	5/12	12/12
OCTOBER 4	13/15	6/6	4/11	11/11
OCTOBER 26	13/15	9/10	4/11	10/10
NOVEMBER 9	13/14	8/9	3/9	10/10

NO. OF POSITIVE SERA / NO. OF SERA TESTED.

TOTAL NO. OF SERA RECEIVED : 640

CHICKEN 1965.

TABLE 4.

HAI antibodies to WE virus in chicken sera from sentinel flocks of capons located in four areas of Lethbridge.

	LUCO	Mc ADOO	NILSSON	8 MILE LAKE
JUNE 7	0/15	0/15	0/13	0/15
JUNE 15	0/15	0/15	0/13	0/15
JUNE 28	0/15	0/15	0/13	0/15
JULY 13	0/15	0/15	0/13	0/15
JULY 27	0/15	0/15	0/12	0/15
AUGUST 10	2/15	0/15	0/12	1/15
AUGUST 24	10/15	9/15	7/12	13/15
SEPTEMBER 8	13/15	14/15	7/12	13/14
SEPTEMBER 23	13/15	14/15	7/12	14/15
OCTOBER 4	12/15	Chicken not bled	7/12	13/14
OCTOBER 26	11/13	13/14	6/11	14/15
NOVEMBER 9	11/13	13/14	5/9	14/15

NO. OF POSITIVE SERA / NO. OF SERA TESTED

TOTAL NO. OF SERA RECEIVED : 661

TABLE 5. CHICKEN 1965.

HAI antibodies to WE virus in chicken sera from sentinel flocks of of roosters put in the field on August 23rd.

	LUCO.	Mc ADOO	NILSSON	8 MILE LAKE
AUGUST 23	0/12	0/12	0/10	0/10
SEPTEMBER 8	1/12	0/12	2/10	0/10
SEPTEMBER 23	1/12	0/12	2/10	0/10
OCTOBER 4	1/12	Chicken not bled	2/10	0/10
OCTOBER 2	1/12	0/12	2/10	0/10
NOVEMBER 9	1/8	0/11	2/9	0/9

TOTAL NO. OF SERA RECEIVED : 245

TABLE 6.

MOSQUITO POOLS 1965.

	JUNE		JULY		AUGUST		SEPT.	
Species	1-15	16-30	1-15	16-31	1-15	16-31	1-15	Total
A. campestris	2	8	-	1	-	-	-	11
A. cataphylla	-	-	5	-	-	-	-	5
A. dorsalis	-	12	8	2	2	3	-	27
A. excrucians	16	32	4	-	15	-	-	67
A. earlei	-	-	-	-	-	1	21	22
A. fitchii	2	5	-	-	-	-	-	7
A. flavescens	4	19	3	-	1	-	-	27
A. nigromaculis	-	-	-	10	-	-	-	10
A. vexans	-	12	36	9	8	3		68
A. spencerii	-	2	-	-	-	-	-	2
C. inornata	-	-	-	-	18	88	6	112
C. tarsalis	-	-	-	-	4	35	4	43
T. campestris	-	-	-	1	-	-	-	1
T. rupestris	-	-	-	-	4		-	4
T. rhumbrica osborni	-	-	-	-	2	-	-	2
Black flies	-	1	-	-	-	-	-	1
Simulidae	-	-	-	-	1	-	-	1
Tabamidae	-	3	-	-	-	-	-	3
	24	94	56	23	55	130	31	413.

TABLE 7.

Mosquitoes collected from mammalian burrows during late summer and fall 1965 and examined for blood contents.

Species	No. examined.	per cent engorged	per cent non-engorged
C. inornata	3,214	50.1	49.9
C. tarsalis	1,141	8.7	91.3
A. earlei	639	0	100.0

0 = None

TABLE 8.

Virus isolations from mosquitoes collected from mammalian burrows in 1965.

Place of collection.	Dated collected	Mosquito species	No. of mosquitoes per pool	Feeding status	Virus isolated.
Brooks	VIII.18.65	C.tarsalis	34	Engorged	WE
Irvine	VIII.18.65	C.tarsalis	30	Non-engorged	WE
Irvine	VIII.18.65	C.tarsalis	52	Non-engorged	WE
Irvine	VIII.25.65	C.tarsalis	30	Engorged	WE
Grassy Lake	VIII.17.65	C.inornata	43	Non-engorged	WE
Irvine	VIII.18.65	C.inornata	39	Non-engorged	WE
Travers	VIII.19.65	C.inornata	32	Engorged	WE
Hays-Wauxhall	VIII.18.65	C.inornata	34	Engorged	Not yet identified
Brooks	VIII.18.65	C.inornata	49	Engorged	CV.

TABLE 9.

Virus isolations from Mosquitoes collected in the Field in 1965.

Place of collection	Date collected	Mosquito species	No. of mosquitoes per pool	Feeding status	Virus isolated.
8 mile Lake	VIII.11.65	C. inornata	42	Non-engorged	WE
Brooks	VIII.24.65	A. vexans	57	Non-engorged	WE

REPORT FROM THE UNIVERSITY OF ILLINOIS CENTER FOR ZOONOSES RESEARCH, URBANA, ILLINOIS

Serologic survey of dogs in McLeansboro, Illinois, following the epidemic of SLE in the human population in 1964, revealed a high incidence of exposure to the virus. A survey of dogs in nine other areas of Illinois showed evidence of WEE infection following epizootics in equines during a two-year period. Evidence of exposure to Powassan, SLE and EEE viruses was inconclusive.

An investigation was undertaken to evaluate the susceptibility of dogs to SLE, WEE and Powassan viruses by experimental infection.

In susceptibility studies, Strains AR 146 and AR 1712 of SLE virus, Strain BFS 1703 of WEE virus and Powassan virus were inoculated intravenously into groups of six purebred Beagles totaling 24 animals. The infective dose administered ranged from 5,200 to 333,000 $ICLD_{50}$ in adult mice.

Clinical observations during the first 14 days after inoculation revealed that, in the case of Powassan virus, febrile response was manifested in four of six dogs on the second and third day post inoculation. Listlessness and injection of mucous membranes were observed in a single case.

Viremia was observed in all dogs except for those individuals receiving BFS 1703. Maximal viremic titers ranged from less than 0.5 $\log_{10}$ $ICLD_{50}$ in adult mice, in the case of AR 146, to 1.83 logs for AR 1712 and Powassan viruses. Initial circulation of virus was seen 24 hours after inoculation in all three groups. Viremia persisted for as long as five days.

Quantitation of immunologic response by HI, CF and NT tests revealed that all animals tested gave measurable antibody responses. Maximal reciprocal HI titers ranged from 1:160 in dogs injected with BFS 1703 to 1:10,240 in dogs given Powassan virus. Peak CF titers varied from 1:16 for BFS 1703 to 1:512 in dogs receiving a second injection of AR 146. Highest NT titers ranged from 2.15 logs in adult mice with AR 1712 to 3.85 logs in two to three-day-old suckling mice with BFS 1703.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF OKLAHOMA MEDICAL CENTER, OKLAHOMA CITY, OKLAHOMA

Sera from three sentinel flocks of white Leghorn chickens (12 chickens per flock) were collected at intervals during two periods (March, 1963 to November, 1963, and April, 1964 to December, 1964) and were studied for the presence of hemagglutination inhibition (HI) antibodies to WEE, SLE, and EEE. The chickens were maintained in sentinel coops at three locations in Central Kansas: Site I was at the Kansas Wildlife Refuge at the Cheyenne Bottoms; Site II at a farm, five miles from Cheyenne Bottoms; and Site III at the Quivera National Wildlife Refuge, 30 miles from the Cheyenne Bottoms. The first two sites were near fresh water, while Site III was located near salt marshes.

Hemagglutinating antigens for WEE, SLE, and EEE viruses were prepared from infected suckling mouse brains according to the acetone-ether extraction method of Clark and Casals (1958) and the sera were treated by the acetone-extraction method of Holden, et al., (1965) to remove non-specific inhibitors. Male goose erythrocytes were used in the test.

In Table I, it can be seen that the last two serum collections of sentinel flocks I, II, and III in 1963 were negative for HI antibodies against SLE and EEE viruses, indicating that none of the chickens had been infected with SLE or EEE viruses in 1963. However, antibodies to WEE virus were detected at Sites II and III. The greatest increase in percent of positive chickens occurred during August. Approximately 90% were positive at collection site II by November, and 80% at collection site III.

In 1964, antibodies to WEE were detected first in July at Sites I and II and in September at Site III (Table II). Peak titers of 80% at Site I in September, 63% at Site II in October, and 80% at Site III in October were noted. Antibodies to SLE were detected first at Site III in July, nine days later at Site I, and 23 days later at Site II. Peak SLE titers of 70% at Site I in November, 25% at Site II in October, and 60% at Site III in October were noted. In 1964, all three sentinel flocks were negative for HI antibodies against EEE virus.

Two hundred and ninety-two wild, long range migratory shore birds (Charadriiformes) were caught in mist nets or traps at Site I and bled from the jugular vein. These sera were treated in a similar manner as the chicken sera and tested for HI antibodies to WEE, SLE, and EEE viruses. HI antibodies for SLE were detected in three wild bird blood specimens. One was a Western sandpiper (titer 1:40) which was bled on September 2, 1964, and the other two were lesser yellowlegs which were bled on October 9, 1964. Both had titers of 1:400. None were positive for WEE and EEE. (L. V. Scott)

ARBOVIRUS ANTIBODY IN SENTINEL CHICKENS

Table I

Serum Collection Date	SLE			WEE			EEE		
	I	II	III	I	II	III	I	II	III
3-29-63	0	0	0	0	0	0	0	0	0
6-24-63	-	-	-	-	-	0	-	-	-
7-8-63	-	-	-	-	0	0	-	-	-
7-26-63	-	-	-	-	9*	9	-	-	-
8-17-63	-	-	-	-	64	55	-	-	-
9-7-63	-	-	-	-	64	55	-	-	-
9-28-63	-	-	-	-	82	82	-	-	-
10-19-63	0	0	0	0	91	82	0	0	0
11-8-63	0	0	0	0	91	82	0	0	0

* - % of chickens with positive titers
I - Site I, Cheyenne Bottoms
II - Site II, Farm
III - Site III, Quivera National Wildlife Refuge

ARBOVIRUS AN TIBODY IN SENTINEL CHICKENS

Table II

Serum Collection Date	SLE			WEE			EEE		
	I	II	III	I	II	III	I	II	III
4-10-64	0	0	0	0	0	0	0	0	0
6-16-64	-	-	-	0	0	-	-	-	-
7-23-64	-	-	-	10*	22	-	-	-	-
8-8-64	-	-	-	20	33	-	-	-	-
8-22-64	-	-	0	30	25	0	-	-	-
9-3-64	0	0	30	40	50	10	-	-	-
9-12-64	20	0	30	80	50	60	-	-	-
9-26-64	40	13	50	80	50	70	-	-	-
10-10-64	60	13	60	80	50	70	-	-	-
10-23-64	60	25	60	80	63	80	-	-	-
11-6-64	70	25	60	80	63	80	-	-	-
11-20-64	70	25	60	80	63	80	0	0	0
12-5-64	70	25	60	80	63	80	0	0	0

* - % of chickens with positive titers
I - Site I, Cheyenne Bottoms
II - Site II, Farm
III - Site III, Quivera National Wildlife Refuge

REPORT FROM THE DISEASE ECOLOGY SECTION, USPHS, COMMUNICABLE DISEASE CENTER, GREELEY, COLORADO

A high percentage of Texas sparrows was found to be infected with WE virus during the 1965 summer transmission season. Virus was isolated during July and August from as many as 10-40 percent of nestling birds collected at weekly intervals. This prolonged WE virus activity of such intensity resulted in a WE HAI antibody rate of only 50 percent in 24 adult sparrows collected in November, immediately after the cessation of detectible virus activity. SLE virus was isolated from six sparrows, but only during late August; eight percent of the November sparrow sample had SLE antibody. Of 140 sentinel chickens held in the same area throughout the summer, 83 percent had WE antibody and 64 percent had SLE antibody. Of 109 one-year-old farm chickens, 82 percent had WE antibody and 48 percent had SLE antibody. Most of the chickens acquired WE antibody during late July and early August when the WE infection rates in _C. tarsalis_ and in sparrows was highest; SLE antibody was acquired in most chickens after mid-August. A second collection of adult sparrows was made in the study area in March of 1966. The antibody rates in 116 sparrows were 72 percent for WE and nine percent for SLE. We are in the process of studying the sparrows which have no detectible antibody to determine if they are refractive to infection or whether this large part of the sparrow population escaped infection in spite of the high _C. tarsalis_ infection rate and the high transmission rates to chickens. Thus far we have found no evidence that these seronegative sparrows are refractive to WE infection.

In April, 1965, it was found that RBC from female geese in Colorado may be significantly less agglutinable than cells from male geese. The results obtained in comparisons of RBC from six male and six female geese are shown in the following table:

PARALLEL HA TITRATIONS IN PRESENCE OF RBC FROM SIX MALE AND SIX FEMALE GEESE, APRIL, 1965

Band Number	Sex	HA Endpoints[a] SLE Parton	WE Olitsky	WE McMillan	EE NJO
1554	♂	12,800	19,200	12,800	160,000
1553	♂	6,400	19,200	12,800	160,000
1135	♂	12,800	19,200	12,800	160,000
2048	♂	12,800	19,200	12,800	160,000
2049	♂	12,800	19,200	12,800	160,000
2050	♂	12,800	19,200	12,800	160,000
1133	♀	12,800	19,200	12,800	160,000
1380	♀	12,800	19,200	12,800	80,000
1134	♀	1,600	< 1,200[b]	< 800[b]	< 10,000[b]
526	♀	6,400	4,800	3,200	80,000
535	♀	6,400	4,800	1,600	40,000
1555	♀	6,400	2,400	800	40,000

(a) Reciprocal of highest dilution containing one full HA unit per 0.5 ml in tube test

(b) Lowest dilution tested. With each antigen tested, the lowest dilution contained 16 full units of HA per 0.5 ml tested in tubes in presence of cells from male geese.

In the above test, little difference was observed in the cells from the six males and two of the females. However, cells from the remaining four females required four-fold or greater increase in the amounts of at least two hemagglutinins used in the test.

We have attempted to determine if the reduced agglutinability of cells from females coincided with the seasonal breeding pattern of geese. Accordingly, cells obtained from the same 12 geese at one to three-month intervals between April, 1965, and April, 1966, were similarly tested. Similar patterns of differing agglutinability occurred in April and June, 1965, and March and April, 1966. Cells from all males and from some of the females were comparable, while cells from three or more females were markedly less agglutinable. However, with two exceptions, cells from all 12 geese were comparable in bleedings made on July 28, October 8, and December 16, 1965. On July 28, the RBC from one male was poorly agglutinable as were the cells from one female on December 16.

During the course of this study, the following observations were made which may be of some value to the investigator: (1) In most instances, the plasma separated from poorly agglutinable cells was almost milky in color. This turbidity was first noted in plasma from the male in July 28 and was not again observed in plasma from it or from any other male. The milky color was frequently observed in plasma from females during the egg laying season and also from the single female with poorly agglutinable cells in December. In one trial, the removal of feed from the female geese for three days neither eliminated the turbidity of their plasma nor increased agglutinability of their cells. (2) Cells with reduced agglutinability were noticeably more fragile than "normal" cells. Hemoglobin staining of the buffy coat on sedimented cells of this type was usually observed after one or two days of undistrubed storage in the regrigerator. Shortly thereafter, the discoloration extended to the DGV suspending medium. This was not observed with "normal" cells.

For the past seven years we have immunized our staff members with inactivated WE and EE chick embryo vaccine prepared and supplied to us by the Walter Reed Army Institute of Research. We have recently tested pre and postinoculation sera from 1964 in both the HAI test and in the duck embryo tissue culture plaque reduction neutralization test. New personnel who were seronegative prior to immunization and who received the complete three

shot series of both WE and EE in 1964 responded with an adequate neutralizing antibody but without HAI antibody. Personnel who had received these vaccines previously and were given a single booster dose showed a significant rise in neutralizing antibody to WE and EE; many showed an HAI titer rise but the post-inoculation HAI titers were very low. Twenty of 24 individuals tested now have neutralizing antibody to WE and 18 of 22 tested have EE neutralizing antibody. Since only six of these people regularly perform relatively hazardous operations with the arboviruses, we believe these vaccines have been responsible for the production of significant neutralizing antibody, and a single booster dose has effectively enhanced the neutralizing antibody titer.

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

IN COLLABORATION WITH THE DISEASE ECOLOGY SECTION, U.S.P.H.S., COMMUNICABLE DISEASE CENTER AND THE CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH

This report reviews field and laboratory studies on arboviruses during the period May 1, 1965, through April 30, 1966.

An eighth year of evaluation was completed on the effectiveness of intensive *Culex tarsalis* control as a means of suppressing transmission of western equine (WEE) and St. Louis (SLE) encephalitis viruses in three rural study areas. The population of adult *C. tarsalis* returned to relatively large numbers within two years of the cessation of intensive control. WEE virus infection rates in *C. tarsalis* and immunologic conversion rates in sentinel chickens reached relatively high levels in the late summer of 1965. Turlock virus also was active but at a lower level. There was no virologic or serologic evidence of SLE virus activity for the second consecutive year in the three study areas.

General epidemiologic observations in Kern County revealed a high level of WEE virus activity in August and September, 1965. Coincidental to an increase of *C. tarsalis* population and WEE virus isolations from the vector there was an epizootic of encephalomyelitis in horses. Of 28 suspected cases in equines 10 were confirmed as WEE. There were no proven cases in humans.

Tests were run on paired sera from 202 human cases of central nervous system disease and/or febrile illness from Kern County and other areas of California. Serologic tests on these sera

with WEE, SLE, Powassan, Modoc, Rio Bravo Bat, California, Bunyamwera group, Buttonwillow, and Turlock antigens have not established a diagnosis. An occasional individual had non-diagnostic rises in titers to California or Powassan antigens.

Serologic tests on sera from 193 domestic mammals from Kern County revealed antibodies to WEE, SLE, California, and Bunyamwera group viruses. There was no evidence of prior infection with Powassan, Modoc, Rio Bravo Bat, Buttonwillow, or Turlock viruses.

A study was begun at 20 localities to correlate mosquito populations, mosquito infection rates, and serologic conversion rates in sentinel chicken flocks. These sites included suburban, rural community, rural agricultural desert, foothill, and mountainous environments. WEE and Turlock viral infection rates were generally highest in rural agricultural environments. However, a high proportion of sentinel chickens developed HAI antibodies to WEE virus in suburban environments where *C. tarsalis* populations were low.

In addition to WEE and Turlock viral isolations from *C. tarsalis*, WEE virus was isolated from *Culex peus*, *Culex erythrothorax*, *Aedes melanimon*, and *Anopheles franciscanus*.

An intensive study of the population dynamics of wild mammals captured on a 40 acre grid in a desert area proximal to an agriculturally developed area was continued for a third year. A total of 3,354 mammals were captured.

There were major fluctuations in populations of the various species over the three year period. The well-known "three-year cycle" of rodent abundance may be occurring on the grid for the major species. *Dipodomys nitratoides* and *Peromyscus maniculatus* populations reached relatively high levels in 1965 and continued to increase in the spring of 1966.

Two additional trapping areas were established in 1965 to supplement data on wild mammals from the grid studies. These areas represent a foothill streamside environment (Poso), and an intensive farming and marginal desert habitat (Tracy Ranch). The species found in these two environments and on the grid were the same except for a few species. At Poso 1,035 mammal captures were achieved, and 1,330 captures at Tracy Ranch. Studies were begun on birds in these two areas and will be carried along in parallel with studies on mammals.

Studies of ectoparasites on mammals have continued to provide information on the prevalence of various species of ticks in different months, but have not led to virus isolations. The predominant Ixodid ticks were *Dermacentor parumapertus* and

Haemaphysalis leporis-palustris; and kangaroo rats, rabbits, and antelope squirrels were the principal hosts of these ticks. The infestation rate of immature stages of D. parumapertus on D. nitratoides at the Lerdo grid was unusually high in the spring of 1966.

A CO_2 trap was developed that effectively collected Ornithodorus parkeri and some fleas from squirrel burrows. These ticks are being tested for arboviruses.

Efforts were made to colonize the predominant ticks found in Kern County, and small colonies of D. parumapertus and O. parkeri were established.

Blood or plasma samples have been tested from 2,040 wild mammals collected on the grid in a three year period. HAI tests on plasmas collected from May, 1965, to November, 1965, indicated the lowest prevalence of arbovirus antibodies in three years of observation. There was no serologic evidence of WEE infection, although one strain of WEE virus was isolated from the blood of a Sylvilagus audubonii. There was a lower prevalence of antibodies to Powassan, Bunyamwera group, Buttonwillow, and California viruses than in two previous years. Powassan antibodies still were prevalent in A. nelsoni. HAI tests on plasmas from 260 mammals from Tracy Ranch and 134 mammals from Poso revealed that group B and Turlock viruses were more active at Poso than at Tracy Ranch or Lerdo Grid; whereas, California, Bunyamwera group, and Buttonwillow antibodies prevailed at Tracy Ranch. In birds the prevalent arbovirus antibodies were to WEE, SLE, Turlock and Bunyamwera group. SLE positive birds were almost all adults and probably were infected in previous years.

Studies of Culicoides were continued and at least 13 species have been identified. The Culicoides variipennis complex predominated in most collections. A study was made of the flying and feeding cycles of C. variipennis by use of truck traps, light traps, and CO_2 bait traps. Flight and feeding activity usually peaked near sunset and sunrise. There were relatively high levels of activity during moonlight periods and almost no activity in full darkness. There was evidence that certain physical conditions may be correlated with periods of C. variipennis flight and feeding: (1) temperatures ranging between 50 and 87F, (2) relative humidity exceeding 25 percent, (3) light intensity exceeding a certain minimum and possibly falling below a maximum, (4) wind velocity below a certain minimum. The flight activity of C. tarsalis and C. erythrothorax usually did not begin until after sundown.

Tests of 37,269 C. variipennis collected in 1965 yielded nine viral isolates that must still be identified.

Studies of the blood-feeding habits of mosquitoes were expanded geographically to include mosquitoes collected in California, Colorado, Utah, Texas, Oregon, Minnesota, Illinois, and the Hawaiian Islands.

At Poso Creek, Kern County, a higher proportion of *C. tarsalis* fed on mammals than at other sites we have studied in this county. *C. erythrothorax* fed almost exclusively on mammals. *Anopheles franciscanus* and *Culiseta inornata* fed mostly on rabbits and cattle.

In the Sacramento Valley *C. tarsalis* fed predominantly on birds with an increase in feedings on columbiform birds and mammals as the summer progressed. *Anopheles freeborni* fed almost entirely on mammals with over 40 percent feeding on rabbits.

Seven species were studied from Colorado. *C. tarsalis* fed in a pattern very similar to that observed in California, exclusive of the Poso Creek area. *Culex pipiens* fed almost exclusively on birds. *C. inornata*, *Aedes vexans*, *Aedes sticticus*, *Aedes dorsalis* and *A. melanimon* fed mostly on domestic mammals.

In Utah 58 percent of *C. tarsalis* fed on mammals, and this is different than the usual pattern in California or Colorado. *C. inornata* had fed mostly on domestic mammals.

In Illinois five species were studied. *C. pipiens*, *Culex restuans* and *Culex erraticus* fed on birds and *Anopheles quadrimaculatus* and *Anopheles punctipennis* almost exclusively on large mammals.

Nine species were collected in Minnesota: *C. tarsalis*, *Aedes cinereus*, *A. vexans*, *A. sticticus*, *Aedes canadensis*, *Aedes stimulans*, *Aedes fitchii*, *C. inornata*, and *Mansonia perturbans*. All species had fed almost exclusively on mammals.

An intensive study of the blood-feeding habits of the four mosquito species found in Hawaii was begun in November, 1965, and over 1,500 engorged mosquitoes have been tested. *Culex quinquefasciatus* was a catholic feeder and its blood-meals reflected the predominant animals at the location collected. This species has fed on the common domestic mammals and birds, and a range of wild birds, including the migratory red-footed booby. Collections of *Aedes vexans nocturnus*, *Aedes albopictus*, and *Aedes aegypti* were few in numbers, and while they had fed principally on mammals the last two species also had fed on birds.

Considerable effort is still being expended in basic immunologic studies to prepare more specific and higher titered precipitin

antisera for the identification of blood-meals from mosquitoes and *Culicoides* gnats.

Considerable progress has been made in the characterization of new arboviruses isolated from Kern County. Buttonwillow virus has been further characterized antigenically as a representative of the Simbu group. Buttonwillow virus multiplied in *A. aegypti*, *C. tarsalis*, and *Culicoides variipennis occidentalis*. *C. variipennis* transmitted this virus biologically. *Oryctolagus cuniculus*, *S. audubonii*, and *Ammospermophilus nelsoni* developed viremias of one to three days duration following peripheral inoculation. Other species of mammals and some birds developed HAI antibodies of short duration, but no detectable viremia after experimental infection. A number of strains of Buttonwillow virus were isolated from *Culicoides* and rabbit bloods collected in 1963 and 1964.

Jerry Slough virus that was isolated from *C. inornata*, was characterized further as a member of the California group. Agents FMS 4332 and A 10452, that were isolated from *C. tarsalis* and a rabbit blood, were further characterized as members of the Bunyamwera sero-group.

Two closely related viruses were isolated from the blood of *A.nelsoni* and *Perognathus californicus*. They were unrelated antigenically to other arboviruses known from California.

A virus isolated from a pool of fleas (CFP 200) does not seem to be an arbovirus and remains unclassified.

The series of 20 viruses reported as isolates from *Culicoides* and rabbit bloods in 1964 were partially characterized. Four were related to Buttonwillow virus. Thirteen viruses were interrelated by complement fixation tests to the prototype (BFS 5015) of a new group, and this group may be related to the Bunyamwera sero-group. Further evaluation of representatives of these 13 new agents by neutralization tests indicated a division into two antigenic groups. Three isolates have not been related to the above agents.

WEE virus was inoculated into *Peromyscus maniculatus*, 30th generation from a laboratory colony source. Only an occasional mouse died and all survivors that became infected developed both HAI and neutralizing antibodies.

The finding of a significant number of wild mammals with HAI antibodies to group B viruses led us to a further study of the immunologic responses of mammals to SLE infection. HAI antibody to SLE virus reached peak titers 14 to 28 days postinoculation. All heterologous reactions except to Powassan virus disappeared within three months.

Studies on the pathogenesis of Turlock virus were begun in 1965. Seven-day-old chicks that were inoculated subcutaneously developed viremias that persisted for seven days. Laboratory mice and rabbits did not develop detectable viremias. Three species of rodents and five species of wild birds were inoculated subcutaneously with the prototype Turlock virus and a recent low passage strain isolated from C. tarsalis. Although 28 of 45 Dipodomys heermanni, D. nitratoides, and P. maniculatus developed neutralizing antibodies, viremia was detected in only three D. heermanni. In contrast, almost all Tricolored and Brewer's Blackbirds, House Finches, English Sparrows, and Mourning Doves developed viremias and neutralizing antibodies. Culex tarsalis was readily infected when fed on chicks with viremias in the range of five to 250 plaque forming units of virus per 0.2 ml of blood. Virus persisted through 21 days extrinsic incubation.

This report represents the summary of an Annual Project Report. A limited number of copies of the detailed report are available upon request. (William C. Reeves)

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

Considerable effort has been devoted to an attempt to isolate strains of dengue virus in suckling mice from the 1964-1965 outbreak in French Polynesia. Although many different techniques were employed, and strains were carried through 11 serial passages, no definite pathogenicity was observed. The strains were serially transmitted, however, as indicated by the resistance of the inoculated mice to challenge with a mouse-pathogenic strain of dengue type 2. Efforts are now being directed to the isolation and typing of the strains in tissue culture. Low-titered interference-producing agents have been recovered, but these have not as yet been typed.

Despite the fact that many persons infected in the outbreak were experiencing their first Group B arthropod-borne virus infection, hemagglutination-inhibition antibody responses were rather broad. Among the dengue antigens, the highest HI antibody titers were usually obtained with type 3. (Leon Rosen)

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

Mosquito Tissue Culture

Ovarian tissues of three species of mosquito, *Culex molestus*, *Aedes aegypti* and *Aedes albopictus*, were cultivated *in vitro* using a further improved medium.

Compositions of the basal salt solution and the final culture medium were modified; sucrose in basal salt solution was reduced to 0.2 g/dl, one-fifth of that reported previously, and calf serum supplemented to final culture medium was doubled. The medium was favorable for the cultivation of hemocyte-like cells adhering to the outer surface of ovary tissues and also useful to study the origin of the migrating and growing cells.

Cells of the ovary sheath did not proliferate under the conditions studied, but their figures were observed clearly and were easily differentiated from cells of the other kinds.

Subcultures of the primarily outgrown cells were attempted, obtaining eight passages so far in the present experiments. (S. Kitamura)

References

Kitamura, S. This Infoexchange No. 12, pp. 114, September, 1965. *Ibid*. The *in vitro* cultivation of tissues from the mosquitoes. Further studies on the cultivation of ovarian tissues of three mosquito species and the examination of the origin of cells grown *in vitro*. Kobe J. Med. Sci. Vol. 12, No. 1, 1966 (in press).

REPORT FROM THE RESEARCH INSTITUTE FOR MICROBIAL DISEASES, OSAKA UNIVERSITY, OSAKA, JAPAN

Photoinactivation of Japanese Encephalitis Virus (JEV)

JEV was photodynamically inactivated by proflavine (PF) and visible light in the extracellular state. The action spectrum for JEV photoinactivation was obtained, and it was suggested that the site of dye-sensitized photoinactivation should be viral RNA.

The virus used was Nakayama strain of JEV grown in adult mouse brains. Infected mouse brains were homogenized with nine volumes of 0.14M NaCl in 0.02M Tris-HCl (pH 7.6) containing 100 ug/ml of cystine (TCS), and spun down at 8,500g for 15 minutes. From this supernatant (S_1), partially purified JEV (U_2), was prepared by two cycles of differential centrifugation (90,000g 90 min. and 2,000g 15 min.). In the successive experiments, U_2 or S_1 was employed.

Infectious RNA of JEV was extracted by cold phenol method according to Gierer and Schramm (1956). Infectivity of JEV and JEV-RNA was assayed on primary culture of chick embryo cells as described previously (1963). Hemagglutinating (HA) activity was estimated by Clarke and Casals' method.

Test tubes containing two ml of JEV or JEV-RNA suspension with or without PF were exposed to a 20 watts DL-fluorescent lamp (FL 20D Toshiba) in the distance of two or ten cm at 4°C. While not being irradiated, samples were handled under the safe light lamp (yellow FL 20 YF National). As shown in Fig. 1, kinetics of photoinactivation revealed a single hit curve in each case of JEV and JEV-RNA, while the photoinactivation velocity of JEV-RNA was slower than that of JEV. No infectious RNA was extracted from photoinactivated JEV by cold phenol method. HA activity was not affected by PF and visible light at all.

The action spectrum for JEV photoinactivation was obtained by using a large quarz-prism Hilger monochromator. The absorption spectra of free PF and of PF-yeast RNA mixture were recorded by a Shimazu recording spectrophotometer, type RS-27. As shown in Fig. 2, the action spectrum for JEV photo-inactivation by PF corresponded approximately to the absorption spectrum of PF-yeast RNA mixture, but not to that of free PF, suggesting that the site of dye-sensitized photoinactivation should be viral RNA.

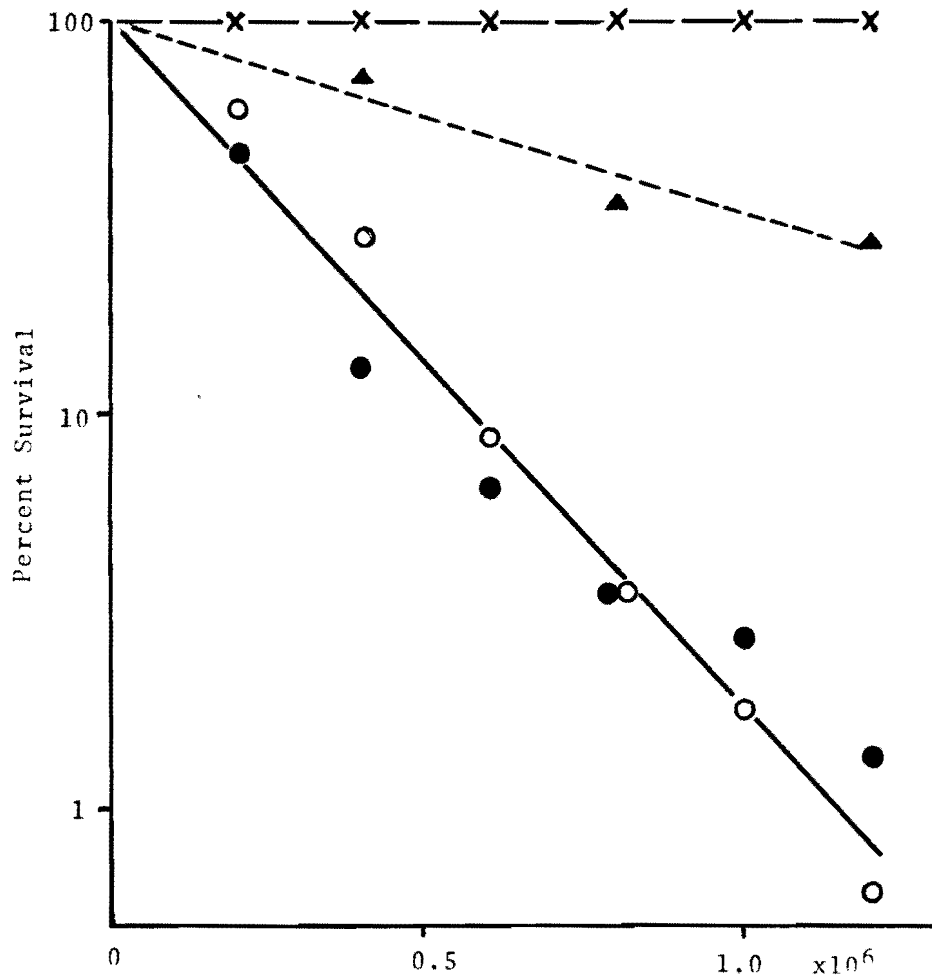
The photoinactivation of JEV was oxygen dependent (Table 1), but showed no dependence to temperature (Table 2). It was also independent of preincubation time of JEV with PF before irradiation (Table 3). When a JEV and PF mixture was filtered through a Sephadex column (Sephadex G-100, eluted with TCS in the dark at 5°C.) and then irradiated, no reduction of infectivity was observed. The re-addition of PF to the filtered JEV fraction, caused the reduction of infectivity by irradiation as in the case of unfiltrated control (Table 4). This seemed to indicate that the dye might reversibly bind to viral RNA.
(Fukai, Igarashi, Fukunaga and Ishikawa)

Reference

Gierer, A., and Schramm, G. (1956) Nature, 177, 702.
Gierer, A., and Schramm, G. (1956) Z. Naturforsch. 11B, 138.
Fukai, Igarashi, and Kitano. (1963) Arbovirus Information Exchange No. 7, p. 72.

Fig. 1

Photoinactivation of JEV and JEV-RNA by Proflavine (PF)



Dosis of DL-Fluorescent Lamp (erg/cm^2)

O———O JEV-PFU

●———● PFU of RNA from irradiated JEV

▲- - - -▲ PFU of free RNA

X— —X JEV-HA

PF: 10μg/ml

Fig. 2.

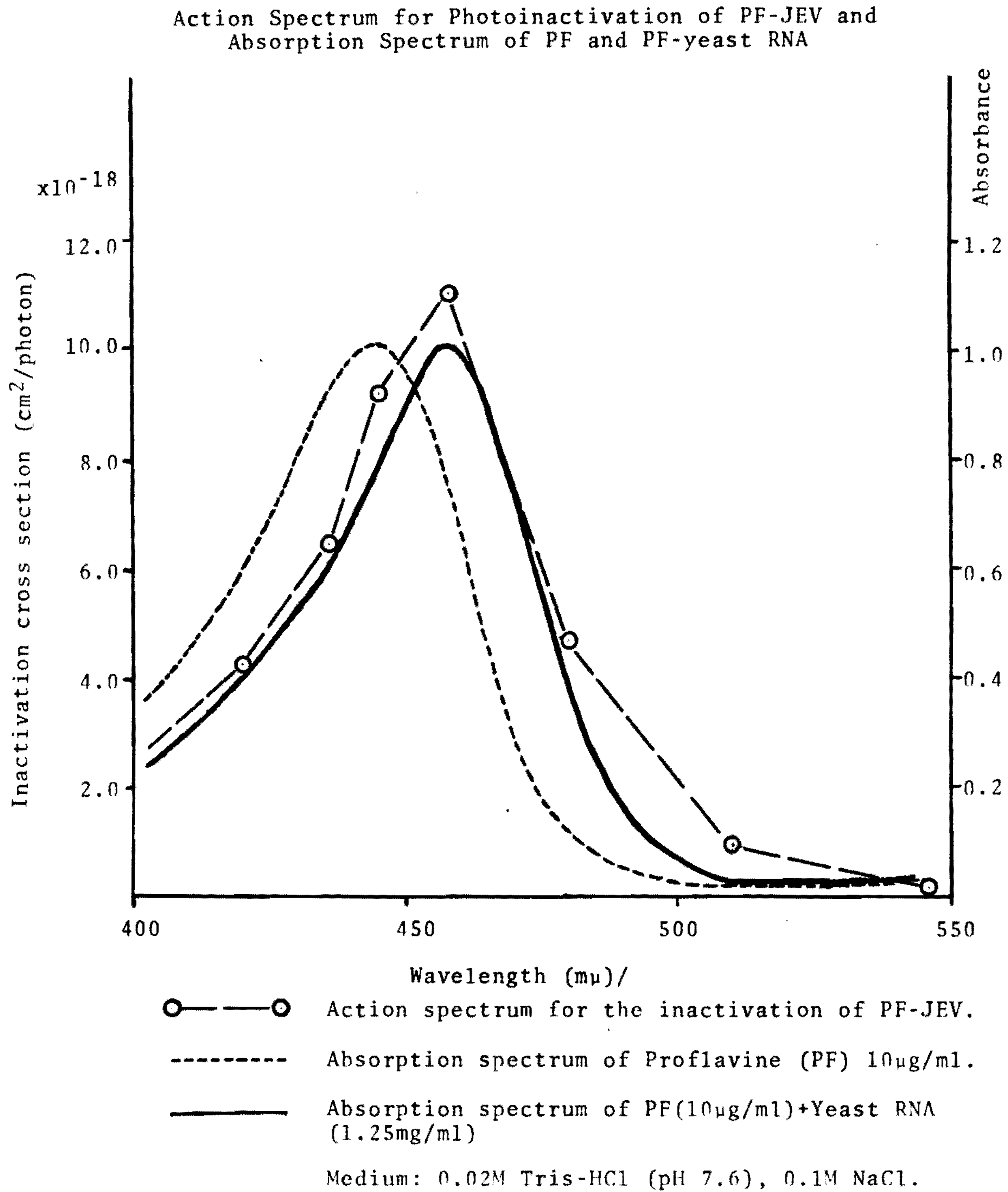


Table 1. Oxygen effect on photoinactivation of JEV

Atomosphere	Reflushed with air	Irradiation	Virus infectivity PFU/ml
N_2	-	-	1.1×10^5
N_2	-	+	3.8×10^4
N_2	+	-	1.5×10^4
N_2	+	+	2.5×10^1
air	-	-	7.0×10^4
air	-	+	2.5×10^1

PF: 1μg/ml, irradiated for 10 min.

Table 2. Effect of temperature

Irradiation with PF at:	Infectivity PFU/ml
4°C	9.2×10^3
R.T (Ca. 25°C)	1.1×10^4
37°C	8.4×10^3
Original sample	1.1×10^5

PF: 10μg/ml, irradiated for 5 min.

Table 3. Effect of preincubation time with PF

Sample	Preincubation time	Infectivity PFU/ml
A	20 hrs. PF(+)	2.0×10^4
B	20 hrs. PF(-)	1.9×10^6
C	30 min. PF(+)	1.0×10^4
D	30 min. PF(-)	1.0×10^6
E	0 min. PF(+)	1.4×10^4
F	0 min. PF(-)	1.5×10^6

PF: 1μg/ml, irradiated for 5 min.

Table 4. Reversible binding of proflavine to JEV

Exp.	Sephadex gel filtration	Re-addition of PF.	Irradiation for 5 min.	Virus activity PFU/ml	HAU/ml
a	-	-	-	6.0×10^6	256
b	-	-	+	4.1×10^4	256
c	+	-	-	1.2×10^6	16
d	+	-	+	9.3×10^5	16
e	+	+	-	7.0×10^5	16
f	+	+	+	1.9×10^3	16

REPORT FROM THE INSTITUTE FOR VIRUS RESEARCH
KYOTO UNIVERSITY, KYOTO, JAPAN

Cure From the Contamination With the Hog Cholera Virus in PS(Y-15) Cells

A clonal line of porcine kidney stable cells, PS(Y-15) cells, has been provided for the study of Japanese encephalitis virus as the most sensitive cell culture system (Info. Exchange, No. 8, 127). In early 1965, Dr. Y. Shimizu, et al (National Institute of Animal Health, Japan) demonstrated the possibility of the contamination in PS(Y-15 cells with an attenuated hog cholera virus. This was suspected from the evidence that pigs showed resistance to virulent hog cholera virus after the injection of culture fluid of PS(Y-15) cells.

PS(Y-15) cells, however, had been cured from the contamination with the hog cholera virus. The cure was done by re-cloning of cells at 41C and the cultivation in the medium containing specific immune serum. It has been confirmed by a test using pigs.

After the cure, there is not any difference of the susceptibility of PS(Y-15) cells to Japanese encephalitis virus. (Y. Kanda Inoue)

REPORT FROM CLARK AIR FORCE BASE, PHILIPPINES

HI Antibody in Human Populations at Clark Air Base, Philippines

Using JBE and chikungunya antigen, sera of Filipino and American residents of Clark Air Base and environs, Pampanga Province, Philippines, were tested for antibody to group A and group B. Posivite reactors are those showing a titer equal to or greater than 1:40. A great disparity between the two populations can be seen in the tables. Data are lacking to completely explain differences in age groups. In Americans the 40-50 year group included many with long term Philippine residence (10-20 years), while the 20-30 year group includes many of short residence (two years) exposed to mosquitoes (guards, airplane mechanics, etc.). Presumably the population differences can be explained on the basis of exposure to mosquito bites. Lenght of stay probably contributes by increasing exposure to biting. Samples of Peace Corps volunteers show a pattern of reaction compatible with this hypothesis. With very few exceptions Americans have received yellow fever vaccination.

TABLE I

PRESENCE OF ANTIBODY TO GROUP A AND B ARBOVIRUS
ANTIGEN IN 682 FILIPINOS BY AGE GROUP, 1964-1965

Age Group	N	Group A	Percentage	Group B	Percentage
0-4	22	0	0	9	41
5-9	70	0	0	48	68
10-14	29	0	0	20	68
15-19	82	4	5	62	76
20-24	169	12	7	132	78
25-29	120	8	7	119	99
30-34	85	10	1	78	92
35-39	49	1	2	41	83
40-49	35	6	17	32	91
50 plus	21	12	57	16	77
Total	682	53	8	557	82

TABLE II

PRESENCE OF ANTIBODY TO GROUP A AND B ARBOVIRUS
ANTIGEN IN 995 AMERICANS BY AGE GROUP, 1964-1965

Age Group	N	Group A+	Percentage	Group B+	Percentage
0-1	10	0	0.0	0	0.0
2-4	9	1	11.1	1	11.1
5-9	24	0	0.0	0	0.0
10-14	69	0	0.0	0	0.0
15-19	156	0	0.0	8	5.1
20-24	477	0	0.0	97	21.2
25-29	110	1	0.9	20	18.1
30-34	48	0	0.0	3	6.2
35-39	21	0	0.0	3	14.3
40-49	34	1	2.9	11	32.3
50 plus	27	0	0.0	9	33.3
TOTAL	995	3	0.3	152	15.3

TABLE III

ARBOVIRUS ANTIBODY STATUS BY PERCENTAGE OF REACTORS
AMONG AMERICANS IN PHILIPPINES BY LENGTH OF STAY

Length of Stay	Population Group	Serological Group	
		A	B
0-3 mos.	141 military & dependents	0.0%	8.0%
	354 Peace Corps volunteers	0.3%	9.0%
3-6 mos.	45 military & dependents	0.0%	7.0%
6-12 mos.	126 military & dependents	0.8%	13.0%
1-2 yrs.	104 military & dependents	2.0%	15%
	95 Peace Corps volunteers	0.0%	34%
10-20 yrs.	61 civilian employees	1.6%	33.%

REPORT FROM VIROLOGY DEPARTMENT
SEATO MEDICAL RESEARCH LABORATORY
BANGKOK, THAILAND

Dengue Hemorrhagic Fever.

Large numbers of hemorrhagic fever cases continue to occur yearly in Thailand with epidemic peaks every other year during the monsoon. In 1965, an "interepidemic" year, 2,228 cases were admitted to hospitals in Bangkok. Over 2,000 cases were reported from other areas of Thailand and a further spread of the disease southward was observed. In the first five months of 1966, 1,270 cases have been admitted to Bangkok hospitals and 49 deaths reported. Four types of dengue virus (1-4) have been isolated from hemorrhagic fever cases in Thailand in 1965-66.

Dengue Virus Identification.

A plaque reduction neutralization test using the LLC-MK-2 cell line has proved to be a very useful method for identification of dengue virus strains. All prototype strains as well as newly isolated strains produce satisfactory plaques under agar in these cells within seven days.

Using monkey antiserum made by single inoculation of live virus, a high degree of specificity was attained. Neutralizing (N) titers of antisera to prototype viruses are given in Table 1. Table No. 2 shows the results of testing 10 representative dengue isolates against the reference sera. No attempt has been made to differentiate between viruses within the 1,6 complex or in the 2,5 complex.

This method is especially useful for identifying newly isolated dengue viruses in second or third tissue culture passage which usually have low titers.

Japanese Encephalitis.

The challenge virus resistance (CVR) technique using BS-C-1 cells has proved to be a sensitive method for isolation of JEV as well as dengue viruses. Two strains of JEV have been isolated from human autopsy specimens by this method and comparative tests indicate that the CVR method is more sensitive than suckling mice or primary hamster kidney tissue culture.

Since 1962 studies on the ecology of Japanese Encephalitis have been in progress at the Red Cross Serum Farm at Bang Phra in cooperation with Dr. Skol of the Pasteur Institute and the entomology department of SMRL. Early studies were confined to

serologic studies of horses at the farm and virus isolation from mosquitoes. The program has been expanded to include detailed ecologic studies of the vertebrate fauna as well as studies of population dynomics of the suspected vector mosquitoes.

Twenty-six virus isolates have been made from mosquitoes since October 1962. Twenty have been identified as JEV. Ten JEV strains were isolated from pools of Culex tritaeniorhynchus and ten from Culex gelidus. One Chikungunya strain, one Sindbis strain and one unknown agent have been isolated from Culex tritaeniorhynchus. One strain of Tembusu, one Sindbis strain and three unknown agents were isolated from Culex gelidus. Infection rates with JEV for both Culex tritaeniorhynchus and Culex gelidus averaged approximately 1/10,000 during 1965.

(Philip K. Russell and Charas Yamarat).

Table 1. Neutralizing Antibody Titers of Monkey Antisera to Reference Strains of Dengue Viruses.

Viruses	Antisera D-1	D-2	D-3	D-4	D-5	D-6
Dengue-1 (Hawaii)	200 (1)	0 (2)	90	10	0	260
Dengue-2 (N.G."C")	0	1400	30	13	1700	0
Dengue-3 (H-87)	0	0	350	0	0	0
Dengue-4 (H-241)	0	0	14	150	0	0
Dengue-5 (TH-36)	0	2500	NT (3)	NT	2500	NT
Dengue-6 (TH-Sman)	40	0	NT	NT	0	240

(1) Reciprocal of 50% plaque reduction titer.
(2) 0 = less than 1:10
(3) NT = not tested

Table 2. Dengue Virus Identification by Plaque Reduction Neutralization Test with Monkey Antisera

Strain No.	Year Isolated	Reference Antisera D-1	D-2	D-3	D-4	D-5	D-6	Dengue type
648	1963	0 (1)	0	150 (2)	0	0	0	3
14670	1964	0	0	140	0	0	0	3
10286	"	-	600	50	10	-	0	2,5
12900	"	60	0	40	0	0	150	1,6
14580	1965	100	20	35	22	15	160	1,6
21868	"	0	190	30	0	100	0	2,5
14962	"	0	0	160	0	0	0	3
11340	"	0	350	0	0	90	0	2,5
16727	"	0	0	0	120	0	0	4
23751	1966	0	0	30	160	0	0	4

(1) 0 = less than 1:10

(2) reciprocal of 50% plaque reduction titer

REPORT FROM INSTITUTE FOR MEDICAL RESEARCH KUALA LUMPUR, FEDERATION OF MALAYSIA

There has been no serious outbreak of haemorrhagic fever in Malaya since the Penang epidemic of 1963-64. A continuing program of surveillance of dengue and other arthropod-borne diseases is being conducted in collaboration with the Hooper Foundation Arbovirus Research Laboratory. Serum specimens from patients with fevers of unknown origin and encephalitis are regularly sent in by the major hospitals for serological examination. Since July 1965, serum specimens from 303 patients were received and tested for HI antibodies against Dengue I, Dengue II, Japanese encephalitis and Tembusu antigens. Paired serum samples suitable for serological analysis were obtained from 137 of these patients. The number of serologically confirmed cases of dengue and JE is shown in Table I.

TABLE I

SEROLOGICALLY CONFIRMED *CASES OF DENGUE AND JE IN MALAYA
(July 1965 - May 1966)

Dengue I	Dengue II	JE	Negative	Inconclusive
7	3	16	105	6

*A four-fold rise in HI antibody and/or an increase in neutralizing antibody from acute to convalescent serum.

Two of the Dengue I cases occurred in Johore Bahru and were clinically diagnosed as haemorrhagic fever. These are the first cases reported from the southern portion of the peninsula. A dengue virus as yet untyped was isolated from one of these patients.

No cases of disease attributable to Tembusu virus were detected.

(Dora Tan).

REPORT FROM THE DEPARTMENT OF BACTERIOLOGY UNIVERSITY OF SINGAPORE, SINGAPORE

Isolation of Dengue Virus Type 3 from a Fatal Case of Fever with Shock Syndrome.

A 12-year-old Chinese girl was admitted to the General Hospital on November 25, 1965 with a history of fever, drowsiness and fits for one day. She was semiconscious when examined and her temperature was 102.2° F. She had a flushed appearance but there was no rash. Her pulse rate was 110 per minute and regular, and blood pressure was 100/70 mm Hg. Her heart and lungs were normal. Her liver, spleen and kidneys were not palpable. Laboratory investigations showed a normal total white count and a platelet count of 170,000 per c.cm. Urine showed albumin present, red blood cells 60-80, epithelial cells 6-8 and a few granular casts. Blood culture was bacteriologically sterile and Widal and Weil-Felix tests were negative. One hour after admission, she had peripheral cyanosis and was in shock with a rapid and feeble pulse and blood pressure of 60 mm Hg. systolic. In spite of therapeutic measures the patient died five hours after admission.

The following specimens from the case were available for virus studies: (1) peripheral blood from the patient before she died, and (ii) autopsy tissues: brain, heart, lungs, liver, spleen, kidneys, and heart blood. From the heart tissue a dengue type 3 virus was recovered by inoculation into 1-day-old mice. Difficulty was encountered in adaptation of this virus in suckling mice. The virus (GH-8/65) was finally adapted to kill 1-day-old mice on the 8th day after 17 passages. Investigations on the other specimens are still in progress.

Studies on Aedes aegypti and A. albopictus.

During the first outbreak of dengue haemorrhagic fever in 1960, six strains of dengue virus type 2 were isolated from mosquitoes, five strains from A. aegypti and one strain from A. albopictus. The mosquito collection and virus isolation were undertaken by Dr. Albert Rudnick of the George Williams Hooper Foundation, San Francisco, who was on a brief visit with us. The mosquitoes were collected during a three-month period (November 1960 to January 1961) from both urban and rural areas. The five virus strains were isolated from pools of 219 A. aegypti and 1,290 A. albopictus mosquitoes.

In 1962 the department made further collection of mosquitoes in urban and rural areas for the purpose of virus isolation. A total of 305 A. aegypti and 36,282 A. albopictus mosquitoes were collected in a 8-month period but no dengue virus was recovered from the female mosquitoes of both species inoculated.

Beginning in January 1966 the department, in collaboration with the Department of Parasitology, initiated a long-term study of A. aegypti and A. albopictus mosquitoes as a part of our haemorrhagic fever study programme. The primary objectives of this study are two-fold: (1) Collection of data on the relative

density and distribution and seasonal fluctuation of the two mosquito species, and (2) evaluation of the vector status of *A. albopictus* in dengue haemorrhagic fever.

In this study, attention is focussed on sections of the island where dense human populations are found and where cases of the disease occur. Weekly collections of adult mosquitoes are carried out at six collecting stations, three stations for each mosquito species, by a team of trained collectors. *A. aegypti* mosquitoes are collected from resting populations inside 16 to 18 houses by aspirator tubes for a period of three hours in the morning at each station and with the collectors working in pairs in one house. *A. albopictus* mosquitoes are collected from the field by human bait using two baits and one collector for each bait. These collections are made in the afternoon for one hour at each station.

In addition to the regular mosquito collections, search is made of the breeding places of *A. albopictus* in areas close to dense human habitation. In the laboratory, virus isolation from mosquitoes and transmission studies are carried out.

The programme is continuing, but studies in the past five months permit the following preliminary observations to be made:

(1) *A. aegypti* is common in all the study areas (aegypti index ranges from 40-68%).

(2) *A. aegypti* population fluctuates with the rainfall. The population increases with the rainfall whenever there was a preceding dry period of more than two weeks and usually lags behind the rainfall by about one week.

(3) *A. albopictus* breeds extensively in areas close to dense human habitation most commonly in discarded motor-car tires and small containers. These breeding sites are found in small, unused plots of land commonly found adjoining to houses. This mosquito species appears to be much more widespread and common in "urban" areas than reported previously.

(4) *A. albopictus* population also fluctuates with the rainfall, but there appears to be no lag between increased mosquito population and peak rainfall.

(5) A few *A. albopictus* mosquitoes, including unfed, fed and gravid ones, can be found inside houses.

(6) *A. aegypti* and *A. albopictus* mosquitoes can occur together in large numbers. In one house, 35 *A. aegypti* (15 females and 20 males) and 95 *A. albopictus* (29 females and 66 males) have

been caught by sweeping within a period of 40 minutes. This house is a converted motor workshop situated among shop-and-living houses.

An agent, as yet unidentified, has been isolated from a pool of A. albopictus inoculated into mice. This agent killed 1-day-old mice in the second mouse passage.

(Drs. Y.C. Chan, B.C. Ho, and K.P. Chan).

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

Studies have been continued in the same area of South Westland New Zealand as in previous years. Mosquitoes for virus isolation attempts were trapped in November and December 1965 and in January 1966. This season the stable traps of previous years were abandoned in favor of two types of portable trap made from 12 gallon cylindrical cans and baited with bantams. A total of 5,519 Culiseta tonnoiri and 3,990 Culex pervigilans were extracted in pools of 100 and tested for virus by plating on duck embryo monolayer tissue cultures under agar, and by intracerebral inoculation of suckling mice. No virus was isolated by either method.

From April 1965 until the present (June 1966), regular mist-netting of birds was carried out on three circuits in the study area. Blood samples were collected, and as with the mosquitoes, virus isolation was attempted on duck embryo monolayer tissue cultures and in suckling mice. No isolation has been made from plasmas collected up to March 1966.

The plasmas collected up until December 1965 have been tested in a plaque reduction test for Whataroa (M78) neutralizing antibodies. On the year's totals the incidence of plasmas containing antibodies is rather similar to that of 1964. However, on examining the monthly totals of the bloods collected from cleared and partly cleared areas (Graham's and Viney's circuits two miles apart), it can be seen that in December there was an increase in the proportion of sera which contained M78 neutralizing antibodies. (Table 1). A number of the bloods tested were from recaptured birds. One or more repeat specimens were obtained from 73 birds during the year. Most of these were negative at both bleedings but some showed changes. Among these were two which lacked antibody when first tested in May and November, but which had titres of 40 and $\geq$ 20 in December. This together with the observation of increased frequency of bloods with M78 virus

antibody in December, suggests that an epizootic of Whataroa virus occurred in the area early in the summer.

The high incidence of bloods with M78 virus antibodies at Graham's in September is rather puzzling. Forty of these bloods were tested by plaque reduction against Murray Valley encephalitis virus. Only one inhibited the virus so it seems unlikely that the inhibitory activity against M78 virus was non-specific. Four of the birds recaptured on this circuit showed changes in their M78 virus antibody level. Two which were bled in August and again in September showed an increase in that time, while another two which were bled in September and November showed a decrease.

It seems improbable that the high incidence of birds circulating M78 virus antibody in September could be due to recent infection because there were very few mosquitoes about at that time. In addition it is not likely that the incidence of bloods with antibody would have dropped from 50% to 3.5% in a period of six weeks. One possible explanation is that the stress of nesting released a burst of antibody in birds which had encountered the virus some time previously. At the time of the September collection many nests had been completed and up to two eggs were found in a few. No nests with more than two eggs were detected.

As in previous years the proportion of bush-dwelling birds with M78 virus antibody was very low.

Studies in Fiji.

The laboratory in Suva, Fiji has now been operating for some 18 months. Eighty-three thousand mosquitoes of nine species, collected within 40 miles of Suva, have been examined for arbovirus by inoculation intracerebrally into suckling mice. No virus has been isolated.

Ninety-one human sera from a district school at Savusavu have been tested for Murray Valley encephalitis (MVE) virus antibodies by haemagglutination inhibition (HI) test. Twenty-three (25%) were positive at a titre of 1/20 or more. A previous survey in this area in January 1963 disclosed that 6.6% of the sera tested for were positive for MVE HI antibodies. Thirty-three individuals were tested in both surveys. Thirteen of these were found to have converted from negatives to positives between surveys. This conversion rate together with the higher incidence of positives this year suggests a marked degree of virus activity in the area since the beginning of 1963. It is hoped to confirm these results in the near future with an extensive serological survey in the Savusavu area with specimens from school children.

TABLE 1

Results of Whataroa (M78) virus neutralization tests on bird bloods.

Month of Capture	Graham's circuit.	Viney's circuit.
1965 April	N.D.	3/33* (9%)
May	N.D.	2/87 (2%)
June	N.D.	1/44 (2%)
August	9/87 (10%)	N.D.
September	21/42(50%)	N.D.
November	2/59(3.5%)	1/29 (3.5%)
December	13/33(40%)	30/88(34%)

* - Number positive/number tested.

N.D. = Not done.

REPORT FROM THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH BRISBANE, AUSTRALIA

Virus strains. The MRM1178 strain was studied at the International Reference Centre, New Haven, Conn., U.S.A. by Dr. Casals, who found no relationship with any of some 30 viruses. It has accordingly been registered with the Catalogue as "Kowanyama" virus.

Two viruses apparently "new" to Australia were isolated during the year - MRM3630, from *Anopheles annulipes* collected at Mitchell River in October, 1965, yields no haemagglutination but by CF and neutralization test is distinct from other viruses known in the area; MRM3929, from the blood of a swamp pheasant

(*Centropus phasianinus*) from Mitchell River in March 1966, is a group B virus related to but distinguishable from MVE.

Antibody Surveys. Twenty-two patients from three areas of Queensland had rising or falling titres indicating infection with Ross River virus in early 1966; all had clinical illnesses similar to those described previously for Australian epidemic polyarthritis.

HI antibody apparently to Edge Hill virus was found in rats and (marsupial) bandicoots in rain forest in northeastern Queensland. Neutralizing antibody to Corriparta, Kowanyama and MRM3630 was found in old aborigines from Aurukun Mission in north Queensland.

Field studies. Collections of mosquitoes and birds for virus isolation and human and domestic fowl sera for antibody studies were made in dry and wet seasons at Mitchell River Mission. Strains of Corriparta and Ross River virus were isolated from birds collected in the wet season of 1965. Evidence of Ross River virus infection during 1965 was found by serological tests of human sentinels; group B virus infection was detected in sentinel fowls in the summer of 1965-1966.

Sentinel fowls exposed in southwest Queensland gave evidence of a very low rate of infection with Sindbis virus in the period October 1965-January 1966.

Studies of reptiles and amphibians. Virus isolation and antibody studies are in progress on 644 reptiles and amphibians collected at several centres in Queensland. Low titre reactions by HI test to several viruses are under study as possible indication of antibody.

Experimental mosquito inoculation. Kowanyama and In1074 viruses were shown to multiply in experimentally inoculated *Culex fatigans* but serial passage (infected salivary gland passed by intrathoracic inoculation) failed after one and two passages respectively.

Pathogenisis of group A arbovirus infection. Suckling mice inoculated with Ross River or Getah viruses developed high titres of virus content in 12 organs tested and in blood; urine contained up to $10^{4.5}$ LD_{50} per ml. Chickens developed low titre viraemia from the second to fourth days following inoculation with Ross River virus, but only a minority developed antibody. (Previous surveys showed little evidence of natural Ross River virus antibody in domestic fowls).

Tissue culture studies. Plaque assay neutralization tests of group B arboviruses in PS cells were evaluated with a variety of field sera. Results suggest that this system will be of value in future studies of dengue immunity.

Biochemical and biophysical properties of arboviruses. Multiplication of Corriparta and Mapputta virus was not inhibited by halogenated derivatives of deoxyuridine, suggesting that they are RNA viruses. Attempts to extract infectious RNA were unsuccessful. Both viruses were studied by electron microscopy, in sections of infected PS cells and mosquito salivary glands and in negatively-stained spray preparations. Both appear to be about 55 mμ in diameter (similar preparations of MVE measured 40 mμ); Corriparta negatively-stained preparations appear to have a regular arrangement of surface sub-units.

Further details of these and other studies are listed in the 1966 Annual Report, Queensland Institute of Medical Research.

(R.L. Doherty, J.G. Carley, H.A. Standfast, E.G. Westaway, R.H. Whitehead, B.M. Gorman, E.J. Wetters, and P.A. Graf).

REPORT FROM THE UNIVERSITY OF QUEENSLAND
ST. LUCIA, BRISBANE, AUSTRALIA

Mild disseminated encephalitis was produced in calves inoculated intracerebrally with Getah and Ross River viruses. Calves inoculated intracerebrally and parenterally with Kunjin virus developed lesions in neural and hepatic tissue. Clinical disease occurred only in young calves inoculated intracerebrally. Viraemia occurred in chickens inoculated subcutaneously or intramuscularly with Sindbis and Murray Valley encephalitis viruses but not after inoculation with Koongal virus. (Spradbrow, P.B. & Clarke, L. (1966) Aust. vet J. 42, 65-69).

Studies have been carried out on the preparation of bovine serum for HI test. Natural agglutinins for goose red blood cells were difficult to remove by adsorption in a high proportion of sera. Non-specific inhibitors were present in some sera after kaolin adsorption.

The acetone-ether extraction was more satisfactory, both from the point of view of non-specific inhibitors and natural agglutinins. Natural agglutinins were inactivated by acetone-ether in 60% of sera, a further 35% were then readily adsorbed.

Natural agglutinins in bovine serum could be removed by heating at 64° C for 30 minutes. This treatment did not affect the antibody titre.

(P.B. Spradbrow and C.J. Anderson)

REPORT FROM THE VIROLOGY SECTION OF THE DIVISION OF ANIMAL HEALTH, C.S.I.R.O. PARKVILLE, VICTORIA, AUSTRALIA

In the Number Four Issue of the Arthropod-borne Virus Information Exchange of October 1961 (see page 54), we reported the results of some experiments with the agent of Ephemeral Fever of cattle. Work with this agent has resumed now that we have insect proof accommodation for cattle.

Known infected blood from experimental cases of ephemeral fever has been used to infect a wide range of bovine cells in both monolayer and Maitland type tissue cultures. No cytophatic or other effect indicating virus growth has been obtained in these tissue cultures. Moreover, when the tissue cultures were inoculated intravenously into cattle, no clinical reaction ensued, and the cattle subsequently reacted typically when challenged by inoculation with blood from an experimental case of ephemeral fever. It has been concluded that either the ephemeral fever agent failed to grow or survive in these tissue cultures, or that it rapidly lost its immunogenicity and so failed to produce resistance to challenge with the virulent agent.

A number of paired bovine sera from animals that had been inoculated with our ephemeral fever agent and had subsequently recovered from the typical clinical disease, were available. Dr. R. Doherty of the Queensland Institute of Medical Research very kindly examined these sera for HI antibody to MVE, Sindbis, Ross River (T48) Getah (N544), Koongal (MRM97) and Wongal (MRM168) but all sera were negative at 1/20. Neutralization screen tests were also carried ou with these sera against Mapputta (MRM186, inoculated I.P.), Corriparta (MRM1, inoculated I.C.) MRM1178 (I.P.) and in 1074 (I.C.). None of the sera caused any significant neutralization of any of the viruses.

Work on ephemeral fever of cattle is continuing in this laboratory.

(J.H. Whittem, N.E. Southern and Eric L. French).

REPORT FROM VIRUS DIAGNOSTIC LABORATORY SHIMOGA, MYSORE, INDIA

Kyasanur Forest Disease (KFD) epidemic, which started in November 1965, lasted through May 1966. The number of cases yielding virus was maximum in 1965-66 for any season so far.

KFD virus has been isolated from 104 cases. There were two reasons for this increase in human cases:

(1) The infected area was very close to Sagar, which has a population of about 20,000. Many people from Sagar visited this area for collection of firewood and were intensely exposed to the KFD virus infection.

(2) There was extensive flowering of bamboos in this area. The bamboo seeds, which are also called "bamboo rice", are used as a substitute for rice. As the villagers visited the infected zone in large numbers for collection of bamboo rice, they became sick.

(Sridhar Upadhyaya).

REPORT FROM THE VIRUS RESEARCH CENTRE, POONA

Field Trip to Kutch District (Gujarat State, India).

During the reconnaissance serological survey undertaken in India in 1952, human sera which could neutralize RSSE virus has been detected in Kutiyana Town of Saurashtra (now Gujarat State). When further surveys were made between 1958 and 1960, after Kyasanur Forest Disease (KFD) had been discovered in South India, human sera which could neutralize KFD virus had been found in several places in the region. However, no disease compatible with RSSE or KFD had been reported.

In Banni, a semi-arid grassland area of Kutch District (north of Saurashtra), serological surveys in 1960 had shown that sera of not only humans but also of several animals (horses, donkeys, and camels, but not of cattle, goat and sheep) could neutralize KFD virus. This area presents ecological features and a tick fauna totally different from those in the semi-deciduous rain forests of Sagar, Mysore State, where KFD virus is known to be active and produces illness in man and monkeys. Therefore, the evidence of the activity of a virus, either KFD itself or a closely related one, in a place nearly a thousand miles away and in a different type of environment, is a matter of great importance needing further investigation.

As a preliminary to detailed investigations, an expedition to the Banni area was made during March 1966, the prime objective being to find out whether the activity of the virus was still present. More than 100 camels, 50 horses and 75 donkeys, apart from a small sample of humans, were bled. Particular efforts were made to collect blood samples from young animals born after 1960. All

the sera have now been tested in HI and only sera from two horses have shown HI antibodies to KFD. The sera are being tested in NT and the preliminary results show that sera of some animals born between 1960 and 1965 can neutralize KFD virus (5 out of 27 donkeys, 7 out of 28 horses and none out of 40 camels). Quite obviously the virus has continued to be active since the time of the previous survey. Further work including attempts to isolate the virus will be undertaken after a final analysis of the results. The absence of HI antibodies in the NT positive sera is interesting and is receiving attention.

An Unidentified Strain of Arbovirus from Blood of a Paddy Bird (*Ardeola grayii*).

As a part of epidemiological studies of Kyasanur Forest Disease (KFD) virus, a large number of birds were shot, their ectoparasites examined and their sera tested for the presence of virus and/or antibodies. From the blood of a pond heron (*Ardeola grayii*), a virus strain was obtained which seemed to be unrelated to any of the arboviruses tested so far.

It was highly pathogenic to infant and adult mice by intracerebral and peripheral routes. Early (1-2 days) cytopathic effect was observed in monkey kidney and chick embryo tissue cultures. Chick embryo inoculated with this agent using allantoic or yolk sac routes were also highly susceptible, dying within 2-3 days. White leghorn chicks (1-3 days), given intramuscular or subcutaneous inoculation of this virus, circulated it up to a titre of 10^5 (approximately) from first to the fifth day after inoculation. The chicks became sick and died by the eighth day.

The strain was DCA sensitive and preliminary attempts of transmission, employing young chicks and *Culex fatigans* mosquitoes were highly successful.

Attempts to prepare hemagglutinins from infected suckling mouse brains or allantoic fluids were unsuccessful, though a workable CF antigen could be obtained by acetone-ether extraction or sucrose acetone extraction of the infected suckling mouse brains. Hyperimmune mouse serum yielding CF titre of 64-128 with the homologous virus, was tested with Japanese encephalitis, West Nile, dengue 1 through 4, KFD, Ntaya, Sindbis, chikungunya, and many other ungrouped virus strains isolated from ticks and mosquitoes from India. No reaction was observed with any of the strains. Similar negative findings were obtained in neutralization tests. HI test on the serum with Newcastle disease virus was found to be negative.

The virus was sent to Dr. Jordi Casals at the Rockefeller Foundation Laboratories, for further characterization. An interim

report received from him indicates it to be unlike any of the following viruses with which they were tested in CF. (In parenthesis are given the homologous titres).

Polyvalent Group A (1:32-1:128), polyvalent Group B (1:32-1:128), Caraparu (1:128) Oriboca (1:128) Ketapang (1:32), Bunyamwera (1:128), Guaroa (1:256), Kairi (1:256), Wyeomyia (1:32), Bwamba (1:256), California encephalitis group (1:64-1:256) Changuinola (titre as yet unknown), Catu (1:64), Akabane (1:128), Oropouche (1:256), Simbu (1:32), Tacaribe (1:256), Cocal (1:512), Anopheles B (1:16), Chenuda (1:32), Kaisodi (1:256), Kemerovo R 10 (L;256), Malaya TP 94 (1:256), Nyamanini (1:256), Quaranfil (1:128), Witwatersrand (1:128), Uukuniemi (1:64) and Newcastle disease virus (1:128).

Two hundred and seventeen human sera and 43 sera from cattle, all collected from the KFD epidemic area of South India, were tested for the presence of neutralizing antibodies to this virus. None of the cattle sera had demonstrable N antibodies; 14 of the human sera showed the presence of N antibodies. All the positive donors came from two out of seven localities tested.

Infection Threshold of Aedes aegypti and Aedes albopictus for Chikungunya Virus.

Studies were undertaken to determine the infection threshold of four strains of *A. aegypti* and a single strain of *A. albopictus* for chikungunya virus. The detailed history of the mosquitoes employed in this study are given in Table I.

Mosquitoes were infected by allowing them to feed through a Bandruch membrane on serial ten fold dilutions of chikungunya virus prepared in defribrinated chicken blood. The fed mosquitoes were tested individually in infant mice to detect the presence of virus after ten days of incubation period.

The results presented in Table II suggest that 5.6 logs or more of virus was required in blood to infect over 50 per cent of *A. aegypti*. With virus titres ranging from 4.6 to 5.5; 3.6 to 4.5; and 2.6 to 3.5 logs in blood, the rate of infection in mosquitoes became correspondingly less. Not a single *A. aegypti* out of 66 fed on blood with 2.5 logs or less of virus became infected. No difference in the infection threshold for chikungunya virus was demonstrated between the four strains of *A. aegypti* tested.

Rate of infection in *A. albopictus* when fed on blood with 6.9, 5.9, 4.9 and 3.9 logs of virus was 12 out of 12, three out of three, 11 out of 12 and two out of seven, respectively. There was no infection in mosquitoes with 2.9 logs or less of virus in blood.

From the results of this preliminary experiment, it seems that A. albopictus have a comparatively lower threshold for chikungunya virus than A. aegypti; 4.5 logs of virus in blood was required to infect 50 per cent of A. albopictus whereas 5.6 logs of the same virus was necessary for A. aegypti.

TABLE I

STRAINS OF MOSQUITOES USED

Strain	Species	Place of origin	History
A	A. aegypti	Vellore, Madras State.	Colony established in 1960 at Poona from field collected adults.
B	A. aegypti	Vellore, Madras State.	Colonized from field collected larvae, used in first laboratory generation (1966).
C	A. aegypti	Rajahmundry, Andhra Pradesh.	Colonized from field collected adults, used in second laboratory generation (1965).
D	A. aegypti	Calcutta, West Bengal.	Colonized from field collected larvae used in eleventh laboratory generation (1964).
E	A. albopictus	Poona, Maharashtra	Colony established in 1953 from field collected adults.

TABLE II

INFECTION THRESHOLD OF AEDES AEGYPTI STRAINS FOR CHIKUNGUNYA VIRUS

	Approximate doses mouse Log LD_{50}/0.02ml	Rate of Infection Strain of *A. aegypti*					Total	Percent
		A	A	B	C	D		
1	$10^{6.6}$ to $10^{7.5}$	7/8*	5/5	3/3	16/16	3/5	34/37	91.8
2	$10^{5.6}$ to $10^{6.5}$	8/10	6/10	9/14	17/20	3/10	43/64	67.1
3	$10^{4.6}$ to $10^{5.5}$	2/9	2/10	6/20	3/20	2/10	15/69	21.7
4	$10^{3.6}$ to $10^{4.5}$	0/7	1/10	1/16	1/16	1/9	4/58	6.9
5	$10^{2.6}$ to $10^{3.5}$	1/10	0/7	0/20	0/18	0/10	1/65	1.5
6	$10^{1.6}$ to $10^{2.5}$	0/10	0/10	0/18	0/18	0/10	0/66	0.0

* Numerator = Number of mosquitoes found infected;

Denominator = Number of mosquitoes processed.

REPORT FROM THE DEPARTMENT OF VIROLOGY
ISRAEL INSTITUTE FOR BIOLOGICAL RESEARCH
NESS-ZIONA, ISRAEL

During the period extending from April 1964 to May 1966, more than 5,000 wild birds were shot or captured throughout the country. These birds belonged to 30 different families and included more than 105 species. The bloods are being processed for virus isolations and serological examinations.

Virus isolations. Two strains of West Nile virus and one strain of Sindbis virus were isolated from turtle doves shot over an area along the coast of the Mediterranean in September 1964. An additional strain of West Nile virus was isolated from the blood of another turtle dove shot in the same area in August 1965. This is the first report of isolation of arboviruses from wild hosts in Israel.

All virus isolations were made in suckling mice, and the strains identified by hemagglutination-inhibition and neutralizing tests using known sera. Of particular interest is the isolation of Sindbis virus, the existence of which in this country was earlier suggested by the finding of specific antibodies in vertebrate bloods.

Serological examinations. So far, 2,843 avian sera have been tested for HI antibodies against EEE, Semliki, Sindbis, RSSE (or Langat), turkey meningoencephalitis and WN antigens. Thirty-three per cent were found to give positive results. Of the positve sera, an insignificant proportion (1.5%) exhibited positive antibodies to Group A antigens only. Most (57.8%) were positive to Group B viruses. Surprisingly enough, the remaining positive sera were found to react with both Group A and B viruses. Investigations are in progress to attempt and find an explanation to this singular phenomenon.

Sentinel chickens. In 1964, we maintained four flocks of 60 chickens at each of four widely separated sites in an attempt to ascertain the activity of arboviruses throughout the year. The chickens were bled at monthly intervals from April through November 1964. The sera obtained are being tested by the HI test against the same antigens as in the wild bird survey.

Other serological studies. In addition to the above, 82 sera samples obtained from frogs (61 Rana ridibunda and 2 Hyla arborea) and turtles (19 Clemmys caspica) were tested for HI antibodies to EEE, Semliki, Sindbis, Langat, turkey meningoencephalitis and WN antigens. These sera were processed with acetone for removal of non-specific inhibitors according to the method of Clarke and

Casals. The results are shown in the following table:

Sera inhibiting hemagglutination of antigens

	EEE	Semliki	Sindbis	Langat	TME	WN	Total tested
Frogs	3	63	0	0	0	0	63
Turtles	5	19	0	0	0	0	19

The results obtained with the EEE and Semliki antigens were suspected as being due to non-specific inhibitors which were not removed by the acetone extraction. We, therefore, followed the acetone extraction with kaolin treatment, and these sera were then found to give negative reactions with both EEE and Semliki antigens. These findings resemble those reported by the Arbovirus Unit from the University of Arizona in a previous report of the Information Exchange concerning the reaction of some chicken sera with WE antigen after acetone extraction.

Mosquito collections. Between May 1965 and May 1966, 21,954 mosquitoes belonging to 17 different species were collected in CO_2-baited light-traps. These were processed in 517 pools containing no more than 100 specimens per pool and inoculated into infant mice. Out of the total number of 3,077 *Culex univittatus* collected throughout the period under study, four pools containing 222 specimens and caught on 10/8/65, 15/9/65 and 4/10/65, yielded strains of virus which appeared to be identical to West Nile virus. Three of these strains were isolated from mosquitoes caught on the Hula reserve in the north of the country. No virus was isolated from 10,359 *C. molestus* processed in 206 pools.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY INSTITUTO SUPERIORE DI SANITA ROME, ITALY

Survey for Antibodies Against Arthropod-borne Viruses in Man and Animals in Italy.

Sera from 371 apparently healthy residents in two areas of Italy and from 153 domestic and wild animals were examined by the hemagglutination-inhibition (HI) test for antibodies against arboviruses. The areas of collection were selected in a northern region at the borders of Yugoslavia (Gorizia province) and in a central region (Fondi).

The 10 arbovirus antigens used were as follows:

Group A - Western Equine Encephalitis (WEE), Sindbis
Group B - West Nile (WN), Yellow Fever (YF), Dengue 1 (De1), Dengue 2 (De2), Tick-borne encephalitis (TBE)
Bunyamwera
Sicilian Phlebotomus fever and Neapolitan Phlebotomus fever.

About 32% of the human sera reacted with one or more antigens (Table 1): 2.6% to TBE, 9.4% to WN, 18.5% to Sicilian Phlebotomus fever, 12.6% to Neapolitan Phlebotomus fever.

Table 2 and Table 3 show the HI patterns of Group B positive sera.

The sera from 153 domestic and wild animals of various species (bovines, goats, sheep, chickens, rabbits, pigs, rats and wild mice) were collected in Fondi and adjacent localities (Latina province). In contrast to the human sera, HI antibodies against Group A viruses were encountered in the animal sera tested (Table 4): 24% of bovine sera, 32% of goat sera and 7% of sheep sera reacted with WEE antigen; 2% of bovine sera, 24% of goat sera and 7% of sheep sera with Sindbis antigen. In regard to Group B antigens, 7% of bovine sera and 44% of goat sera reacted with TBE; 5% of bovine sera, 32% of goat sera, one rabbit serum, four rat sera and a pool of six sera of wild mice were positive to WN.

Some of the human and animal HI positive sera were further tested with the corresponding viruses by mouse neutralization (NT) test (Table 4 and Table 5). The presence of TBE neutralizing antibodies ($NI_{50} > 100$) was confirmed in human and goat sera; WEE and WN neutralizing antibodies were not evidenced (except in one serum), indicating perhaps the presence of viruses closely related; NT antibodies to Sicilian Phlebotomus fever virus were detected only in two sera among the HI positive tested, and that can be explained by the limited and not durable antibody response following infection with Phlebotomus fever viruses.

(P. Verani and M. Balducci).

Table 1 - Summary of HI antibodies in 371 human sera from 2 areas in Italy to 10 arthropod-borne viral antigens

GROUP	VIRUSES	Area of collection				TOTALS	
		Gorizia (166 sera)		Fondi (205 sera)			
		No. sera positive	Per cent of positives	No. sera positive	Per cent of positives	No. sera positive	Per cent of positives
	Any virus	58	35.0	60	29.2	118	31.8
GROUP A	Sindbis	1	0.6	0	–	1	0.2
	WEE	0	–	0	–	0	–
GROUP B	TBE	6	3.6	4	1.9	10	2.6
	WN	18	10.8	17	8.3	35	9.4
	YF	9	5.4	7	3.4	16	4.3
	De 1	6	3.6	2	0.97	8	2.1
	De 2	6	3.6	2	0.97	8	2.1
BUNYAMWERA GROUP	Bunyamwera	0	–	6	2.9	6	1.6
UNGROUPED	Sicilian Phlebot.fev.	27	16.2	42	20.5	69	18.5
	Neapolitan Phlebot.fev.	27	16.2	20	9.7	47	12.6

TABLE 2 - HI antibody titers against group B viruses found in 166 Gorizia province residents.

Serum No.	Antigen 4-8 Units					Age	Sex	Time person has been living in Gorizia (in years)	YF Vaccine	Travel abroad
	TBE	WN	YF	De1	De2					
L 2	0	40+	40	20	20	87	f.	57	no	no
L 3	0	20	0	0	0	76	m.	36	no	no
L 12	640	0	0	0	0	71	f.	46	no	Yugoslavia (1889-1929)
L 16	0	20	40	0	0	76	f.	76	no	no
L 22	0	20	0	0	0	69	m.	4	no	no
L 24	20	640	160	160	160	74	m.	6	yes	Ethiopia
L 28	320	0	0	0	0	71	f.	71	no	no
L 30	0	160	0	0	0	71	f.	1	no	Egypt (1931-1964)
L 35	0	40	0	40	80	80	f.	51	no	no
L 39	0	20	0	0	0	80	f.	38	no	no
L 60	0	20	20	0	0	84	m.	28	no	no
L 65	80	1280	1280	81820	1280	65	f.	3	no	Egypt (1926-1962)
L 84	0	20	0	0	0	Personal data not available				
A 248	0	40	0	0	0	47	m.	47	no	no
G 2	0	20	0	0	0	56	f.	56	no	no
G 10	20	40	40	20	40	69	f.	5	no	no
G 11	0	80	40	0	0	50	f.	43	no	Austria (1944)
G 16	0	80	160	0	0	54	f.	24	no	no
G 28	80	1024	320	160	160	56	f.	45	no	Austria (1914-1918)
G 46	0	40	0	0	0	52	f.	14	no	no

+40 indicates that the titer of the serum was 1:40. 0 = no inhibition at dilution. 1:20, lowest used.

Table 3 - HI antibody titers against group B viruses found in 205 Fondi residents.

Serum No.	Antigen : 4 - 8 units							Time person has been living in Fondi(in years).	YF vaccine	Travel abroad
	TBE	WN	YF	De1	De2	Age	Sex			
7	0	20+	0	0	0	37	m.	14	no	no
21	0	20	0	0	0	44	m.	44	no	no
23	40	20	0	0	0	18	m.	18	no	no
56	0	20	0	0	0	54	f.	six months yearly	no	Germany
60	0	40	40	0	20	28	f.	28	no	no
93	80	20	0	0	0	40	f.	40	no	no
113	0	40	20	0	0	22	f.	22	no	no
117	20	160	160	80	40	57	f.	57	no	no
122	0	20	0	0	0	43	f.	43	no	no
154	0	20	20	0	0	29	m.	26	no	Austral.(1959-1962)
157	0	0	20	0	0	31	m.	31	no	no
161	0	20	0	0	0	48	f.	48	no	no
164	0	20	0	0	0	30	m.	30	no	no
174	0	20	0	0	0	58	f.	58	no	no
177	0	20	20	0	0	55	f.	55	no	no
185	0	20	20	0	0	44	f.	38	no	no
190	20	0	0	0	0	23	f.	23	no	no
269	0	40	0	0	0	66	f.	66	no	no
270	0	20	0	20	0	37	f.	37	no	no

+ 20 indicates that the titer of the serum was 1:20; 0 = no inhibition at dilution 1:20, lowest used.

Table 4 - Results of HI and NT tests in domestic and wild animal sera collected in Fondi region.

No.sera examined	No.sera reacting	Titers of sera reacting by HI test with arboviruses										Titers of sera reacting by NT test with arboviruses		
		Group A		Group B					Bunyamw.	Phlebotomus fever		WEE	TBE	WN
		Sindbis	WEE	TBE	WN	YF	De1	De2		Neap.	Sic.			
41(bovine)	13		40+									180++		
				40									50	
			40											
			20											
		20		20	20							56	50	
			20											
			20											
			20											
			20	20									50	
			20											
			20											
			40											
					80									22
25(goat)	17	40	40	320	40							56	≥320	22
			40	40								32	220	
			80	80								18	3,200	
		40	40	160	20	20							4,700	
			20			40								
		40	40	160	20	20						18	4,700	
		40	80		20							32		
				160									≥5,000	
				20									≥5,000	
				40									1,600	
					40									
			80		40			20						
				40									≥5,000	
					20									
					20									
				40				20					≥5,000	
				20									50	
15(Sheep)	1	20	40									5.6		
12(chicken)	0													
9(rabbit)	1				40	40		20						
2(pig)	0													
19(rat)	6						40							
					40		40	20						
							20							
					40									
					20									
					80									
30(wild mice)	1 pool of 6 sera				40									

+ reciprocal of serum dilution. ++ NI50.

Table 5 - Neutralization test with 23 human sera against TBE, WN and Sicilian Phlebotomus fever viruses.

Area of collection	Serum No.	TBE HI titer 1 :	TBE NI_{50}	WN HI titer 1 :	WN NI_{50}	Sicilian Phlebotomus fever HI titer 1 :	Sicilian Phlebotomus fever NI_{50}
Gorizia	L 2	neg.	-	40	3	neg.	-
	L 4	neg.	-	neg.	-	20	56
	L 12	640	≥32,000	neg.	-	neg.	-
	L 20	neg.	-	neg.	-	20	100
	L 24	20	10	640	4	neg.	-
	L 28	320	≥32,000	neg.	-	neg.	-
	L 30	neg.	-	160	4	neg.	-
	L 35	neg.	-	40	-	320	56
	L 39	neg.	-	20	4	neg.	-
	L 55	neg.	-	neg.	-	640	31
	L 63	neg.	-	neg.	-	320	100
	G 10	20	3	40	-	neg.	-
	G 67	neg.	10	neg.	-	neg.	-
	G 28	80	-	1024	1,000	neg.	-
Fondi	7	neg.	-	20	3	20	-
	23	40	≥32,000	20	-	neg.	-
	32	neg.	-	neg.	-	20	3
	60	neg.	-	40	3	neg.	-
	67	neg.	-	neg.	-	80	15
	93	80	4,700	20	-	80	-
	113	neg.	4	40	3	neg.	-
	117	20	-	160	3	160	3
	173	neg.	-	neg.	-	40	3
	190	20	≥ 320	neg.	-	neg.	-

neg. = no inhibition at dilution 1:20, lowest used.

REPORT FROM THE INSTITUTE OF HYGIENE
UNIVERSITY OF VIENNA, AUSTRIA

Investigations on the Ecology and Epidemiology of Tahyna Virus.

In 1965, field investigations on the circulation of Tahyna virus were carried out in two quite different areas in the east of Austria, namely, in the meadows along the Danube River near Fischamend (about 15 miles east of Vienna) and in the steppe-biotope east of the Neusiedlersee near the Hungarian border.

In Fischamend, a total of 143,720 mosquitoes was collected during the period May to October; from these, 109,440 individuals were tested for virus from which four strains of Tahyna virus were isolated on August 4 and August 25 from pools of unidentified mosquitoes and from a mixed pool of Aedes cantans and Aedes annulipes. In addition, one strain was isolated from the blood of a sentinel rabbit on July 5, thus indicating that rabbits actually act as vertebrate hosts during the summer. It was, however, not possible to demonstrate the incidence during May and June, although the potential vectors, i.e., the Aedes-species, reached their maximum of population density during the period.

In the area of Neusiedlersee, 30,550 mosquitoes were collected during the period from the last third of June to the last third of July; from these, 28,050 individuals were tested for virus, from which 26 strains of Tahyna virus were isolated from pools of unidentified mosquitoes, from mixed pools of Aedes caspius and Aedes dorsalis, from Aedes flavescens and from Taeniorhynchus richiardii. In particular, Aedes flavescens - hitherto unknown to transmit the virus - apparently plays an important role in the circulation of the virus. It was, however, not possible to isolate any virus strain from 8,000 hibernating female mosquitoes (mainly Culex pipiens) collected in cellars in the same area during the winter, thus indicating that the virus probably does not hibernate in mosquitoes.

Comparing the infection indices and the time of the incidence of the virus in the Neusiedlersee area with the data obtained in the meadows along the Danube River, it appears highly probable that the virus is regularly introduced from the Neusiedlersee to the regions flanking the Danube River and probably other rivers, but cannot be maintained there in permanent foci. As such regular introduction can neither be traced back to mosquitoes nor to any mammal-speices, and as birds apparently do not play any role in the circulation of the virus, it seems to be a reasonable assumption that Tahyna virus is spread over from the Neusiedlersee by man every year.

Fluorescent Antibody Studies.

Tahyna virus.

Identification of 24 of the virus strains isolated from mosquitoes was attempted with the direct fluorescent antibody technique in smears prepared from brains of baby mice. This method had already been applied to the differentiation of viruses of the California encephalitis group (Ch. Kunz, Zbl. Bakt., I. Orig., 198, 171-174, 1965). Nineteen strains were in the first, four were in the second and one was in the third baby mouse passage. The globulin fraction of three-injection mouse sera was labeled and used for this study. Out of 21 strains, which were stained with the Tahyna conjugate, the fluorescent antibody test was negative with only three strains. A California encephalitis conjugate failed to react with most of the antigens tested, whereas a Lumbo conjugate stained the virus-specific antigens in the smears with about the same intensity as the homologous conjugate.

CEE virus.

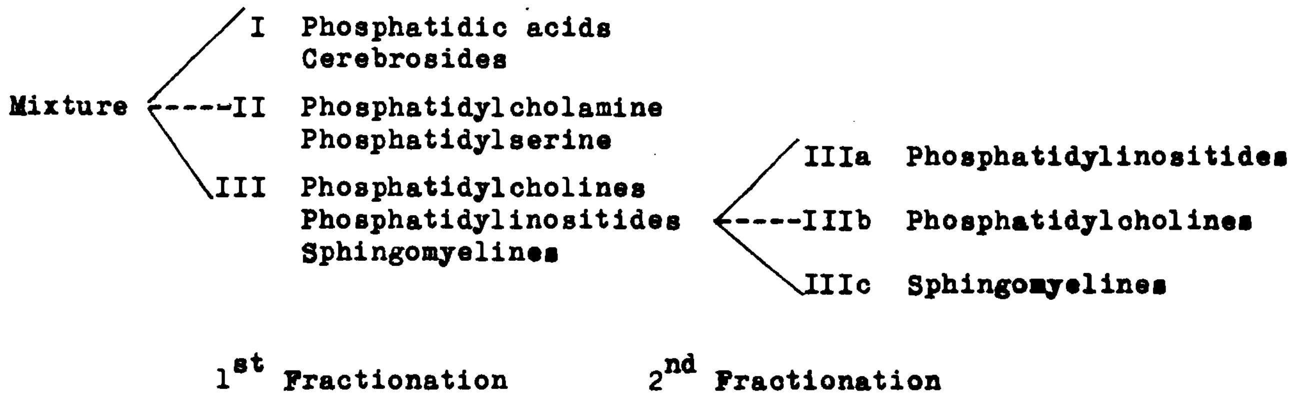
The fluorescent antibody test was also used for the identification of strains of TBE virus subtype CEE isolated from ticks. Whenever at least two mice of a litter, which was injected with material from ticks, showed evidence of a possible virus isolation (3-7 days p.inf.), one mouse was sacrificed and an imprint of the brain was stained with a CEE conjugate. Thus, all 45 CEE strains isolated during 1965 and 1966 were readily identified in the first baby mouse passage.

Receptor Substances for TBE Virus

A technique was developed to perform HI tests at the pH-optimum of the hemagglutination reaction of arboviruses. This allows one to test substances for their action as competitive inhibitors of this reaction. (W. Frisch-Niggemeyer, 1966).

Homogenates from mouse brains, when tested at pH 6.4, were more than 100 times as effective as inhibitors for the hemagglutination of TBE virus, subtype CEE, than when tested at pH 9.0. The active principle could be extracted with chloroform-methanol 2:1 and was found to be present in the alcohol soluble phosphatide containing fraction. For further fractionation of this lipid mixture, column chromatography on silica gel was applied. The chromatography was monitored by phosphorus estimation. The composition of the resulting fractions was investigated by two-dimensional thin layer chromatography.

By running two consecutive chromatograms on silica gel with different activities, the lipid mixture could be separated according to the following scheme:



None of the fractions was able to inhibit hemagglutination of TBE virus. However, the HI-capacity was not lost during preparation, because a mixture of all fractions was again active. By testing all possible combinations of our products, it was found that the phosphatidyl-inositides, together with phosphatidyl-cholines (lecithines) or sphingomyelines, were inhibiting the HA capacity of our virus preparation. A weaker action could be observed from the substances of peak I together with lecithines. The inositides, together with lecithines, were able to inhibit competitively the hemagglutination of TBE virus down to 0.05 ug/0.4 ml = 0.00001 %. Therefore, we regard this combination as a possible receptor substance for arboviruses.

(Drs. Christian Kunz, Horst Aspock and Walter Frisch - Niggemeyer).

REPORT FROM THE INSTITUTE OF VIROLOGY CZECHOSLOVAK ACADEMY OF SCIENCES BRATISLAVA, CZECHOSLOVAKIA

Isolation of Tickborne Encephalitis Virus from the Blood and Milk of Sentinel Pastured Goats.

Fourteen goats as sentinel animals have been pastured from May 25 to July 13, 1965 in a known natural focus of tickborne encephalitis in west Slovakia. Blood samples, milk and ticks from these goats have been collected for virus isolation experiments.

Two virus strains were isolated from the blood, one strain from the milk and two strains from different stages of *Ixodes ricinus* ticks. The isolated virus strains were identified in virus-neutralizing tests as tickborne encephalitis virus.

Three goats from 14 possessed virus-neutralizing antibodies against tickborne encephalitis virus (Table 1).

These findings show that under certain conditions, the goats can participate in the circulation of the tickborne encephalitis virus in the nature and that they can play a role in the infection of the men when drinking unboiled milk, as it was proved in previous laboratory experiments.

Table 1. Isolation of the TBE Virus from the Blood and Milk of Sentinel Pastured Goats

No of goat	Virus isolation from the			Neutralizing antibodies titre
	blood	milk	ticks	
7	+	0	+	1:32
9	+	+	+	1:16
11	0	0	0	1:64

Reference: Ernek, E., Kožuch, O., Nosek, J.: Isolation of the Tick-borne Encephalitis Virus from the Blood and Milk of Sentinel Pastured Goats in Tribeč Region, J. Hyg. Epid. Microb. Immun. /in press/.

Study on Vector of Tickborne Encephalitis (TBE) Virus.

The chief vector of TBE virus in Central Europe is Ixodes ricinus. However, the ticks Haemaphysalis inermis, Ixodes trianguliceps and Ixodes hexagonus can participate in the circulation of the virus under natural conditions. In the present study, experiments with Haemaphysalis inermis were carried out.

In May 1965 we succeeded in isolation of tickborne encephalitis virus from Haemaphysalis inermis ticks (Gresikova and Nosek 1966). The suckling mice inoculated intracerebrally with isolated strain exhibited hyperirritation, paresis, paralysis, and death in nine days in subpassages. The virus was reisolated from original material and subjected to identification procedures. Haemagglutination inhibition and complement fixation tests with tickborne encephalitis antiserum confirmed that agent isolated from Haemaphysalis inermis ticks is tickborne encephalitis virus.

Isolation of tickborne encephalitis virus from H. inermis ticks from nature showed that these ticks may be potential vectors for TBE virus. In view of this fact, the multiplication and transstadial transmission of TBE virus was studied. The larvae were infected on white mice. The titre of virus in H. inermis ticks was $10^{4.2}$ intracerebrally mouse LD_{50} per 0.01 ml. The premolting period from larvae to nymphs lasts 38 days, the prefeeding period of nymphae 35 days. The transmission of TBE virus by infected nymphae was proved. The virus was found in the blood of white mice on 3rd, 5th and 7th day after tick-bite. At the autopsy the virus was recovered from the brain. The titre of infected individual nymphs varied from $10^{3.6}$ to $10^{5.7}$ on 3rd day after feeding.

The results of interstadial virophory of larvae to nymphs of H. inermis ticks proved the validity of isolation experiments. On the grounds of this results H. inermis ticks can be considered as a vector of TBE virus in xerothermic regions predominantly in Southern Europe. (Ref.: Gresikova, M.; Nosek, J. 1966: Isolation of tickborne encephalitis virus from Haemaphysalis inermis ticks, Acta. virol. in press).

REPORT FROM THE VIROLOGICAL DEPARTMENT
RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY
BRATISLAVA, CZECHOSLOVAKIA

The multiplication of Tahyna virus-extraneural variant of 236 strain in cell cultures used for neuroadapted variants up to the present was not accompanied with cytopathic effect. In recent experiments, where the African green monkey kidney cell line

(GMK-AH-1) was used, (this cell line wad developed in the Research Division of Infectious Diseases, Boston and was kindly supplied by Dr. A. Swedmyr, Stockholm), the extraneural variant of Tahyna virus showed complete cytopathic effect which was inhibited in the presence of hyperimmune anti Tahyna serum. In parallel titrations in GMK cells and in i.c. tests in infant mice, comparable titre values were observed. The cytopathic changes appeared regularly in successive virus passages in this culture. Virus titre in medium 48 hours after inoculation showed values 5.5 - 6.5 log, CPD_{50}/ml.

Fluorescent antibody technique was also successful in demonstration of virus antigen of extraneural variant of Tahyna virus in GMK cells. At low input, multiplicity of infection (Mi = 0.3 CPD_{50} per cell), the viral antigen was detected as soon as 24 hrs. post infection (p.l.) as brightly fluorescent granules in the cytoplasm of 30-50% cells. The nuclei were negative. Titre of infectious virus in the medium at this time was 5.5 log. CPD_{50}/0.1 ml and titre of cell-associated virus was about 4.7 CPD_{50}/0.1 ml. All infected cells showed fluorescent antigen in their cytoplasma 48 hrs. p.i. At this time also the degeneration of the cells and their separation from the glass wall into medium began.

The neuroadapted strains of Tahyna virus in GMK cells were also successfully cultivated with regular CPE.

The susceptibility of the diploid cell strain LEP_{13} derived from the human embryo lungs (Institute of Sera and Vaccines, Prague) to infection with the Tahyna virus was studied. The multiplication of virus line of the "92" strain adapted to the chick embryo cell primary cultures (92 CEC_{50}) was accompanied by a cytopathic effect, the titres CPD_{50} were 10-100 times lower than the CPD_{50} titres obtained in chick embryo cell primary cultures. The neuroadapted line 92 M_{40} and the neuro-adapted variant of 236 strain (236 M_{17}) of Tahyna virus showed cytopathic activity only at low virus dilutions corresponding to a high multiplicity of infection (10-100 LD_{50} per cell). No cytopathic activity was observed with the extraneural variant of the 236 strain (236 H_8) of Tahyna virus. The kinetic study of reproduction of these virus strains in LEP_{13} cells revealed that virus formation reached the maximal values between 24 -48 hours after the inoculation of cell culture with both lines of the 92 strain of the Tahyna virus, when the multiplicity 1-10 LD_{50} per cell was used. The neuroadapted variant of the 236 strain and the extraneural variant of 236 strain also multiplied well in human diploid cell strain, when the multiplicity of infection 0.01 - 10 LD_{50} per cell was used.

The susceptibility of some strains of mice (A/L, CBA, C_3H, CFW, CF_1, $C_{57}BL$, BALB.C, DBA-1, DBA-2, AsW - inbred, H "specific

pathogen free" and H conventional random bred to the virus Tahyna was studied on the basis of the dynamics of their decay after inoculation (IC, IN, SQ) of two different variants of the virus. There was no significant difference in the susceptibility to both of the "neuro-adapted" and "extraneural" variants of the 236 strain of Tahyna virus among these strains of inbred and random bred mice. In all strains of inbred mice was found, in contrast to the random bred mice, a parallel dependence on the amount of virus inoculum, the incubation period which testifies to the homogenity of inbred mice groups, and to the greater suitability of using them in experiments. Viremia in mice (CBA, A/L, C_{57}BL - inbred mice, H "specific pathogen free" and H conventional - random bred mice) was followed after IN inoculation of two variants of the Tahyna virus. It was found that after inoculation of the "extraneural" variant of the 236 strain of Tahyna virus, viremia persisted longer than after the application of "neuroadapted" variant of the same virus. No difference was found among the strains of mice used.

In the study of experimental Tahyna virus infection in suckling mice by the fluorescent antibody technique, the following results were obtained. No viral antigen was detected in kryostat sections at 24 hrs. post subcutaneous inoculation of 1.5 - 5.7 log. LD_{50} of extraneural variant of Tahyna virus. Forty-eight hours after p.i., specific antigen, demonstrated by immunofluorescence, was found in nervous cells of the brain and medulla spinalis, in special sensory nerve cells in the retina and the tongue, in intervertebral spinal ganglia, in intramural ganglia of the alimentary tract - plexus myentericus Auerbachi, and in the muscle fibres of oesophagus below the diaphragm. Scattered specific fluorescence was regularly found in the striated muscle fibres in the whole body and in the renal tubular epithelium in the subscapular part of the kidney. The small islets of positive cells were detected irregularly in the adventitia of some vessels in the heart and muscles of the neck, and sometimes in the muscle of the myocardium. Values of infectious virus titres in organs and blood examined were dependent on the quantity of inoculated virus.

When an approximately equal dose of neuroadapted variant of the same strain of Tahyna virus was inoculated subcutaneously, the specific fluorescence was found after 48 hrs. in the nervous cells of the brain and spinal cord. The small islets of viral antigen were irregularly detected in the striated muscles of the back.

For the study of experimental pathogenesis in primates - Macaca rhesus, Macaca radiata monkeys and chimpanzees, the extraneural variant of Tahyna virus strain 236 was used. Eighteen rhesus monkeys (M. rhesus) were exposed to various virus aerosol concentrations. None of the monkeys showed signs of disease, no virus could be demonstrated in either blood, in nose mucosa membrane

or in tissues from these animals at any time during the course of experiments. Virus neutralizing and complement fixing antibodies were not detected in any of the monkeys during three months after the virus inhalation. Seventeen rhesus monkeys (M. rhesus and M. radiata) and five chimpanzees were inoculated subcutaneously with doses varying from 2.7 to 7.7 log i.c. mouse LD_{50} of virus. In each animal viremia was detected, the maximal level of virus was 1.5 to 3.5 log per 0.03 ml of blood. The duration of the viremia (one to seven days) and the antibody responses were dependent on the size of virus inoculum. While the M. rhesus and M. radiata monkeys showed no signs of clinical symptoms during the course of infection, in all chimpanzees, fever, and in two chimpanzees, acceleration of the red bloods cells sedimentation, were observed.

(V. Bardos, A. Simkova, L. Sefcovicova, E. Cupkova, Z. Wallnerova, I. Schwanzerova and V. Schwanzer.

REPORT FROM THE INSTITUTE OF VIROLOGY
SCHOOL OF VETERINARY MEDICINE
HANNOVER, W. GERMANY

Studies on the Inactivation of a Group A Arbovirus (Sindbis) by Formalin and Hydroxylamine.

Sindbis virus was grown in chick embryo tissue cultures. The medium used for virus multiplication consisted of Earle's solution supplemented by 0.5% Lactalbumin hydrolyzate and 0.1% yeast extract; serum was omitted. Media of virus-infected tissue cultures were treated either with formalin (Formaldehyde 35% w/v) or hydroxylamine (NH_2OH). Figure 1 demonstrates that inactivation of Sindbis virus by formalin follows first-order kinetics only, if the formalin-concentration is 0.3% and if inactivation occurs at pH 8.8 From this result it can be concluded that - in the Sindbis virus system - inactivation according to a first order reaction depends to some degree on the concentration of formalin and to a great part on the pH at which inactivation occurs.

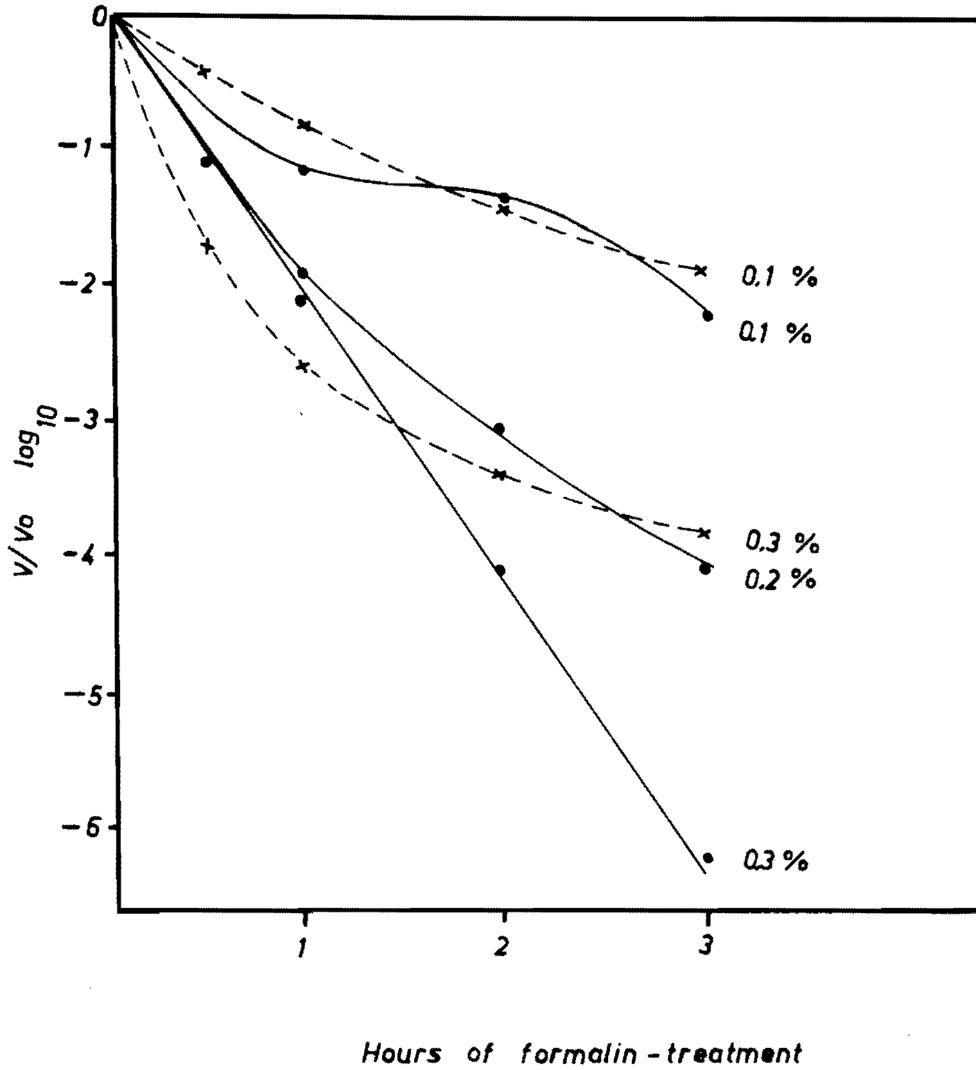
The next substance tested was hydroxylamine. According to Schuster, inactivation of RNA viruses by hydroxylamine may be explained in the following way: a) at pH 6.0, a two-step reaction probably occurs; one hydroxylamine molecule adds to the 4,5 double bond of cytosine (saturation of the 4,5 double bond probably favors formation of the imino groups at C-6), then, a second hydroxylamine molecule substitutes the imino group at C-6, yielding 4,5 hydroxylamino-N6-hydroxycytosine. This alteration leads to mutations. b) At pH 9.1, hydroxylamine treatment results in the splitting of the base uracil. This has a lethal effect.

In our studies with Sindbis virus, we found no difference in the inactivation of Sindbis virus at pH 8.7 and pH 6.3, and it was demonstrated - as shown in Figure 2 - that inactivation at hydroxylamine concentrations of 1.0 m, 0.5 m and 0.25 m followed first-order kinetics. The inactivation curve of 0.5 m hydroxylamine corresponded to that of a 0.3% formalin concentration at pH 8.8.

These results may be of some value in connection with questions which arise in the development of killed arbovirus vaccines. It would be interesting to know, whether similar inactivation curves are obtained with other arboviruses, and whether there are differences in the potency of vaccines prepared either by treatment with 0.3% formalin at pH 8.8 or 0.5 m hydroxylamine at pH 6.8 or 8.7 - 9.0.

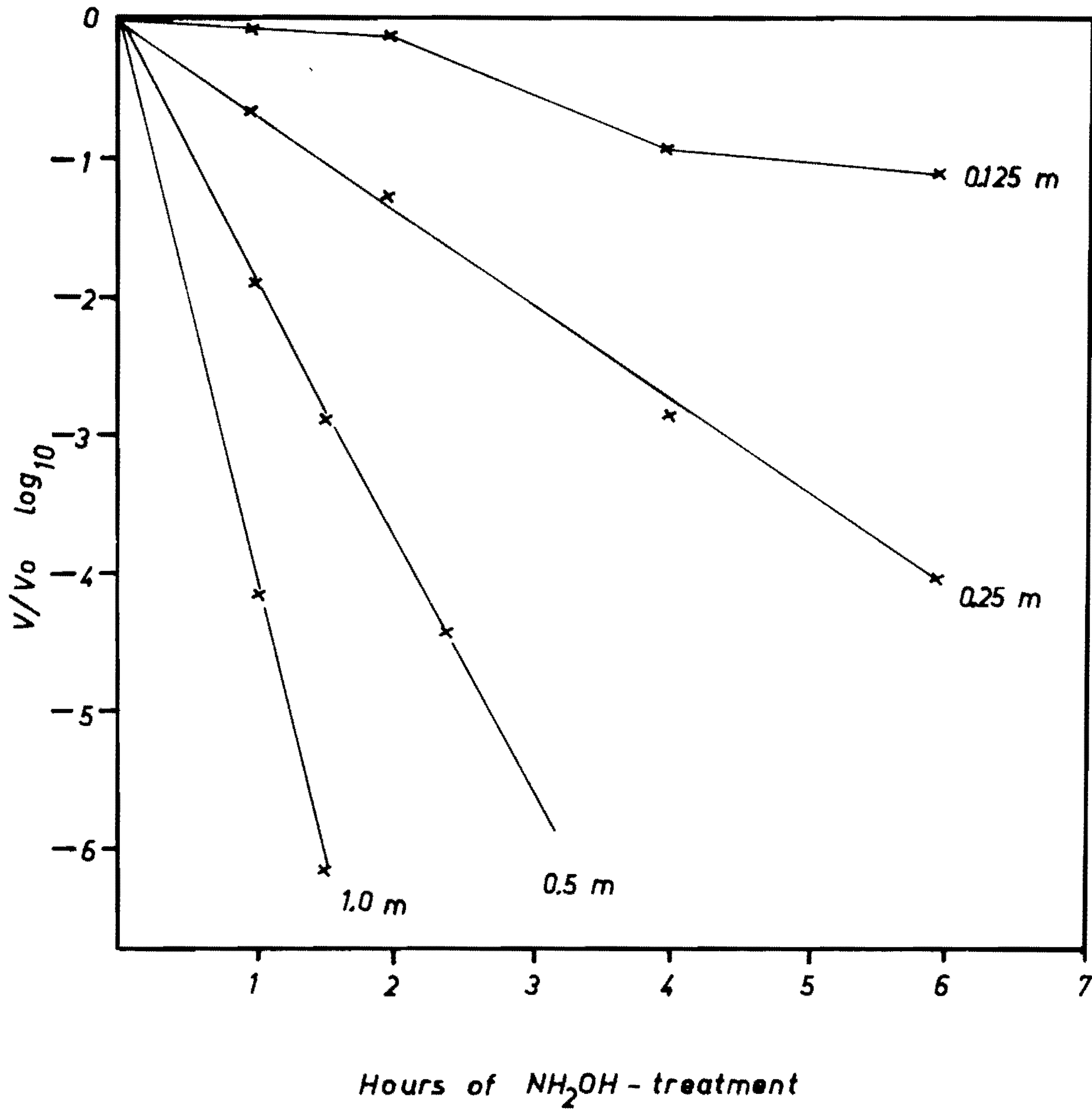
(M. Mussgay).

Figure 1



Inactivation of Sindbis virus by formalin-treatment at 22° C. •—• at pH 8.8; x—x at pH 7.0. Concentration of formalin in the reaction mixture was 0.3, 0.2 and 0.1 per cent, respectively. V/Vo is the ratio of final to initial plaque count.

Figure 2



Inactivation of Sindbis virus by NH_2OH-treatment at pH 7,0 and 22° C. Concentration of NH_2OH in the mixture was 1.0 m, 0.5 m, 0.25 m and 0.125 m, respectively. V/Vo is the ratio of final to initial plaque count.

REPORT FROM THE VIRUS DEPARTMENT OF THE CENTRAL BACTERIOLOGICAL LABORATORY STOCKHOLM CITY, SWEDEN

Elks (*Alces a. alces*) from the endemic region of southeastern Sweden have neutralizing antibodies to TBE virus strikingly often than have domestic cows. A strain of TBE virus was isolated from the brain and blood of an encephalitic elk calf. (A. Svedmyr, G. von Zeipel, K. Borg and H. J. Hansen: Acta path. et micribiol. scandinav. 1965, 65:613).

Investigations of birds trapped at the Ottenby Bird Station on the iland of Oland in the Baltic Sea demonstrated that ticks are carried by numerous bird species, particularly Passeriformes. The resident bird population as well as birds undertaking migration were found to be infested. So far infestation of birds undertaking long-range migration was made probable only for birds coming in from the south in spring. The tick species most commonly encountered was *Ixodes ricinus*, while *I. arboricola* and *Hyalomma marginatum* were occasionally represented, the last mentioned in but a single case.

Infestation rates during autumn migration were: in early August, 0% (62 birds of 7 species examined), in September 8.8% (136 birds of 14 species), and in October 5.4% (93 birds of 11 species). In most cases only one or a few ticks were taken off each bird. The maximum numbers were as follows: 49 *I. ricinus* on a Blackbird, 54 *I. arboricola* and 13 *I. ricinus* on a Starling and 65 *I. ricinus* on a Blackbird. Only larval and nymphal ticks were encountered, except 1 ♀ of *I. arboricola*.

Examination of blood samples to demonstrate presence of tickborne encephalitis virus was negative and only one of 291 serum samples neutralized this type of virus; it was collected from a Garden Warbler (*Sylvia borin*). The individuals of this bird species passing Ottenby on their way to tropical and southern Africa originate from the Baltic Islands on the coastal areas around the northern and central Baltic Sea.

A series of investigations on experimental infection with TBE virus in small wild rodents has been performed (G. von Zeipel, Z. Heigl):

The pathogenicity, following subcutaneous inoculation of two TBE virus strains, one never being brain passed, was tested in a study involving 177 wild rodents of the following species: *Cl. glareolus*, *A. flavicollis*, *A. sylvaticus* and *M. musculus*. Most animals were inoculated with a dose of about 100 s.c. LD_{50} for white mice.

In the majority of the animals of all species the infection was inapparent. The etiology of three cases of paralysis is discussed, as well as the cause of death in 27 animals. Ten of which succumbed during exposure to cold, and twelve in direct connection with anesthesia and blood sampling with no other obvious cause.

The presence of TBE virus was almost always demonstrated in the brain of animals that died from one to three weeks after inoculation of virus.

The duration of viremia based on titration of plasma was found to last for five to nine days, the shortest period being recorded in A. sylvaticus and the longest in M. musculus. Virus could, however, be recovered from extracts for a still longer time. High virus titers of the blood were recorded in animals of all species, the maximal values per 0.02 ml being $10^{3.3}$ in A. flavicollis, $10^{4.1}$ in A. sylvaticus, $10^{4.5}$ in Cl. glaerolus and $10^{4.7}$ in M. musculus. The mean titers of viremia were, however, higher in M. musculus and Cl. glareolus than in the Apodemus species.

The species A. flavicollis, A. sylvaticus and M. musculus were infected by about one subcutaneous LD_{50} unit of virus for white mice.

The antibody response in the four species of wild rodents was followed over a period of six weeks after the subcutaneous inoculation of TBE virus.

The response was similar whether the animals were kept at room temperature or at +4° C.

A total number of 19 Cl. glareolus, 27 A. flavicollis, 31 A. sylvaticus and 45 M. musculus were examined during this experiment.

The quantitative evaluation by neutralization (NT), complement fixation (CF) and hemagglutination-inhibition (HI) tests of blood samples drawn serially from each of the animals, revealed significant differences in antibody titer between the species. Generally, the lowest values were found in A. sylvaticus, the highest in Cl. glareolus and M. musculus. A. sylvaticus and M. musculus could be infected with about one s.c. LD_{50} for white mice, the subsequent antibody response being similar to that of animals receiving 300 LD_{50}.

Neutralizing antibodies were demonstrated in all animals of the four species, CF antibodies in all but 30% of A. sylvaticus. A varying proportion of animals belonging to the three species other than M. musculus did not develop HI antibodies under the

conditions of the tests. This, together with a subsequent drop of the CF and HI titers, occurring mainly within the species A. sylvaticus, A. flavicollis and Cl. glareolus, resulted in a high proportion of such animals negative in these tests six weeks after inoculation of virus. By that time the approximate frequencies of animals negative in CF and HI tests were as follows:

A. sylvaticus, CF 63%, HI 79%; A. flavicollis, CF 30%, HI 19%; Cl. glareolus, CF 5%, HI 21%. There was no significant drop of NT titres over the time intervals studied, i.e. up to 110 days.

The NT reaction, being a reliable test for past infection with TBE virus in these rodents, appears to be the method of choice for most ecological work.

REPORT FROM THE BACTERIOLOGY DEPARTMENT PRINS LEOPOLD INSTITUUT VOOR TROPISCHE GENEESKUNDE ANTWERPEN, BELGIUM

The program on the search of possible latent infections in animals after experimental inoculation of arboviruses has been stopped after only negative results were obtained.

These experiments are summarized briefly. Animals were injected s.c. with arboviruses, and after intervals some of their organs tested for the presence of virus by two methods:

a) "apparent" virus was detected by inoculation of organ suspensions into mice or tissue cultures;

b) "latent" virus was looked for by establishing tissue cultures of these organs and subinoculation of the tissue culture maintenance fluid, at each change, if possible during a period of nine months, into mice or baby mice.

The following combinations were tested:

Newborn chick - Semliki Forest virus - tissue cultures of kidney and spleen at days 10, 16, 23, 30, 37 after virus injection. Cultures from crop performed at days 10 and 24 also remained negative for virus.

Newborn chick - Sindbis virus - 10, 14 days after virus injection.

Newborn chick - RSSE - viremia lasts 9-10 days. At days 11 and 14 "apparent" virus was detected in some organs. Tissue cultures

started at days 28 and 43 remained negative for virus.

Newborn chicks - West Nile virus: no virus obtained in cultures started at days 12 and 26.

Newborn chicks - EEE: from a few surviving animals tissue cultures were performed at days 9 and 22 without appearance of virus.

Newborn chicks - Uruma: no virus obtained from tissue cultures of kidney at days 15, 30, 50.

Newborn chicks - Getah: no virus obtained from kidney tissue cultures.

Rats - RSSE: no virus obtained from kidney tissue cultures at days 16, 23, 30.

Rabbits - RSSE: no virus obtained from kidney and spleen tissue cultures at days 8 and 15.

Hedgehogs - RSSE: no virus obtained from kidney tissue cultures.

Australian authors have since found latent infections in about 5% of inoculated baby chicks. Our negative findings may be the results of using too small numbers of animals since we inoculated chicks in groups of 10-12.

The study of the small and large plaque variants of Middelburg (Group A) virus was completed (Strain AR 749).

The following conclusions could be drawn:

Agar (Difco Noble) contains substances which act in this system at three different levels:

a) on the virus: MB-s virus is partly and immediately inactivated by agar-extract;

b) on the cell virus union: Agar-extract inhibits MB-s virus adsorption of CETC cells. This action is immediate and increases with time of action of agar extract. After removal of agar extract, the cells are able to "recover".

c) on virus synthesis: MBl virus is synthesized in increased amounts in the presence of agar.

Agarose (Industrie Bioligique Francaise, Gennevilliers, France) was not free of inhibiting substances versus MBs virus. Behaviour in embryonated eggs and newborn chicks was not significantly different. In newborn mice MB-s virus constantly produced titers that were 1-2 logs lower than MB-1 virus.

From strain AR 2196 small and large plaques could also be obtained in pure culture.

The effect of dextran incorporated in agar and agarose overlay, is now being quantitatively investigated upon several arboviruses and their small and large plaque variants.

During our work with MB virus on CETC, we compared the following media for virus adsorption on CE monolayers in petridises: Hanks BSS, Hanks BSS + 1% bovine albumin, Hanks + 10% calf serum and BGT (buffered gelatin tris solution).

Adsorption in BGT produced the highest number of plaques. In view of these results, the effect of gelatin containing media was studied on virus and CPE production of some arboviruses in CETC tubes.

In tubes containing 2% gelatin in the maintenance medium, higher virus titers, respectively 0. 5 to 1.5 log, were obtained in the case of chikungunya, Mayaro, Uruma and Getah-viruses. CPE was definitely enhanced in the cases of chikungunya, Mayaro and Getah viruses.

Unfortunately gelatin was without effect in CETC inoculated with Group B, RSSE and dengue viruses.

The technique described by J.O. Mason *et al* of induced feeding of mosquitoes through the presence of ATP was successful after some initial difficulties. We concluded that ATP by itself is not sufficient to induce artificial feeding but that temperature conditions are also important.

We now work currently in a room maintained at 24° C, 85-90% humidity, the fluid presented for feeding being maintained by a heating device at about 35-37° C.

Virus is suspended in Hanks-lactalbumin solution with 20% pooled human serum. ATP is incorporated at a concentration of 0.005 M. Mosquito feeding is obtained in 80-90% of female *A. aegypti* in 30 minutes. Preliminary experiments with SF-virus have been done.

(S.R. Pattyn).

REPORT FROM THE PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER RIO DE JANEIRO, BRAZIL, AND THE ANIMAL VIRUS RESEARCH INSTITUTE, PIRBRIGHT, WOKING, SURREY, ENGLAND

Vesicular Stomatitis Virus: Relationship between Strains of the Indiana Serotype.

Recently there have been recovered from the field a number of strains of vesicular stomatitis virus showing some relationship to the classical Indiana serotype and completely distinct from the New Jersey serotype. The three strains which have been examined and compared with Indiana C are as follows:

1. Cocal virus (Jonkers, Shope, Aitken and Spence, 1964).
2. Argentina (Salto) (Garcia Pirazzi, Gaggiano and Fernandez, 1963), associated with an outbreak in horses in the Province of Buenos Aires.
3. Brazil (Alagoas) (Andrade, Rozas, Ferreira dos Santos, Teixeira and Porfirio da Mota, personal communication, 1964), from an outbreak in mules, with associated cases in horses and cattle. In this outbreak a number of plantation workers were affected with fever, headaches and malaise and their sera were shown to contain strain-specific antibodies.

Complement fixation tests and cross neutralization tests demonstrated that the Argentina and Cocal strains could not be differntiated one from the other but each differed from the classical Indiana strain, Ind. C, to the same degree as from the Brazil strain. The Brazil and Ind. C strains were the most widely different of the pairs examined.

The Cocal virus was shown to be pathogenic for cattle and pigs, although the disease produced was not so severe as with the other strains. Electron microscopy has demonstrated the bullet-shaped particle characteristic of vesicular stomatitis virus in tissue cultures infected with all three strains described.

On the basis of the serological findings, it is considered that these strains should be classified as subtypes of the Indiana serotypes rather than as new types of vesicular stomatitis virus. It is proposed that they are classified as Indiana 1 for the classical strains, Indiana 2 for the Argentina and Cocal viruses, and Indiana 3 for the Brazil virus. The difference between these strains is sufficient to make it advisable to use subtype specific serum in screening samples for the identification of vesicular stomatitis.

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Garcia Pirazzi, A.J., Caggiano, G.H., and Alonso Fernandez, A. (1963). Publicacion Tecnica No. 2, CANEFA, Ministry of Agriculture, Buenos Aires, Argentina.

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Personal communication.

Table 1. Results of Complement Fixation Tests using Constant Antigen and Variable Serum Dilutions in the Examination of the Cocal, Argentina and Brazil Strains of Vesicular Stomatitis Virus

Serum	Virus and year of isolation							
	Cocal 1961	Argentina 1963	Brazil 1964	Indiana USA 1959	Indiana Mexico 1952	Indiana Venezuela 1962	Indiana Ecuador 1961	New Jersey Costa Rica 1961
Cocal	1/200*	1/250	1/10	1/25	1/20	< 1/10	< 1/10	< 1/10
Argentina	1/180	1/100	<1/10	1/10	1/15	1/10	< 1/10	< 1/10
Brazil	<1/10	<1/15	1/320	1/10	1/15	1/10	<1/10	< 1/10
Indiana USA	1/10	1/10	1/10	1/350	1/200	1/400	1/300	< 1/10
Indiana Mexico	1/15	1/10	1/40	1/80	1/120	1/200	1/160	<1/10
Indiana Venezuela	<1/10	<1/10	<1/10	1/40	1/40	1/280	1/120	<1/10
Indiana Ecuador	1/15	1/15	1/10	1/180	1/150	1/250	1/300	<1/10

* Serum dilution endpoint

Table 2. Results of Virus Neutralization Tests in Young Adult Mice (Intracerebral Inoculation) using Constant Serum and Variable Virus Dilutions in the Examination of the Cocal, Argentina and Brazil Strains of Vesicular Stomatitis Virus

Serum*	Virus and year of isolation							
	Cocal 1961	Argentina 1963	Brazil 1964	Indiana USA 1959	Indiana Mexico 1952	Indiana Venezuela 1962	Indiana Ecuador 1961	New Jersey Costa Rica 1961
Cocal	> 5.0**	> 5.0	2.0	3.75	3.25	3.25	3.0	1.0
Argentina	4.5	> 5.0	2.75	4.0	4.0	3.5	3.25	1.25
Brazil	1.75	1.75	4.0	1.5	2.25	2.0	2.25	0.5
Indiana USA	2.25	2.5	1.5	4.5	4.5	4.25	4.0	1.0
Indiana Mexico	2.0	2.0	1.0	4.75	4.5	4.25	4.0	0.7
Indiana Venezuela	3.25	2.5	1.75	4.75	4.75	4.25	4.0	1.25
Indiana Ecuador	2.5	2.5	2.0	5.0	4.5	4.5	4.25	1.25

* Initial serum dilution 1 in 10

** Virus neutralization index

SPECIAL NOTICE

Change of Address for Communications To the Information Exchange

In view of the current move of the editor to Los Angeles, to become Professor of Infectious and Tropical Diseases, all further correspondence, inquiries, communications, and contributions relating to the Arthropod-borne Virus Information Exchange should be addressed to:

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