ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number Thirteen March 1966

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REPORT OF THE CHAIRMAN OF THE SUBCOMMITTEE ON ARBOVIRUS INFORMATION EXCHANGE

Since the Special Report (September 1965) sent out with the No. 12 issue of the Information Exchange, the number of Catalogues mailed out has increased to 121; 51 within the continental United States and 70 to foreign lands representing 40 countries.

The total of registered viruses in the Catalogue is now 181.

The number of abstracts and personal communications that have been reproduced on 3 x 5" slips, coded and distributed for inclusion in the current information file now totals 3,339.

It is the intention to distribute another "Special Report" on the Catalogue with the next (fall) issue of the Infoexchange.

REPORT FROM SUBCOMMITTEE ON BIRDS AND OTHER VERTEBRATES IN RELATION TO ARBOVIRUSES

Since our last report, the subcommittee met March 14, 1965 at Tampa, Florida in conjunction with the annual meeting of the American Mosquito Control Association, where subcommittee members participated in a panel discussion regarding birds and other vertebrates in relation to arthropod-borne viruses. Many favorable comments were received following the panel. The panel topics and the following discussion provided the American Mosquito Control Association vector control workers the opportunity to obtain information they desired on vertebrate host-arbovirus relationships.

Additional progress has been made towards publication of the manuscript entitled, "Relations of Birds to Arboviruses". The manuscript was revised, up-dated, and resubmitted to the editor of The Auk. The paper was published in the January 1966 issue of The Auk. Reprints may be requested from the Communicable Disease Center, Atlanta, Georgia 30333.

The March 1965 subcommittee meeting discussed the ACAV Executive Council proposal that the subcommittee itself act on the recommendation made last year regarding formulation of a report on standard procedures for collecting and processing field specimens for

laboratory tests. It was agreed that the survey should be undertaken by arbovirus laboratory workers rather than by field biologist. Therefore, no action was taken on the Executive Council proposal.

The subcommittee is currently considering the possible use of grant funds to support graduate students, or others, interested in analyzing Fish and Wildlife Service bird banding data in relation to bird migration and arbovirus dissemination. The possibility of grant support for other subcommittee activities is also being investigated.

(Subcommittee Members: Drs. D. E. Davis, R. W. Dickerman, R. R. Lechleitner, R. J. Newman, M. W. Provost, C. B. Worth, R.O. Hayes)

EDITORIAL NOTE

The following notice is reproduced with regrets and extension of sympathy to the colleagues of the late Professor Hans Moritsch.

In deep sorrow we send news that

Univ. Prof. Dr. Hans Moritsch
Ordinarius for Hygiene at the
University of Vienna
Director of Institutes of Hygiene
of Univ. of Vienna

has left us forever on November 12, 1965.

We mourn an always kind and just director who stood helpfully by the side of his co-workers.

The funeral of the dear desceased takes place on November 18, 1965 at Well Cemetary, Vienna XXIII. The Mass of the Holy Soul will be celebrated on November 19, 1965 in the Antonius Chapel of Holy Trinity Church.

The Co-workers of Institutes of Hygiene.
Vienna

REPORT FROM PUERTO RICO NUCLEAR CENTER
SAN JUAN, PUERTO RICO

The Puerto Rico Nuclear Center, which is operated for the U.S. Atomic Energy Commission by the University of Puerto Rico, has been studying the effects of gamma radiation from a stationary 10 kilo curie Cesium 137 source on a tropical mountain rain forest. The study area consists of two adjoining 160 m diameter circles, at the center of one of which the source was located and exposed for three, one-month periods, with two 24 hours "down" periods to allow access to the irradiated area to make observations which could not be achieved by telemetric methods. Part of the project consisted of a program directed towards an investigation of natural virus cycles and any changes which might result in them during or immediately following the irradiation.

Although we were interested in all viruses, our facilities are limited, and the program was directed towards arthropod borne animal viruses and any others detectable by the standard procedures employed in arbovirology. Preliminary study of the area allocated for study (in a Federal Forest Reserve) revealed that birds were so few in numbers that they were unlikely to be of any importance in virus cycles, and the only mammal present in any numbers was Rattus rattus. There are several species of bats reported from the area, but as rabies occurs in Puerto Rico and we did not have the services of anyone experienced in trapping and handling bats, no attempt was made to study them.

The virus program was activated in August 1963, the source was exposed from January 19 through April 24, 1965, and the observations reported here continued until January 31, 1966. In this period 12 CDC miniature light traps were set on 181 nights and caught a total of 19,695 female mosquitoes, the distribution by species is shown in Table I. Havahart rat traps were set weekly; prior to radiation, ninety traps were used, during radiation seventy and after radiation one hundred forty traps were set. At the time of radiation those trap lines outside the irradiated areas were augmented with a further fifty traps.

On 130 trap nights a total of 615 Rattus rattus were caught 1,807 times. From them 733 blood specimens were obtained for attempted virus isolation (diluted in 5 volumes of B.P.A.) and 400 for HI testing (diluted in 5 volumes of B.S.B.). No viruses were obtained by inoculation of infant mice (CF1) with either mosquito suspension or rat material (serum or occasional tissue specimens) until February 11, 1965, but between then and October 31, 1965, 41 strains of virus were recovered from rat serum, 1 from brain, 1 from liver, 1 from leg muscle and 3 from mosquito suspensions.

All these were strains of Cox-A10, our prototype being identified for us at CDC.

No arbovirus was recovered, although 14 of the 400 rat sera tested by HI reacted with antigens from St. Louis and Murray Valley encephalitis viruses, one reacted with Dengue III. One to Chikungunya, one to California encephalitis and one to Tahynia - the latter four were all minimal reactions to single antigens. The HI results are set out in Table II and the virus isolations by month and origin in Table III.

One interesting finding in the mosquito fauna is that although Aedes aegypti cannot regularly be trapped using light traps or human, rodent, or avian bait, they can readily be reared from ova deposited in "artificial tree holes". We designed these to attract the tree hole breeders to lay in them so that we might assemble adequate numbers of Strontium 89 tagged adults to release for flight range and pattern studies. This is, as far as we have been able to ascertain, the only instance in which A. aegypti have been recovered from a New World forest, although a virtually identical situation to that described above was obtained in East Africa.

(Dr. Paul Weinbren)

Table I

Mosquitoes caught in 12 CDC miniature light traps on 181 nights at El Verde study area.

Species	
Aedes (Finlaya) mediovittatus (Coquillett)	804
Aedes (Ochlerotatus) taeniorhynchus (Wiedemann)	63
Aedes (Ochlerotatus) serratus (Theobald)	145
Culex (Culex) nigripalpus Theobald	5,422
Culex spp.	12,077
Wyeomyia spp.	34
Uranotaenia lowii Theobald	68
Mansonia (Mansonia) flaveolus (Coquillett)	1,042
Anopheles spp.	35
Aedes (Stegomyia aegypti (Linnaeus)	5

Table II

Positive reactors in HAI tests on rodent sera from El Verde - corrected to 4 units. Serum treated with Kaolin and goose cells.

No.	EEE	WEE	VEE	Chikungunya	SLE	MVE	Ilheus	Langat	Dengue II	Dengue III	Caraparu	Oriboca	Catu	Bunyamwera	Cache Valley	California	Guaroa	Tahynia
161				20														
21										20								
188																20		
189																		20
317					80	80												
318						40												
326					80	20												
327					80	20												
328					80	20												
330					20	80												
337					40	20												
338					80	20												
343					80	20												
345					80													
346					40	20												
351					40	80												
377					80													
378					20													

Table III

Sources of Coxsackie A10 Virus from El Verde Field Station - 1965.

Month	No.	Source
January	0	
February	1	Rat Serum
March	2	Rat Serum
April	2	Rat Serum
May	2	Rat Serum
June	3	Rat Serum
	1	Rat Brain
July	12	Rat Serum
	1	Rat Liver
August	12	Rat Serum
	1	68 *Culex spp.*
	1	14 *Mansonia flaveolus*
September	4	Rat Serum
	1	Rat Muscle
October	3	Rat Serum
	1	62 *Culex spp.*
November	0	
December	0	

Table III

Month	No.	Rat Serum	Organ	Mosquito
January	0			
February	1	1		
March	2	2		
April	2	2		
May	2	2		
June	4	3	1 Brain	
July	13	12	1 Liver	
August	14	12		2
September	5	4	1 Muscle	
October	4	3		1
November	0			
December	0			

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

In the early part of 1965 we were all very busy finishing the new virus building which was inaugurated on May 7, 1965. The move and the settling in the new quarters took considerable time, and somewhat delayed current investigations.

Dr. Halldor Thormar from Iceland joined our staff on August 1, 1965, succeeding Dr. R.F. Sellers.

Dr. Albo Saturno returned in January 1965 from two years training at Yale with Dr. J. Henderson and Dr. G.D. Hsiung, and was put in charge of all serological investigations of this department. He prepared hemagglutinating, complement fixing and

neutralizing antigens against some 30 arboviruses as well as 10 different immune sera. One hundred twenty eight human and some animal sera were checked for presence of antibodies against 13 different antigens of group A, B, and C viruses.

The mosquito survey in Venezuela carried out in part as our contribution to the National Commission for the Study of VEE and Other Arboviruses, commenced its operation under the direction of Dr. O. Suarez, with generous help in personnel of the "Direccion de Malariologia". Some 56,000 mosquitoes were collected; of which about 1,100 were classified. Steps are being taken to train personnel for classification of mosquitoes. Dr. O. Suarez is also participating in a survey of mosquitoes in Venezuela assisting Dr. J.N. Belkin of the University of California at Los Angeles in his survey of mosquitoes of central and northern South America.

Four viruses from the Bunyamwera group, that is Cache Valley, Kairi, Guaroa and Wyeomyia as well as Melao (California complex) were purified, concentrated and investigated with electron microscope. The spherical particles having a diameter of 50, 50, 56, 45, and 45, are presently investigated in detail (G.H. Bergold, Th. Graf). Purified preparations of all these viruses contained consistently spherical or disc-like particles with a diameter of about 11 mμ which frequently have a hollow center. Guaroa producing consistently plaques of two different diameters could readily be separated in two viruses by cloning with either large or small plaques, which are presently being identified.

(G.H. Bergold)

REPORT FROM GORGAS MEMORIAL LABORATORY PANAMA, R.P.

Previous survey work conducted in Almirante, a tropical rainforest area located in northwestern Panama, indicated that many arboviruses were highly prevalent in certain species of mosquitoes and in cotton and spiny rats. A long-range study was initiated in this area in June 1964 aimed at determining the natural cycle of certain arboviruses in rodents and mosquitoes and their relation to human infections. The program included the capture, bleeding, release and recapture of wild rodents in a designated area. Such a program has been successfully performed in Brazil and Trinidad.

During the first year of the study, a total of 724 rodents and 17 marsupials were captured and bled for virus isolation. From

these specimens, 468 cotton rats, 127 spiny rats and 10 black rats were tagged and released. One hundred and fifty-six of these rodents were recaptured. A total of 17 arboviruses was isolated from these small mammals, all of them from either cotton or spiny rats. The cotton rats yielded 10 agents, 2 Venezuelan equine encephalitis (VEE), 2 Nepuyo, 1 Patois (a new group C agent), 1 Guama and 4 unidentified viruses. Spiny rats were found to harbor 2 VEE, 1 Nepuyo, 1 unidentified group A, 2 unidentified group C and an unidentified virus.

When serological comparison of paired sera of recaptured rodents obtained during the year is completed, information will be available on activity throughout the year of different arboviruses which infect the wild rodent population. Preliminary HI tests performed on nearly 400 sera of wild rodents (Table 1), indicate a great deal of activity of VEE and group C viruses in both cotton and spiny rats. The spiny rats also show a high rate of serological response to group B antigens, whereas this response is very low in cotton rats. Thus far, only two members of group B arboviruses have been isolated in Almirante, namely, Ilheus and Bussuquara. The latter has been frequently found infecting rodents.

Results of the exposure of sentinel suckling mice show that from a total of 176 litters exposed during the year, 20 yielded viruses. Of these, 4 have been identified as VEE, 1 as Zegla (a new member of group C arbovirus), 2 other group C isolates and 5 as Guama group agents. Eight isolates await identification.

From a total of more than 33,000 blood-suckling insects collected from different baits, 708 pools of 30 mosquitoes or less per pool have been inoculated from virus isolation attempts. Culex vomerifer and C. taeniopus and/or C. opisthopus represented more than 50% of the total pools. Two VEE isolates were obtained from Culex vomerifer and one from Culex (Melanoconion) sp. An unidentified group A and a group C agent were isolated from Culex taeniopus and/or C. opisthopus and Culex vomerifer respectively. Three Guama group viruses were obtained from Culex vomerifer. Viruses not grouped as yet were 2 from Culex vomerifer, 2 from Aedes (Ochlerotatus) sp., 1 from Aedes angustivittatus, 1 from Culex taeniopus and/or C. opisthopus and 1 from Psorophora cingulata.

Studies of virus activity in the human population revealed 3 isolates of VEE, 1 each in the month of May, July and December. These isolates were from 43 cases who reported sick at the Almirante Hospital with fevers of unknown origin. No other viruses were obtained from these blood specimens. Antibody surveys were performed in 1964 among inhabitants of Almirante selected at random (Table 2). Some 347 sera were tested by HI with

7 antigens in group A, 5 in group B and 3 in group C. Although results have not been thoroughly analyzed as yet, it may be said at this time that 26.8% of the samples were positive to group A antigens. Most of the positive samples gave highest titers when reacted with VEE antigen. Of the samples tested, 24.8% were positive for group B antigens, and 5.2% positive specimens were obtained when tested with certain group C antigens.

In mouse neutralization tests, screening was performed with approximately 100 LD_{50} doses of VEE in group A, Ilheus in group B, and Guama group viruses. Of 100 samples tested with VEE, 33 were positive. Nineteen positive samples were detected from 70 sera tested for Ilheus encephalitis antibody. Eight out of 65 were positive for Guama virus. No isolations of Guama-group viruses have ever been obtained from man in this area.

In summary, viruses isolated from various sources, as shown in Table 3, indicated that VEE was the most important virus in the area and was detected from all sources tested. Group C arboviruses were widespread among cotton and spiny rats and often infect sentinel mice. Guama group virus was detected in cotton rats, sentinel mice and mosquitoes. It is impossible at this time to analyze the annual cycle of virus activity of individual arboviruses, since many agents await identification. However, general conclusions can be arrived at if we consider arboviruses as a whole. As shown in Figure 1, there seems to be a relationship between rainfall and monthly rate of virus isolations.

Analysis of the partially completed data presented here points to cotton and spiny rats as important sources of VEE, Guama and group C arboviruses in the Almirante area. It appears that the mosquito Culex (Melanoconion) vomerifer Komp and perhaps other Melanoconion mosquitoes are important vectors of VEE and Guama group viruses.

(Sunthorn Srihonges and Pedro Galindo)

TABLE 1

Preliminary Results of HI Tests for Arbovirus Antibodies in Cotton and Spiny Rats

Almirante, Panama, 1964-65

Antibodies to		Cotton rat		Spiny rat	
		No. tested	% positive	No. tested	% positive
Group A	VEE	298	23.2	84	44.0
	others	298	6.4	84	15.5
Group B		298	3.3	84	57.1
Group C	Caraparu-Apeu	298	2.0	84	17.9
	Patois	270	15.0	76	21.1
	Zegla	280	24.0	77	13.0
	others	298	5.0	84	16.7
Group Bunyamwera		196	0	60	0
Group Turlock		26	0	2	0
Group Phlebotomus Fever		274	0.4	67	1.5

TABLE 2

Preliminary Results of Arbovirus Antibody Surveys in Man

Almirante, Panama, 1964

Virus	HI antibody		Virus	Neutralizing antibody	
	No. tested	% positive		No.tested	% positive
Group A	347	26.8	VEE	100	33
Group B	347	24.8	Ilheus	70	27
Group C	347	5.2	Guama	65	12

TABLE 3

Arboviruses Isolated from Field Specimens in Almirante, Panama
June 1964 - June 1965

Virus	Rodent		Sentinel mice	Mosquito	Man	Total
	Cotton rat	Spiny rat				
VEE	2	2	4	3	3	14
Other gr. A	-	1	-	1	-	2
Caraparu	-	1	2	-	-	3
Nepuyo	2	1	-	-	-	3
Patois	1	-	-	-	-	1
Zegla	-	-	1	-	-	1
Other gr. C	-	1	-	1	-	2
Guama gr.	1	-	5	3	-	9
Unidentified	4	1	8	7	-	20
Total	10	7	20	15	3	55

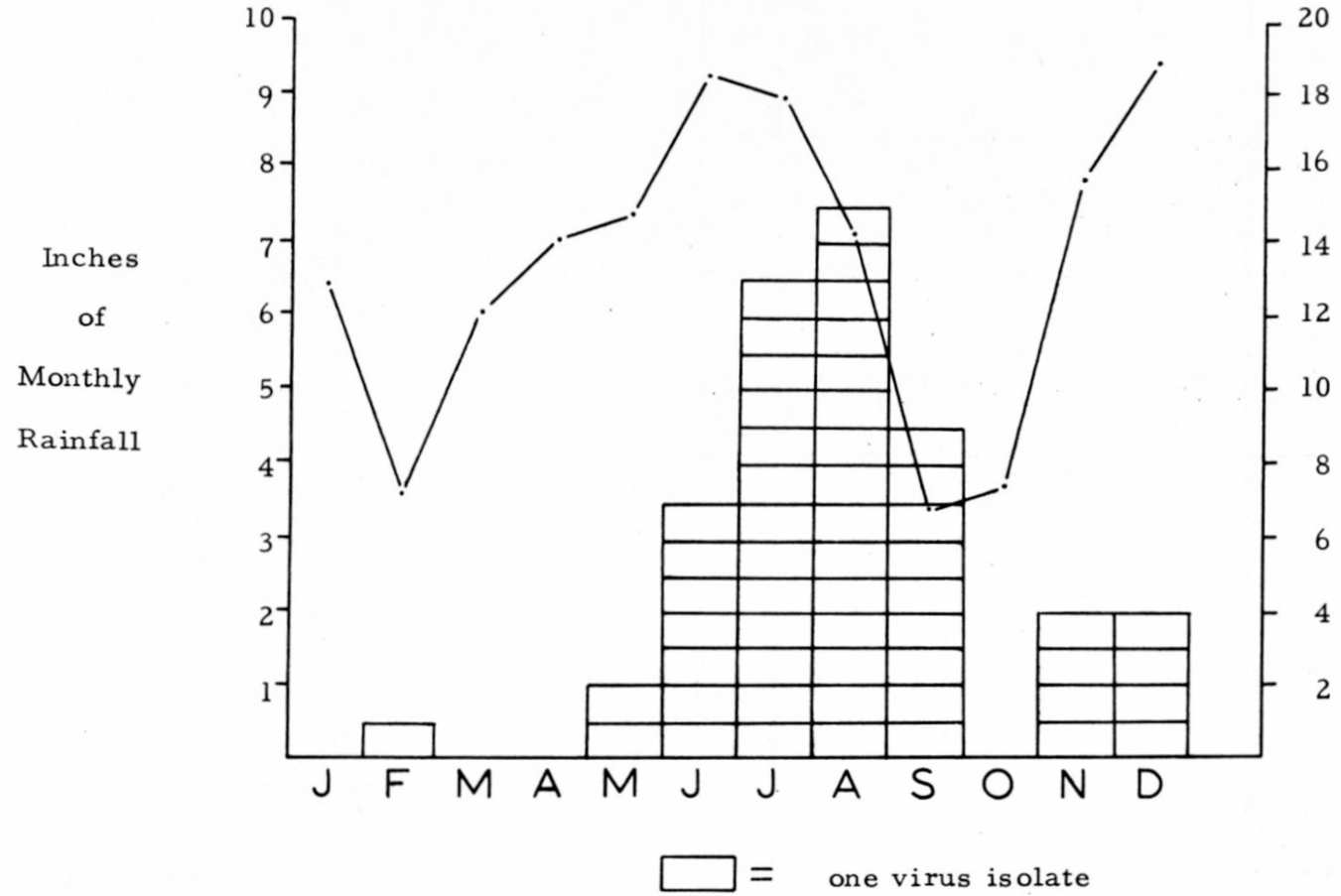


Figure 1: Comparison of Monthly Rainfall and Number of Arbovirus Isolates Obtained. Almirante, Panama.

REPORT FROM THE MIDDLE AMERICA RESEARCH UNIT (NIAID)
PANAMA

Plaque Neutralization Tests.

Using either embryonic rabbit kidney (strain MA-111 or green monkey kidney (VERO) cells grown in disposable metabolic-inhibition trays (plastic-LINBRO Co.) many of the arboviruses known to occur in Panama and neighboring countries have successfully been shown to produce readable plaques under agar. Comparative sensitivity to infant mouse titrations are shown in Table 1. Homologous, specific plaque neutralization using a fixed virus dose and serum or ascitic fluid dilutions has been readily demonstrated for many of the viruses when hyperimmune reagents were used. A study to compare this method with the LNI test in mice for measurement of antibodies to VEE virus in human sera showed complete qualitative correlation. There was also qualitative agreement and significant quantitative correlation between the plaque neutralization test and the HI test on these sera (Table 2). The economy of this technique in terms of materials and test sera is evident. Studies to fully characterize the method for use with other viruses forming plaques and with sera from various animal species are in progress.

Experimental Infection of Wild Rodents of Panama with VEE Virus.

Serum surveys and numerous virus isolations from different geographic regions of Panama and the Canal Zone have implicated the spiny rat (Proechimys semispinosus) and the cotton rat (Sigmodon hispidus) as reservoirs of VEE virus.

To elucidate the potential role of these rodents in the natural cycle of infection, ten Proechimys and twelve Sigmodon were inoculated with approximately 2.5 logs plaque forming units (PFU's) of the 3880 strain of VEE. Table 3 shows the results of virus assays in VERO (African green monkey kidney) cells by the plaque method. Titers in serum and throat were highest on the second day after inoculation. The values for Sigmodon were higher than for Proechimys both in serum and throat secretions. The duration of viremia, usually 4-5 days, was similar for both species. Proechimys shed virus in the throat for a shorter time than in the serum; whereas in Sigmodon, virus persisted longer in the throat than in the serum, and in one case was present at least seven days after inoculation.

Ten Proechimys had detectable neutralizing antibody* by three to five days following inoculation. In all cases this preceded the disappearance of circulating virus by one or more days. Titers of neutralizing, HI and CF antibodies reached maximum values by 18 days. CF titers had already declined by day 35, while NT and HI titers were unchanged at 128 days.

Attempts to demonstrate chronic infection by late virus isolations from serum and throat up to 35 days after infection were negative. Furthermore, virus could not be isolated from urine or feces either during the acute infection or up to 78 days later.

* $\geq$ 80% reduction of 50-100 PFU's

Hemorrhagic Fever.

Disease surveillance and rodent control activities were maintained in San Joaquin, Bolivia, throughout 1965. Sporadic cases were recorded from the environs of San Ramon about 20 miles from San Joaquin, and from the village of San Joaquin itself. Preliminary serologic testing indicates the number did not exceed 30 for the entire year. Strong house focality was again observed and several further Machupo virus isolations were made from a small number of Calomys callosus captured in "infected" houses.

A team of seven military and civilian personnel studied 17 areas in Bolivia, Brasil, Paraguay and Peru during the period 3 April to October 20, 1965. Small mammals were live-trapped from the major habitat types in each locality. Animals were killed by exsanguination in the field laboratory and the spleen and kidneys removed aseptically and preserved in liquid nitrogen for virus isolation attempts. Ectoparasites were collected for taxonomic study and a museum specimen was prepared from the skin and skull of each host animal. Human sera were collected in nine of the areas studied as indicated in Table 4.

The house mouse, Mus musculus, and the roof rat, Rattus rattus, were much more widely distributed in South America than was originally supposed. Houses infected with these introduced rodents were found in all of the areas studied except for two remote military posts in the Paraguayan Chaco. Calomys callosus, a reservoir of hemorrhagic fever virus in Bolivia, has not been collected in houses infected with Rattus or Mus.

A total of 196 Calomys callosus and eight specimens of Calomys laucha were collected during the survey. Of the total of 344 spiny rats (Proechimys sp.) collected this year, 74 were trapped in the Amazonian rain forest well beyond the northern limits of Calomys distribution. Virological and serological studies of the specimens collected during the survey will help to establish the geographic distribution of Machupo virus infections in man and animals and clarify many points concerning the ecology of the disease in South America.

(E.M. Earley, K.M. Johnson, M.L. Kuns, P.H. Peralta, P.A. Webb, N.A. Young)

TABLE 1

Comparative Infectivity Assays of Assorted "Arboviruses" in Infant Mice and MA-111 Cell Cultures

Group	Virus	Reciprocal Log_{10} Titer in:		Plaque Description Temporal development,		
		Infant Mouse	Cell Culture	Size,	dextran	effect*
A	EEE	9.3	8.3	1[x]	L[**]	-[+]
	VEE	9.0	9.3	1	L	-
	UNA	7.1	6.8	1	M	-
	Pixuna	8.5	8.5	2	M	-
	Mucambo	8.5	8.6	2	L	-
B	Ilheus	8.3	8.2	4	S	D
	YF	9.1	8.8	5	M	D
	SLE	10.2	9.2	4	S	D
	Bussaquara (GA 7)	7.5	6.3	4	M	D
Bunyamwera	Guaroa (BT-1122)	6.1	6.6	4	S	D
	Cache Valley (BT-2368)	7.3	7.4	2	M	D
	Wyeomyia (BT-219)	5.6	5.3	7	S	D
California	B-878	7.1	6.6	6	S	D
	Melao (B-1113-14)	7.3	6.3	5	S	D
Sandfly Fever	Changuinola (BT-104)	6.2	5.5	4	S	D
	Chagres	6.6	6.2	5	S	Na
Guama	BT-640	7.8	7.1	5	S	D
Vesicular Stomatitis	Indiana	7.2	8.0	1	VL	-
	New Jersey	7.0	6.8	1	VL	-
	Cocal	8.7	8.6	1	VL	-

[x]No. days required for optimum plazue detection

**VL, >3 mm., L, 2-3 mm., M, 1-2 mm., S, <1 mm.

[+]D, plaque size and/or number enhanced by DEAE-dextran: Na, plaque size enhanced by sodium-dextran-sulphate: -, no effect of additives.

TABLE 2

Quantitative Comparison of VEE Virus Antibody Titers in 46 Human Sera by Plaque Neutralization and Hemagglutination-Inhibition Techniques

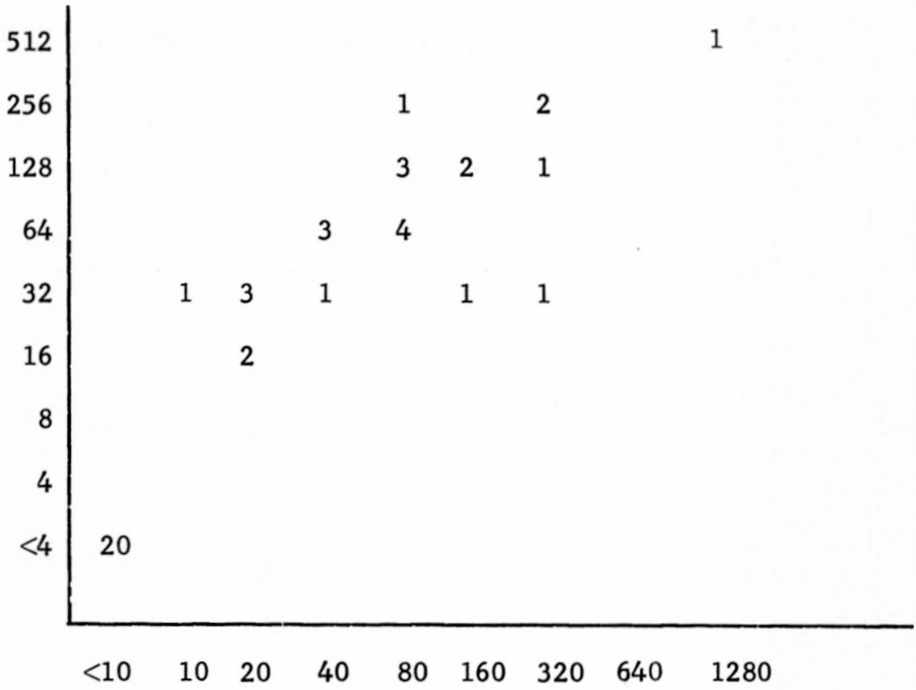


TABLE 3

MULTIPLICATION OF VEE IN RODENTS*

Days After Inoc.	SERUM (10)			THROAT (10)			SERUM (12)			THROAT (11)		
	No Pos.	Med. Titer	Max. Titer	No. Pos.	Med. Titer	Max. Titer	No. Pos.	Med. Titer	Max. Titer	No. Pos.	Med. Titer	Max. Titer
0	0			0			0			0		
1	8	2.9	3.9	2	2.0	2.0	12	5.1	5.9	6	2.3	3.8
2	10	5.3	5.8	7	2.8	3.4	12	7.1	>7.3	11	5.1	5.9
3	10	4.7	5.1	5	2.5	3.0	12	6.0	7.1	11	3.2	5.0
4	9	2.8	4.6	2	3.3	3.6	5	5.1	6.8	8	3.6	5.7
5	2	2.5	2.6	0			3	2.3	2.8	7	2.6	5.1
6	1	2.3	2.3	0								
7							0			1	3.0	3.0
8	0			0								

* Titers expressed as $\log_{10}$ PFU's per ml. of serum or throat secretions.

TABLE 4

SUMMARY OF SPECIMENS COLLECTED

1965 BHF Field Research Program

COUNTRY	State	Locality	Human Sera	Animal Specimens
Bolivia	Beni	San Joaquin	-	131
"	"	Riberalta	182	79
"	"	San Ignacio de Moxos	184	128
"	Pando	Cobija	0	15
"	Sta. Cruz	San Ignacio de Velasco	302	415
"	" "	Warnes	195	112
Brasil	Acre	Rio Branco	0	17
"	Guapore	Puerto Velho	0	19
"	Mato Grosso	Cuiaba	0	23
"	"	Caceres	285	102
"		Corumba	224	103
"	"	Campo Grande	0	32
"	"	Dourados	0	30
Paraguay	Boqueron	Fortin Guachalla	244	68
"	"	Mariscal Estigarribia	0	9
"	Missiones	San Ignacio	175	68
Peru	Madre de Dios	Puerto Maldonado	195	100
		TOTALS	1986	1451

REPORT FROM THE LABORATORY OF TROPICAL VIROLOGY
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
N.I.H., BETHESDA, MARYLAND

Since our last report (Infoexchange Number 11), Dr. Bunsiti Simizu completed his year at N.I.H. and moved to the Beth Israel Hospital, Boston, Massachusetts (June 1965) under sponsorship of the Harvard Medical School. Dr. Johng S. Rhim renewed his appointment as Visiting Scientist until September 1966. Dr. William D. Hann joined the laboratory as a Staff Fellow in October, 1965. Dr. Wiebenga spent 35 days in the U.S.S.R. as a member of the Delegation on Hemorrhagic Fever. The activities of the Delegation were reported elsewhere.

The laboratory program of hemorrhagic fever studies continued with emphasis on Machupo, Junin and Tacaribe viruses, and included characterization of virus infection of cell cultures, hemagglutination and pathogenicity of virus infections in guinea pigs.

Tissue Culture.

The MA-111 line of continuous rabbit kidney and the Vero line of continuous monkey kidney cell cultures were in routine use for virus assays by plaque counts. Simultaneous assays indicated that the sensitivity of plaque forming unit (PFU) titrations were comparable in both lines and compared with ID_{50} titrations in either suckling mice (SM) or hamsters (SH).

Thirty rapid serial passages of Machupo virus were accomplished in MA-111 cell cultures but changes in cell culture morphology (CPE) were not discernible. By contrast, Tacaribe and Junin virus infected Vero cell cultures developed CPE. Tacaribe virus infection and CPE were directly related but insufficient evidence was obtained to demonstrate Junin virus etiology of CPE.

Preliminary studies of primary human embryonic kidney (HEK) cell cultures suggested that there was a CPE response 7-10 days after Machupo virus infection but results were not reproducible with different kidney source lots. Only one of 8 lots tested was positive and the CPE-endpoint titrations were 2 or 3 $\log_{10}$ units less than parallel ID_{50} assays in SH. Continued study is stimulated by the persistent absence of CPE associated with Machupo virus infections of cell cultures in fluid medium and by the expense of plaque assays in cells under agar.

Hemagglutination.

Hemagglutination (HA) of mouse, chick, goose and sheep RBC was observed when crude alkaline extracts of Tacaribe, Junin and

Machupo virus antigens (infected SM or SH brain) were further extracted with chloroform. Antigens prepared by similar treatment of uninfected SH or SM brains did not hemagglutinate. However, specific inhibition (HI) of the HA by paired and serial convalescent human sera was not demonstrable. Furthermore, the occasional variability of HA titrations is not understood and further study of the hemagglutinin is in progress.

Pathogenicity.

Reports of Junin virus pathogenicity for adult guinea pigs (Parodi and Tauraso) described deaths and hemorrhagic lesions due to Junin virus infection. We have inoculated Machupo or Junin virus into a variety of guinea pigs without demonstrating a direct relationship between infection and death of any histopathological lesion. The absence of any definable hemorrhagic lesion was especially noteworthy. Testing included the NIH and Hartley strains of GP, the American inbred "Peruvian" line and non-hybrid animals imported from Lima, Peru, and from Buenos Aires, Argentina. Scattered spontaneous deaths in the two groups named last limited the adequacy of testing. Histological sections of organs were obtained from all animals sacrificed at selected intervals after virus inoculation, and uninoculated animals were included for histological controls. There was no histological variation seen exclusively in the virus inoculated animals. Serological response by complement fixation (CF) tests of sera taken from sacrificed or surviving animals was irregular, ranging from CF titers of $<$1:4 to 1:64 in 30-day survivors of a constant virus dose.

Recent perplexing results are shown in Table 1 as a partial summary of repetitive tests that include two serial passages of Machupo or Junin virus in guinea pigs. Guinea pig lung suspensions from animals sacrificed two weeks after virus inoculation consistently killed GP 2 or 3 weeks after ip inoculation, with an LD_{50} titration endpoint of $10^{-6.0}$. If these deaths were due to Junin or Machupo virus, it should have been recoverable from the lethal inocula by established assay techniques. As shown in the table, the 10^{-3} through 10^{-6} dilutions were negative when tested in SM and SH for lethal effects and in two cell lines for plaque formation, even though the same dilutions were lethal for the GP. The absence of CF antibody in hamsters surviving challenge with the 10^{-3} through 10^{-7} dilutions support the conclusion that they were not infected with a Tacaribe group virus. Even though the results of assaying the 10^{-1} and 10^{-2} dilutions indicated the presence of a Tacaribe group virus, the specificity of an LD_{50} assay in G P for Junin or Machupo virus must be questioned. Attempts to demonstrate the presence of interferon or virus inhibiting tissue enzyme in the GP lung suspension were negative.

Since contamination of reference antigens with extraneous murine or hamster agents could not be excluded, our neutralizing antisera seemed inadequate to resolve a question of specificity. Plans for additional studies include preparation of suitable antiserum and attempts to isolate and identify extraneous contaminants. Host-dependent variation of virus infectivity and antigenicity was considered to be an improbable explanation under the conditions observed.

Table 1. Summary of Bio-Assay Characteristics of Guinea Pig Lung Suspensions Harvested at Time of Illness (2 weeks) after I,P. Inoculation of Either Junin or Machupo Virus Subcultures[1]

Inoculum[1] Log_{10} Dil.	DEATHS			CF Serum Titer[2] in 30-day SH Survivors	Virus Plaque Counts	
	Guinea Pig	Suckling Mouse	Suckling Hamster		MA-111 Cont. Rabbit Kidney	Vero Cont. Monkey Kid.
10^{-1}	Not Done	Some	Some	Pooled >1:16	0-3	0-3
10^{-2}	All	Some	Some		0-1	0-1
10^{-3}	All	None	Some	<1:4	0	0
10^{-4}	All	"	None	<1:2	0	0
10^{-5}	All	"	"	"	0	0
10^{-6}	Some	"	"	"	0	0
10^{-7}	None	"	"	"	0	0

[1] Virus passages = SM or SH → GP/1 → GP/2. GP/1 and GP/2 lung harvests tested as inoculum.

[2] 2 units of homologous virus (Junin or Machupo) antigen used in complement fixation tests. Results expressed as serum dilution showing less than 50% hemolysis.

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

Summary of south Florida studies, 1963-1964.

Serological evidence of past VEE infection in the Seminole Indians of the Brighton and Big Cypress Reservations in south Florida was obtained by T. H. Work in 1960. Attempts were made in 1961 to isolate the virus from mosquitoes and wild vertebrates collected on the Big Cypress Reservation but with negative results. Reasoning that the main infection focus might be still farther south, a search was made for the virus in the Everglades National Park, about 80 miles south of the Reservation on the tip of the Florida mainland.

This search was productive, yielding VEE virus from mosquitoes, primarily Culex (Melanoconion) species, as shown in Table 1 (1963) and Table 2 (1964). Studies for 1965, still incomplete, confirm continued infection of C. (Melanoconion) species with VEE virus in the park area. The VEE isolates were from mosquitoes captured on hardwood-palm hammocks with the exception of one from Aedes taeniorhynchus, which was taken in more open terrain. In addition, numerous isolations of virus in the California group were obtained, as well as of Tensaw virus (Bunyamwera group), a new member of the Guama group, and a number of unknown viruses still under study.

A high VEE antibody rate was found in cotton mice (Peromyscus gossypinus) and cotton rats (Sigmodon hispidus) captured in association with infected Culex (Melanoconion) species, indicating a basic host role (Table 3). The isolation of VEE from Aedes taeniohynchus, an abundant Everglades tidewater mosquito capable of traveling many miles, suggests a means of epidemic spread from the rodents to man and other animals.

The isolation of SLE virus in 1964 from Culex nigripalpus is of interest as being the first made in the state of Florida since the 1962 St. Petersburg epidemic.

All of the mosquito isolates of the new Guama group virus were from Culex (Melanoconion) species. Another was also made from the blood of a cotton rat. Preliminary studies have been conducted by Dr. S. Ryder with nine of these agents (eight from the mosquitoes and the rat isolate). Thus far, serological studies have been confined to CF and neutralization tests because of the difficulties encountered in producing suitable hemagglutinins.

By CF, the nine isolates appear to be identical to each other. Strain FE 4-2s, the prototype strain, has been antigenically compared with known members of the Guama group and appears to be closely related but not identical to any of the previously described agents within the group (Table 4). The new agent has been tentatively called Mahogany Hammock virus after one area of the Everglades where isolates were made.

In an effort to determine the northward extension of recent VEE activity, a search for virus and antibodies in mammals has been initiated in the area between the Big Cypress Indian Reservation and the Everglades National Park. A north-south transect is being conducted extending from the Reservation through the center of the Everglades. To date, 292 animals have been taken during two, 2-week trapping trips covering about 16 miles of the transect. Thus far, no VEE virus has been identified from the animals (principally rodents, raccoons and opossums). Antibody tests for VEE have not been completed. Five virus strains have been isolated from cotton rats and one isolate obtained from a raccoon. Of these isolates only one has been sufficiently studied to report. Preliminary serological studies of this isolate, from the lung of a mature cotton rat, indicate that this agent is a new member of the group B arboviruses. It has tentatively been designated the name Cowbone Ridge Virus after the locality in which the infected cotton rat was captured.

Summary of southeast Georgia studies, 1964.

The area in the vicinity of Waycross, Georgia, had been studied in August-October, 1963, in conjunction with an outbreak of EEE in horses. The study was highly productive, with a total of 167 virus isolations from 60,000 mosquitoes tested (35 of EEE, 15 of WEE, 42 of Tensaw, 31 of Flanders and 44 of California group). Because of the large numbers of California-like isolates, all from Aedes atlanticus, the area was considered worthy of follow-up studies in 1964.

The results of tests on 60,150 mosquitoes collected between April and November, 1964, are shown in Table 5. The results are not unlike those of 1963, considering that the collections were commenced earlier in the season when virus activity was generally at a lower ebb than the restricted August-October period sampled in 1963. They show the recurrence of all the virus types present the previous year.

During 1964 and the winter of 1965, sera of 125 rodents and 26 penned ducks were collected in the Waycross area and tested for evidence of plaque neutralizing antibody. The results (Table 6) reveal California and Tensaw antibodies in a high proportion of the rodents. A high rate of EEE antibodies was found the ducks.

Summary of studies in the Atlantic Coastal area of U. S.

Extensive activity of eastern encephalitis virus occurred in 1965 in the Atlantic coastal states. One human case was diagnosed at the CDC. Five of 18 horse brains from North Carolina and 7 of 9 horse brains from South Carolina yielded EEE virus. Members of the CDC Arbovirus Unit, in cooperation with State personnel, made mosquito collections at various periods in the vicinity of Sylvania, Georgia, several counties in eastern North Carolina, and parts of the Tidewater area of Virginia as far north as the Potomac River in association with encephalitis in horses; and in the Eastern Shore district of Maryland in relation to an outbreak in commercially reared Chinese pheasants. Results of testing 70,801 mosquitoes from these various areas are summarized in Table 7. At least 119 arbovirus isolations were made. In addition, a number of "toxic" pools of Culex salinarius were encountered in Virginia which killed suckling mice within one day but which would not pass.

Five virus isolations were also made from 497 birds collected during what appears to be the latter stages of the epizootic: EEE from two moribund house sparrows, and a 4-week-old game farm bobwhite quail from North Carolina, WEE from a bluejay from North Carolina, and WEE from a red-eyed vireo from Virginia.

Summary of studies in South West area of U. S., 1965.

An outbreak of vesicular stomatitis in cattle began in July of 1965 in northern New Mexico and southern Colorado, and was confirmed as VSV - Indiana type by isolation of virus from vesicles of cattle by Dr. Jenney of the National Animal Disease Laboratory, Ames, Iowa. As the epizootic progressed, suspected human cases were reported. As a result of the human involvement, the New Mexico State Health Department requested epidemic assistance from the CDC in August. A team from the Laboratory and Epidemiology Branches investigated the human cases and conducted an ecological survey.

A total of 24 possible human cases was investigated. Clinical findings were variable, including pharyngitis (with or without vesicles), flu-like syndrome with fever and chills, headache and non-descript febrile illness. Specimens were also collected from 16 people without symptoms but in similar ecologic settings (asymptomatic family members, veterinarians, etc.). Of the 24 people with symptoms, 7 had serologic evidence of disease (Table 8). The other 17 ill patients and the 16 without symptoms had no serologic evidence of infection with VSV. The 7 positives were distributed in 4 different ranches, 4 in one family unit, the other 3 in different ranches. The four involved ranches were located in 3 adjacent counties on the New Mexico-Colorado border.

Interestingly, as shown in Table 8, when CF tests were done utilizing sucrose acetone antigen prepared from an isolate of the epizootic, sera from the seven cases reacted weakly or not at all. This same antigen gave high titers with homologous laboratory produced sera. However, when the same virus was used to prepare a crude antigen utilizing ultracentrifuged veronal buffer preparations of infected suckling mouse brains, diagnostic CF titers were obtained in most instances. Studies are in progress to determine if this is a chance phenomenon or if it is of real significance in preparing antigens for diagnostic work with this group of viruses.

Mosquito collections were made in the Abiquiu, Taos and Aztec areas. A total of **24** virus isolations were made from 10,895 mosquitoes (Table 9). These included one isolation of vesicular stomatitis virus from Aedes species, probably dorsalis, the first of this virus from a mosquito in the U. S.

During the epizootic, a total of 127 vertebrates, primarily small rodents, was collected from two sites in North Central New Mexico. All specimens are awaiting laboratory processing for isolation attempts and serology.

TABLE 1. Arbovirus isolations from Florida Everglades mosquitoes, 1963.

	VEE	Calif.	Tensaw	Unident	TOTAL
Aedes infirmatus		2	1		3
Aedes taeniorhynchus	1	27	47	3	78
Anopheles crucians	1		24	2	27
Culex nigripalpus		1		2	3
Culex (Melanoconion)sp.	4	1		4	9
Psorophora confinnis			4		4
TOTAL	6	31	76	11	124

TABLE 2. Arbovirus isolations from Florida Everglades mosquitoes, 1964

	EEE	VEE	SLE	Cal.	Ten.	Guama	FE3-71h	not typ	not tes	TOTAL
Aedes atlanticus		1		2						3
Aedes infirmatus				6				4	1	11
Aedes taeniorhynchus				7	4		1	1	1	14
Anopheles crucians		1		1	65			2	1	70
Culex nigripalpus		1	1		1					3
Culex (Melanoconion)sp.		14		3		8	1	33	5	64
Culiseta melanura	1									1
Psorophora confinnis					1					1
TOTAL	1	17	1	19	71	8	2	40	8	167

TABLE 3. VEE antibody rates, based upon HI titers of 1:40 or greater, in Everglades rodents captured in January and May, 1964.

Month	Rodent Species*	Area: Pa-hay-okee	Mahogany	Royal Palm	Corkscrew	TOTAL
Jan.	O. p.		0/1			0/1
"	P. g.		16/26 (62)**		0/16 (0)	16/42 (40)
"	S. h.		3/14 (21)			3/14 (21)
	TOTALS		17/41 (46)		0/16 (0)	20/57 (35)
May	P. g.	2/9 (22)	2/10 (20)	1/13 (8)		5/32 (16)
"	S. h.	0/1	0/2	1/2		1/5 (20)
	TOTALS	2/10 (20)	2/12 (17)	2/15(13)		6/37 (16)
Jan. +May	P. g.	2/9 (22)	18/36 (50)	1/13 (8)	0/16 (0)	21/74(29)
	S. h.	0/1	3/16 (19)	1/2		4/19 (21)
	TOTALS	2/10	21/52 (40)	2/15 (13)	0/16 (0)	25/93 (27)

* O.p. - Oryzomys palustris (rice rat)
P.g. - Peromyscus gossypinus (cotton mouse)
S.h. - Sigmodon hispidus (cotton rat)

** percentage

TABLE 4. Serological relationship of Mahogany Hammock virus (FE 4-2s) with Guama group arboviruses.

Hyperimmune Ascitic Fluids	Mahogany Hammock Antigen CF H_t/H_o	Neut. H_t/H_o	Viruses	Mahogany Hammock Ascitic Fluids CF H_t/H_o	Neut. H_t/H_o
Mag. Ham. FE 4-2s	128	$\geq$4.1	Mag. Ham. FE 4-2s	128	$\geq$4.1
Guama BeAn 277	256/512	3.8/$\geq$3.6	Guama BeAn 277	32/128	2.2/$\geq$4.1
Bimiti Tr 8362	64/256	1.7/$\geq$3.5	Bimiti Tr 8362	32/128	3.3/$\geq$4.1
BeAn 20525	256/512	3.0/$\geq$3.5	BeAn 20525	32/128	2.3/$\geq$4.1
Moju BeAr 12590	32/64	3.2/$\geq$2.0	Moju BeAr 12590	64/128	2.0/$\geq$4.1
Catu BeH 151	256/512	0.3/$\geq$5.3	Catu BeH 151	32/128	0.9/$\geq$4.1

TABLE 5. Arbovirus isolation from Waycross, Georgia mosquitoes, 1964.

	EEE	WEE	Cal.	Flan.	Ten.	Unident	TOTAL
Aedes atlanticus			20				20
Aedes (prob. atlanticus)			1				1
Anopheles crucians	1				25	1	27
Culex salinarius						1	1
Culex (Melanoconion)sp.	1						1
Culiseta melanura	20	7		13		1	41
Mansonia perturbans	7						7
TOTAL	29	7	21	13	25	3	98

TABLE 6. Results of plaque neutralization test of small mammal and duck sera from Waycross, Georgia, 1964-1965.

	Virus Strains Used				
Species	Calif.	EEE	Tensaw	WEE	SLE
Sigmodon hispidus	35/66*	0/67	6/65	0/67	0/67
Peromyscus gossypinus	3/38	0/38	0/38	0/38	0/38
Peromyscus nuttalli	0/4	0/4	0/4	0/4	0/4
Didelphis virginiana	0/1	0/1	0/1	0/1	0/1
Sciurus niger	0/2	1/2	0/2	1/2	0/2
Sciurus carolinensis	0/1	0/2	0/2	0/2	0/2
Sylvalagus floridanus	3/3	0/3	3/3	0/3	0/3
Mallard ducks	0/25	14/25	0/26	1/26	0/26

*Number positive/number tested. A serum was considered positive if it neutralized 90% or more of the plaques produced in control virus titrations. Utilization 50 to 150 PFU.

TABLE 7. Arbovirus isolation from mosquitoes collected in Atlantic Coastal States, 1965, in association with outbreaks of Encephalitis in horses or pheasants.

Location	Dates Collected 1965	No. of Mosquitoes Tested	Positive Mosquito Species	Virus Type & No. of Isolates* CE	EE	FV	TV	WE	Un	TOTALS
Georgia (Screven County)	6/28	2,051	C. melanura					2		2
North Carolina (East)	8/3-10, 9/16-19	39,183	A. atlanticus	15						15
			C. melanura	1	19	15		13	5	53
			P. confinnis		1			1	1	3
Virginia Tidewater	8/19-25, 9/13-16	22,466	A. atlanticus	5						5
			infirmatus		1					1
			An. crucians				1			1
			C. restuans			1				1
			salinarius		1					1
			C. melanura		5	2		2		9
Maryland (E. Shore)	9/11-12	7,111	A. atlanticus	1						1
			canadensis	3						3
			vexans	1						1
			C. melanura		14			8	1	23
	TOTAL	70,801		26	41	18	1	26	7	119

*CE California encephalitis group
EE Eastern encephalitis virus
FV Flanders virus
TV Tensaw virus
WE Western encephalitis virus
Un Unidentified

TABLE 8. Summary of clinical and laboratory data on patients with suspected vesicular stomatitis infection

Patient	Age	Sex	Clinical Syndrome	Onset 1965	Date of Collection 1965	Complement Fixation SA Antigen VSV(NJ)	Complement Fixation SA Antigen VSV(IND)**	Complement Fixation Crude Antigen VSV(IND)**	Neut. Index
1. RLG	28	M	Flu*, oral vesicles	8/5	8/11	<8	<8	1:16	1.3
					9/3	<4	<8	----	3.7
					11/10	<4	<8	<8	---
2. DA	34	M	Flu, pharyngeal vesicles, 20 lb. weight loss	7/23	8/12	<4	<8	1:64	≥3.9
					9/3	<8	<8	----	≥3.9
					11/10	<4	<8	1:8	----
3. RB	28	F	Flu, headache, sore throat, cervical adenopathy	8/3	8/19	<4	<8	1:64	≥2.65
					11/9	<4	1:8	1:32	-----
4. TB	10	M	Flu, headache, sore throat, cervical adenopathy	8/4	8/19	<4	1:32	1:128	≥2.15
					11/9	<4	1:8	1:16	-----
5. DaB	7	M	Flu, headache, sore throat, cervical adenopathy	8/4	8/19	<4	<8	1:32	≥2.65
					11/9	<4	<8	<8	-----
6. DiB	11	M	Flu, headache, sore throat, cervical adenopathy	8/3	8/19	<4	<8	1:32	≥2.15
					11/9	<4	<8	<8	-----
7. PC	42	M	Flu, fever blister	8/9	8/13	<8	<8	<8	0.43
					11/8	<8	<8	1:16	----

* Flu - includes generalized malaise, myalgias, fever, and variable gastrointestinal symptoms.
** Isolate of VSV - Indiana type from mosquitoes collected during the epidemic.

TABLE 9. Arbovirus isolations from New Mexico mosquitoes, August,1965.

	VSV*	WEE	Calif.	Turlock	Unident.	TOTAL
Aedes dorsalis		4	1		1	6
Aedes vexans		1	2			3
Aedes sp.(prob. dorsalis)	1		1			2
Culex tarsalis		5		4	1	10
Culex sp.(prob. tarsalis)				1		1
Culiseta inornata			2			2
TOTAL	1	10	6	5	2	24

*Vesicular stomatitis virus, Indiana serotype.

REPORT FROM THE DEPARTMENT OF PATHOBIOLOGY JOHNS HOPKINS UNIVERSITY SCHOOL OF HYGIENE AND PUBLIC HEALTH BALTIMORE, MARYLAND

Experimental Infection and Serology of the Indian Fruit-Bat Pteropus giganteus, with chikungunya and dengue 2 viruses.

The Indian fruit-bat Pteropus giganteus is a large bat and is widely distributed on the Indian subcontinent. Bat colonies vary in size from 20 to 6,000 bats per colony and are found close to human communities.

Results of experimental infection of bats with early mouse brain passages of chikungunya and dengue 2 viruses are given in Tables 1 and 2. The lowest dilution of serum tested for viremia was 1:10. Test for chikungunya virus was performed by inoculation of primary hamster kidney cell cultures, and for dengue 2 virus by inoculation of weanling mice, followed by IC challenge of these mice with a mouse-adapted dengue 2 virus.

After subcutaneous inoculation of four bats with 6.5 log units of chikungunya virus, there was transient viremia in one of four bats. Three developed HI and neutralizing antibodies by day 14. The neutralizing antibodies persisted through the observation period of 14 to 27 weeks for the three bats. HI antibodies were detectable at 5 but not at 17 weeks after infection. Transmission from infected bats to an uninoculated cage-mate was not demonstrated. Inoculation of 3.5 log units of chikungunya virus produced neither viremia nor antibody response in any of the four bats.

After inoculation of 4.0 log units of dengue 2 virus, viremia was not demonstrable. In the bleedings of day 14, HI titers of 1:20 and 1:80 were found in two of six bats. Neutralizing antibodies were not detected in these or in any other sera of the inoculated bats.

The results of antibody survey of two collections of bat sera are given in Table 3. HI and/or neutralizing antibodies to rabies, dengue 2 and chikungunya viruses, and myxoviruses parainfluenza 1, parainfluenza 3 and Newcastle disease were not detected in any of the sera. In the collection of 1962-63, HI antibody titers to dengue virus ranging from 1:20 to 1:320 were detected in eight of 52 sera; in neutralization test with dengue 1 virus, five of 34 sera were partially protected with mortality ratios of 2/6, 3/6 or 4/6. The distribution of the partially protective sera by their HI titers is given in Table 4. It is probable that the two partially protective sera with HI titers of 1:160 and 1:320 were previously infected with a dengue virus.

The results of this study did not reveal any positive evidence which would suggest that the Indian fruit-bat was an important reservoir from which mosquitoes would transmit chikungunya and dengue 2 virus infections to man.

TABLE 1

Viremia and Antibody Response of the Indian Fruit-Bat Pteropus giganteus

After Subcutaneous Inoculation of Second Infant Mouse Brain Passage Chikungunya Virus

Dose	Bat Number	Viremia — Days Post Inoculation										Antibody — HI — Days Post Inoculation								Antibody — Neutralizing — Days Post Inoculation							
		1	2	4	5	6	7	9	12	14	28	Pre	14	28	35	87	112	126	168	Pre	14	28	35	87	112	126	168
6.5 log units	64-475	N		N		N		N		N		N	80	40		N				N	P	P		P			
	64-476	P*		N		N		N		N	N	N	160	80		N	N	N	N	N	P	P		P	P	P	
	64-482		N		N		N		N	N	N	N	N	N		N	N	N	N	N	P	N		N	N	P	N
	64-485		N				N		N	N	N	N	80		20	N	N	N	N	N	P		P	P	P	P	P
Uninoculated Cage Mate	64-483											N	N	N		N	N	N	N	N	N	N		N	N	N	N
3.5 log units	64-479	N		N		N		N		N	N	N	N	N						N	N	N					
	64-480		N		N		N		N	N	N	N	N	N		N	N	N	N	N	N	N		N		N	N
	64-481	N				N		N		N	N	N	N	N	N	N	N	N	N	N	N		N	N			N
	64-486		N		N		N		N	N	N	N	N	N						N	N	N					
Uninoculated Cage Mate	64-484											N	N	N						N	N	N					

N = Negative

P = Positive

* Titer $10^{4.7}$/ml.

TABLE 2

Viremia and Antibody Response of the Indian Fruit Bat Pteropus giganteus

After Subcutaneous Inoculation of Third Infant Mouse Brain Passage Dengue 2 Virus

Dose	Bat Number	Viremia																Antibody							
																		HI				Neutralizing			
		Days Post Inoculation																Days Post Inoculation							
		1	2	3	4	6	7	8	10	12	14	17	21	24	28	35	70	Pre	14	28	70	Pre	14	28	70
4.0 log units	64-475	N		N		N		N		N								N				N			
	64-476		N		N		N		N		N	N		N	N	N	N	N	N	N	N	N	N	N	N
	64-480	N		N		N		N		N	N		N		N	N	N	N	80	N	N	N	N	N	N
	64-481*	N		N		N		N		N	N		N		N	N	N	N	N	N	N	N	N	N	N
	64-482		N		N		N		N		N	N		N	N	N	N	N	20	N	N	N	N	N	N
	64-483		N		N		N		N		N	N		N	N	N	N	N	N	N	N	N	N	N	N
	64-485*		N		N		N		N		N		N		N	N	N	N	N	N	N	N	N	N	N

N = Negative

* Brain, kidney, liver, spleen and lung processed for virus isolation on day 70 after inoculation; all negative.

TABLE 3

Prevalence of Antibodies to Some Arbo, Myxo and Rabies Viruses in Bat Sera

Year of Collection	Virus	Proportion with Antibodies	
		HI	Neutralizing
1962-63	rabies		0/39
	dengue 1	*8/52	**5/34
	chikungunya	0/70	
	parainfluenza 1	0/42	
	parainfluenza 3	0/42	
	Newcastle disease	0/42	
1965	dengue 1	0/43	0/43
	dengue 2	0/43	0/43
	chikungunya	0/43	0/43

* HI titers of 8 positive sera ranged from 1:20 to 1:320

** Five sera partially protective in neutralization test with survival ratios between 16 and 84%

TABLE 4

Correlation Between Results of HI and Neutralization Tests of Bat Sera with Dengue 1 Virus

HI Titer	Proportion Partially Protective in Neutralization Test
< 20	2/26
20	1/5
40	0/1
80	-
160	1/1
320	1/1
Total	5/34

REPORT FROM THE VIRUS AND RICKETTSIA DEPARTMENT
U.S. ARMY BIOLOGICAL CENTER
FORT DETRICK, FREDERICK, MARYLAND

Immunological Studies on Arboviruses*

Recent investigations by Dr. W.P. Allen have been oriented toward the development of methods for producing broadly cross-reactive mouse immune sera or ascitic fluid against group A arboviruses. Approximately 80 different pools of immune mouse sera were compared for HI activity against 11 different group A virus antigens. These pools represented an equal number of different combinations of immunizing agents or immunization schedules. Immunizations included the use of one or more of the following viruses: Western equine encephalitis (WEE), Eastern equine encephalitis (EEE), Venezuelan equine encephalomyelitis (VEE), Semliki Forest, Sindbis, Mayaro, Chikungunya, Getah, Bebaru, Una, Middelburg, and O'nyong-nyong. Of the combinations tested, five were selected that yielded HI titers of 1:80 or greater against all 11 antigens. These five methods had the common denominator of using VEE virus as one of the immunogens. Either attenuated VEE virus (Hearn 11t strain) was used for a primary immunization and followed in 3 to 4 weeks by a second virulent group A virus, or a nonlethal group A virus was used as a primary immunogen and followed in 3 to 4 weeks by a fully virulent VEE virus. The best combinations observed are summarized as follows:

	Primary virus	- Route	Secondary virus	- Route	Days to Bleeding
1.	Semliki Forest (killed)	i.p.	virulent VEE	i.p.	11
2.	Mayaro	i.c.	virulent VEE	i.p.	14
3.	Sindbis	i.c.	virulent VEE	i.p.	13
4.	attenuated VEE	i.p.	WEE	i.p.	14
5.	attenuated VEE	i.p.	Semliki Forest	i.p.	16

Aside from virus strains used as immunogens, at least two other parameters were noted that frequently caused profound effects on cross-reactivity and antibody titers of immune sera. The first

* In conducting the research reported herein, the investigator adhered to 'Principles of Laboratory Animal Care' as established by the National Society for Medical Research.

of these parameters was the route of administration of the secondary immunization. Generally, the intraperitoneal (i.p.) route was superior to the intracerebral (i.c.) route. Apparently, the i.p. route allowed sufficient systemic multiplication and infection to provide adequate secondary antigenic stimulus without killing the host.

Another parameter was the time interval between the secondary immunization and bleeding. Optimal titers and greatest cross-reactivity were obtained when animals were bled 11 to 17 days after the secondary immunization. This interval apparently corresponded with the maxima of the anamnestic response. Sera from later bleeding had high homologous HI antibody titers but progressively less cross-reactivity with related viral antigens. This observation is compatible with the hypothesis that different antibody molecules are responsible for homologous and heterologous reactivity to these viruses.

The use of mouse ascitic tumor to produce large quantities of broadly reactive fluid for use in the HI test has not been successful as yet in our hands. Large quantities of ascitic fluids were obtained from relatively few immunized mice, but the HI antibodies in these immune fluids reacted mainly with antigens homologous to the immunogens and seldom reacted with antigens of distantly related heterotypic viruses. It was noted that the initial tap of ascitic fluid generally yielded higher titers of HI antibodies than subsequent taps. It appeared that a more intensive immunization regimen is required to produce high quality, broadly reactive HI antibodies by the ascitic fluid technique.

(A.N. Gorelick)

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

Continuing Arbovirus Survey in New York State.

Isolation

Processing of specimens from live-trapped wild animals and arthropods collected from June, 1964, through September, 1965, in St. Lawrence County is continuing.

Infectious agents with properties similar to those described previously (Arbovirus Information Exchange, 1965, 12, 34) from three *Microtus pennsylvanicus* have been isolated from three additional animals #498, #599, and #603 of the same species, two of which were caught in March 1965, one on the mainland, and the other on Barnhart Island. An agent was also isolated from mites from animal #603.

From two other animals, Clethrionomys gapperi #452 and #497, trapped on October 29 and November 17, 1964, infectious agents similar to #138 previously described (Arbovirus Information Exchange, 1965, 12, 34) were recovered.

Serologic Evidence of the Bunyamwera Group of Arboviruses in Deer Sera in New York State.

Previous studies have shown evidence of the Bunyamwera group of arboviruses in human sera from residents of Suffolk, Schuyler, St. Lawrence counties and in cow sera from dairy herds in St. Lawrence County. Schuyler and St. Lawrence counties are more or less rural districts while Suffolk County is suburban to urban. The present study deals with serologic evidence of Bunyamwera group agents found in deer sera collected in January, 1965, from Erie County on the western border of New York State and Delaware County in the southeastern section, and discusses previous findings.

Fifty-five deer sera were examined by the Clarke and Casals hemagglutination-inhibition (HI) method adapted to the microtechnic. Antigens were prepared with five arbovirus strains representing two group A (Eastern and Western encephalomyelitis), two group B (Powassan and St. Louis encephalitis), and one of the Bunyamwera group (the strain from Belem now known as Maguari). Evidence of activity of the Bunyamwera group was found in both counties but with a marked difference in the frequency of reactions: 9 of 25 sera (36.0%) from Erie County reacted but only 1 of 30 sera (3.3%) from Delaware County reacted. No evidence of the presence of group A or group B arboviruses was detected by the in vitro test.

The same sera were examined in neutralization tests using 3-day-old suckling mice and the prototype strain of Cache Valley virus originally isolated by Holden and Hess. The intracerebral route of inoculation was employed. All the sera reacting in HI tests and eight additional sera from Erie County demonstrated neutralizing antibodies for Cache Valley virus. Thus the percentage of reactivity for the Bunyamwera group in Erie County noted by the neutralization technic (66%) was twice that noted by the HI technic.

Similar results were obtained with the cow sera from dairy herds in St. Lawrence County. On Farm No. 1 in the western part of the county, no activity was noted by the HI test but by neutralization, 1 of 30 sera (3.3%) reacted; on Farm No. 18, however, in the eastern section of the county, Bunyamwera group antibodies were detected in 11 of 40 sera (27%) by HI and in 20 of 40 sera (50%) in neutralization tests. Neutralizing antibodies were found in all sera which reacted in the HI test.

In a serologic survey of 899 wild animal sera collected in St. Lawrence County examined by the HI method, no reactions with the Bunyamwera group antigen were obtained. No neutralizing antibodies have been found in the 116 sera so far tested.

Earlier screening of sera from residents of Schuyler, St. Lawrence and Suffolk counties have shown neutralizing antibodies for the prototype strain of Cache Valley virus, as follows: 13 of 188 sera (6.8%), 11 of 139 sera (7.8%), and 10 of 337 sera (3%), respectively. It should be noted that Suffolk County is suburban to urban in character while the other two counties are rural.

Serologic Studies.

Wild animal sera. Hemagglutination-inhibition (HI) tests on 899 wild animal sera collected in St. Lawrence County from June, 1964, to September, 1965 have been completed. Five hundred and ninety-five sera were from 23 mammalian species, 201 were from 6 amphibian species, 8 were from 6 avian species, and 95 were from 4 reptilian species. All sera were acetone-treated to remove nonspecific inhibitors and were examined with 5 arbovirus antigens: Eastern and Western encephalomyelitis, group A; Powassan and St. Louis encephalitis, group B; and Cache Valley of the Bunyamwera group. Group A reactivity was markedly less than group B and only one serum reacted with Cache Valley antigen. Group A activity with Eastern encephalomyelitis antigen was shown in 14 sera (1.5%), 10 of which were from the cold blooded groups; Western encephalomyelitis reactions were noted in 22 sera (2.4%), 19 of which were from Tamias striatus (chipmunks). Group B reactions were equally divided between Powassan (147 sera, 16.3%) and St. Louis encephalitis (156 sera, 17.4%). The single Bunyamwera group reaction was from Chrysemys picta. Cross reactions occurred in 88 sera in group B, but in only one sera in group A. No reactions whatsoever were found in 5 of the avian, 5 of the mammalian, and 1 of the reptilian species.

Neutralization tests in suckling mice with the five arbovirus antigens were completed for all sera collected in 1964 but are only partially complete for the sera collected in 1965. Of 252 sera examined with Eastern encephalomyelitis, detectable neutralizing antibodies were found in one serum of Chrysemys picta; increased average survival time was noted on one serum from a Lepus americanus. Of 193 sera examined with Western encephalomyelitis, 2 of 3 Vulpes fulva and one of Lepus americanus showed antibodies; prolonged average survival time was noted for 5 Marmota monax and 1 Procyon lotor.

In Powassan neutralization tests with 396 sera protection was noted only in the following mammalian sera: 1 Erethizon

dorsutum, 1 Lepus americanus, 51 Marmota monax, 1 Mephitis mephitis, 1 Mustela erminea, 2 Mustela frenata, 1 Napaeozapus insignis, 5 Procyon lotor, 2 Sciurus carolinensis, 2 Tamiasciurus hudsonicus, and 2 Tamias striatus. Increased survival time was noted for 2 Peromyscus maniculatus and 2 Tamias striatus. Neutralizing antibodies for St. Louis encephalitis, however, were detected in only 5 of the 235 sera, 3 Marmota monax, 1 Lepus americanus and 1 Chrysemys picta. No neutralizing antibodies for the Bunyamwera group were found in 116 sera tested.

Correlation between the HI test and the suckling mouse neutralization test with Powassan was excellent for the Marmota monax sera, the only group in which both methods have been completed for all survey samples. Results are shown in the table. Activity with St. Louis encephalitis antigen may be due to group B cross reactions.

Combination	Group B			
	Powassan		St.Louis encephalitis	
	Number	Per cent	Number	Per cent
HI + * and Neut. +**	39	55.9	2	2.9
HI - and Neut. + or ? ***	12	17.1	1	1.4
HI + and Neut. -	2	2.8	34	48.6
HI - and Neut. -	17	24.2	33	47.1
Totals	70	100.0	70	100.0

*titer reproducible, 10 or higher.
**75 per cent or more of animals survive.
***Less than 75 per cent of animals survive.

Human sera.

One hundred and two sera from 61 residents of several other counties in New York State who had been suspected of having encephalitis were examined by HI tests. Two convalescent sera from two patients, and paired sera from a third had reproducible titers of 10 with St. Louis encephalitis antigen. Neutralization tests with Powassan and St. Louis encephalitis viruses were done on five sera from three patients and on one of these patients, paired sera were tested also with the group A and Cache Valley viruses. Neutralizing antibodies for Cache Valley virus were noted.

Twenty-eight samples from healthy members of the collection team for the St. Lawrence County survey, the St. Lawrence Power Authority personnel on Barnhart Island, and staff members of Massena Hospital, were also examined, none of which reacted by the HI test, but neutralizing antibodies for Eastern and Western encephalomyelitis were found in the serum from one person who was known to have previously been immunized against group A arboviruses.

(Elinor Whitney)

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

Studies on Neutralization Test with Sindbis and Whataroa Viruses.

During investigations into serological relationship among several strains of Sindbis virus isolated in various parts of the world and between them and a newly described agent, Whataroa (M 78) virus, marked differences were observed - see Table I - in the values of the neutralization index of a serum when the same serum-virus mixture was injected simultaneously into three separate host systems, namely: primary chick-embryo monolayers for plaque inhibition, baby hamster kidney cell (BHK 21) cultures for CPE inhibition, and 2-4 day newborn mice for intracerebral (IC) neutralization test. All three systems were equally sensitive to infection with the virus strains used, the mouse LD_{50}, the TCD_{50} and the plaque titers for all strains being between $10^{8.3}$ and 10^{10} per ml. The virus stocks used were 20% suspensions of infected newborn mouse brain in 7.5% bovine plasma albumin in buffered saline: the immune sera were produced in adult mice by giving them an intraperitoneal (IP) injection of formalin inactivated virus stock followed two to three weeks later by a second IP injection, of fully active virus in dilution 10^{-1}. Later serum samples and ascitic fluids from the same mice also gave similar differences in neutralization index values. The diluent in all the tests was 0.75% bovine plasma albumin in buffered saline. The serum-virus mixtures were incubated either at 4^{o} C overnight or at 37^{o} C for one hour; the same differences were noted with either method. The diluted virus-constant serum technique was used throughout.

In later studies and following the suggestion of Dr. P.H. Coleman, CDC, Atlanta, Ga., similar comparative neutralization tests were carried out with Sindbis (strain Ar 1055) virus in the presence of different diluents, as follows: a/, 10% normal unheated rabbit serum in phosphate buffered saline; b/, 10% unheated newborn calf serum in Hanks' balanced salt solution; c/ 0.75% bovine plasma albumin in phosphate buffered saline; and d/, undiluted, fresh, unheated rhesus monkey serum. It can be seen from Table II that in each host system that fresh monkey serum diluent was superior to the others in enhancing neutralization, but that, as was seen previously, the mouse test was superior to the other two with all the diluents.

These results have been tentatively interpreted as indicating that Sindbis virus is very sensitive to an accessory factor in fresh serum that promotes neutralization of the virus by immune sera, and that after inoculation of serum-virus mixtures into newborn

mice, enough of the factor is supplied by the mouse itself to give superior neutralization than in either tissue culture system. The observation that accessory factors increase neutralization of a virus by a serum is not new, but if this interpretation of the results shown proves to be correct, this would make Sindbis an excellent agent with which to study the nature of these serum factors, about which little is known at present.

(T. Maguire, S.M. Buckley, J. Casals)

Table I

Neutralization Indices of Sera Against Sindbis and Whataroa Viruses in Different Host Systems

Virus	Sera	Neutralization Indices		
		BHK 21	Chick embryo monolayers	Mice
Whataroa (M 78)	Whataroa, M78	1.0	1.6	> 5.5
	Sindbis, C 263	1.0	1.1	> 5.5
	MRM 39	1.0	1.2	4.6
	C 377	1.0	2.1	> 5.5
	P 886	0.0	1.0	3.6
Sindbis (EgAr 339)	Whataroa, M 78	0.0	1.8	3.0
	Sindbis, C 263	0.4	2.1	3.1
	MRM 39	0.2	2.2	3.7
	C 377	0.0	2.5	4.4
	P 886	0.0	2.2	3.7
	EgAr 339	0.0	2.2	4.0

Table II

Neutralization of Sindbis Virus (Ar 1055) by Homologous Serum in the Presence of Various Diluents

Diluent	Neutralization Indices		
	Chick Embryo Monolayers	Hamster Cells BHK 21	Mice
a. 10% rabbit serum	1.5	1.2	3.5
b. 10% calf serum	1.4	1.2	2.1
c.0.75% BPA	1.5	1.8	3.9
d. 100% monkey serum	4.2	3.0	> 4.4

REPORT FROM ENCEPHALITIS FIELD STATION MASSACHUSETTS DEPARTMENT OF HEALTH

Testing of the 1965 arthropod pools has been completed. WE virus was isolated from one pool of 50 Culiseta melanura collected on August 15 and 16 at Pine Swamp, Site I in Raynham, Massachusetts. This was the only isolation from the 435 pools tested.

A total of 429 wild mammals were sampled in 1965. All of the samples were tested for EE and WE virus and antibody. All were negative except one cottontail rabbit which was previously reported positive for EE antibody.

A total of 609 residents and migratory birds were captured at Site I, Site IV and the Dike for the months of July through October. EE virus was isolated from a chickadee netted at Site IV on October 19, 1965. This was the only wild bird collected in 1965 which was in viremia when bled.

A total of 330 blood samples were taken during the 1965 survey of immature migratory birds at Duxbury Beach. All were negative for EE and WE virus. Antibody tests on these blood samples have not been completed.

A total of 117 blood samples from 8 species of reptiles were taken in 1965. All of the samples were negative for EE and WE virus and antibody.

The results of the 1964-65 Virus Overwintering Study in Turtles indicates that three spotted turtles, experimentally infected with EE virus, were in viremia for a period of 209 days when tested previous to and following winter hibernation. Another experimentally infected spotted turtle circulated WE virus for this same period.

(Robert J. Tonn)

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

By inoculation of suckling mice, Powassan virus strains were isolated from 7 of 122 pools of Ixodes cookei ticks removed from groundhogs (Marmota monax) which were collected in northern Ontario between 8 May and 9 September 1965. Virus isolations were achieved in 5 of 7 pools collected during late May and early June. Virus titers in tick suspensions after storage at -70° C for approximately one month ranged from 2.0 to 5.5 $\log_{10}TCD_{50}$ per ml., when tested by inoculation of monolayer cultures of primary swine kidney epithelial cells. Neutralizing antibody to Powassan virus was detected in 148 of 350 groundhogs. The incidence of antibody in groundhogs born during 1965, which increased steadily from zero in May to 25% during August, in contrast to the slight change of antibody prevalence in animals born during previous years (54% in May and 42% in August), suggested that especially the current season's animals acquired active infections during spring and summer.

Silverwater virus was isolated from one of 13 pools of Haemaphysalis leporispalpustris ticks removed from snowshoe hares (Lepus americanus), and sera from 3 of 9 hares neutralized this virus.

(Donald M. McLean)

REPORT FROM THE VIROLOGY SECTION, ANIMAL HEALTH DIVISION NATIONAL ANIMAL DISEASE LABORATORY, AMES, IOWA

A non hemagglutinating virus isolated from a pool of Aedes trivittatus collected at Ames during the summer of 1964 has been identified by the CDC Arbovirus Laboratory as Trivittatus virus. Mosquito collections were made at the NADL Animal Production Unit at Ames during the summer of 1965. These are being identified, pooled and ground but mouse inoculations cannot be made until cage space is available.

Fifteen small pools of arthropods collected in Mesa County, Colorado, during the 1965 Indiana type vesicular stomatitis outbreak are being prepared for inoculation.

An outbreak of Indiana type vesicular stomatitis virus involving horses and cattle was first noted near Espanola, Rio Arriba County, New Mexico, on July 25, 1965. This spread throughout western New

Mexico and southwestern Colorado during the summer. One case was diagnosed in adjacent Utah and several in Arizona. Indiana type VSV was diagnosed in 236 herds involving 18 counties. Human infection was suspected in New Mexico and investigated by CDC.

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

Thirteen suckling-mouse-lethal agents were isolated from arthropods captured in Wisconsin during 1965. Although not as yet identified, these agents appear to be unrelated serologically to Eastern, Western, St. Louis and California encephalitis. Twelve of the thirteen killed adult mice and none of the cultures so far tested have been cytopathic for HeLa cells or lethal for chicken embryos.

	No. Pools	No. Isolations
Aedes vexans	173	1
A. trivittatus	99	2
A. sticticus	88	1
A. stimulans	77	4
other mosquitoes	361	0
Culcoides sp	7	1
Simulium meridionale	93	1
other black flies	150	0
Chrysops celeris	9	1
other deer flies	104	0
Hybomitra lasiophthalma	247	1
other horse flies	743	0
Haemaphysalis leporis-palustris	28	1
other ticks	32	0
	2211	13

In 1964 and again in 1965, blood and tissues were obtained from reptiles and amphibians collected in Wisconsin. In 1964 the inocula were given to 10-day-old chicken embryos. In 1965 three day old suckling mice were injected. No isolations were recovered from 225 turtles, 198 snakes, 14 lizards and 15 amphibians. In 1965 some specimens were subjected to temperature fluctuations (4^{o} C to 25^{o} C etc.) and blood repeatedly drawn. All blood samples were negative.

Sera collected from cold blooded animals were also tested for neutralizing antibodies in tissue culture using the metabolic

inhibition test. Seven viruses were employed: Eastern, Western, St. Louis, California and Venezuelan encephalitis, Indiana and New Jersey vesicular stomatitis virus. Only 14 reactors were found and these were to St. Louis encephalitis--10 of 436 painted terrapins, 3 of 22 blanding turtles and 1 of 2 leopard frogs.

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

Serologic tests were conducted with five arbovirus antigens (Eastern, Western, St. Louis, California and Powassan virus) on sera from 220 patients hospitalized with a variety of febrile illnesses throughout Wisconsin during 1965.

There was no antibody evidence of Eastern, St. Louis or Powassan encephalitis virus in the sera of these 220 patients.

Fourteen cases of California encephalitis were identified with an HI antibody titer of 1:20 or more in their convalescent serums and corresponding rises in HI or CF antibody levels in their acute and convalescent phase serums. One case (an 8-month-old boy) had a 2-fold rise in antibody level while the other 13 cases each had a 4-fold or greater rise in California antibody titers; each convalescent serum also neutralizing 2 or more logs of the virus in mice. (California encephalitis virus, Hammon and Reeves' BFS283 strain, was used as an antigen in the HI tests and the La Crosse human isolate strain of California group virus was used in CF and neutralization tests).

Twelve of the 14 cases had histories of exposure in forested areas of western or northern Wisconsin, and the other two cases were from similar rural areas in SE Minnesota and NE Iowa, hospitalized in La Crosse. These cases include 13 boys and 1 girl, 8 months to 16 years of age, who were hospitalized with a diagnosis of meningitis or encephalitis during July (4), August (4), September (5), and October (1). All have recovered with no evidence of paralysis or other readily apparent sequelae. The clinical and epidemiologic aspects of these cases are similar to those of the 23 cases reported during the previous years (1960-64) in Wisconsin, indicating continuing endemic virus activity in the area. Epidemiologic evidence so far indicates exposure primarily on farms in forested areas, however 4 of the cases this year occurred in children playing around their suburban homes recently built in the forested hills around La Crosse.

One case of Western equine encephalitis occurred in a 15-year-old boy hospitalized with encephalitis during the last week in August, with probable exposure either around his home in Packwaukee, a small town in central Wisconsin, or during a trip to South Dakota about 12 days before his illness. This is the first known serologically confirmed case of Western encephalitis to be reported from Wisconsin.

Western equine encephalitis virus activity was also found in horses.

Tests were conducted on sera sent in for diagnostic tests by practicing veterinarians from 40 horses which had clinical encephalitis this summer and fall. Sera from 26 of these horses had an HI antibody titer of 1:40 or more to Western encephalitis virus. In CF tests of paired sera received from 18 of the 26 positive horses, 10 had 4-fold or greater increases in antibody, 6 had 2-fold increases, and 2 had CF antibody with no change in titer. Eight of these horses died. Three of the 40 horses had antibody to California virus in their serums, but there were no rises in titer to associate this infection with encephalitis. Hemagglutination-inhibition tests with Eastern, St. Louis, and Powassan encephalitis virus antigens were negative.

Field studies of the circumstances of infections with California encephalitis group virus are under way on several farms where children live who have been infected with California encephalitis virus. One study area is a dairy farm with wooded hill land in Iowa County, southwestern Wisconsin, and the other one is a dairy farm with wooded hill land in Spring Valley Coulee 12 miles south of La Crosse. Antibodies were present in some of the dairy cattle on these farms. Approximately 100 small wild mammals were collected on these farms. Antibodies to California virus were detected in sera from 4 of 12 large chipmunks (Tamias) and 4 of 22 tree squirrels (Sciurus) collected from a forested area. Sera from five other species so far including 29 ground squirrels (Citellus tridecemlineatus), 6 field mice (Peromyscus), 6 rabbits (Sylvilagus), 2 small chipmunks (Eutamias), and 2 opossums (Didelphis) were negative in tests for California group virus antibodies. Two sentinel rabbits placed in this same forested area during August and September acquired antibodies to California group virus.

REPORT FROM TEXAS STATE DEPARTMENT OF HEALTH LABORATORY AUSTIN, TEXAS

In 1965, a total of 42,710 mosquitoes, most of them Culex quinquefasciatus and C. tarsalis, were tested in 735 individual pools. From this material 64 virus isolates were made, viz., 41 Hart Park, 10 WEE, 6 SLE, 4 Turlock, 1 California, and 2 as yet unidentified.

Most of the Hart Park isolates were obtained from C. quinquefasciatus mosquitoes collected in Houston - 1 isolate in January, 28 in June, 2 in July, and 2 in August. No mosquitoes were collected in Houston after September 1st. Additional Hart Park isolates include 5 from C. quinquefasciatus collected in Fort Worth in July and August, 1 each from C. tarsalis collected in Wichita Falls (June), and Lubbock (September), and a mixed Hart Park-SLE isolate from a pool of 14 C. tarsalis collected in Hale County in August.

Ten WE isolates from C. tarsalis were made, 9 of them from Hale County (August) and the tenth from Cameron County in April. Intensive collection of mosquitoes was carried on in the Lower Rio Grande Valley (Cameron County) during the winter and early spring, this being the only time of year when C. tarsalis is found there in appreciable numbers. In addition to the WE isolate previously mentioned, 4 Turlock isolates were obtained from C. tarsalis collected in February, March and April and 1 California virus isolate from Psorophora confinnis collected in July. This isolation of CEV is the third recovery of this virus from Texas mosquitoes, the other two having come from Anopheles pseudopunctipennis (Tom Green County, 1958) and P. confinnis (Lubbock County, 1961).

Fort Worth (Tarrant County) experienced a high population index of mosquitoes, particularly C. quinquefasciatus, during the summer and fall months. In addition to the five Hart Park isolates previously mentioned, 5 SLE isolates were made from pools of C. quinquefasciatus collected there in July and August.

Serological tests on sentinel chicken sera from Orange, Harris, Cameron, and San Patricio counties in 1965 were entirely negative, as were tests on non-sentinel chickens from scattered areas including Dallas County. Two of six duck sera from Dallas were reactive in WE HI tests. Of more than 200 pigeon sera principally from Lubbock not more than 5% mostly WE were reactive in HI tests. It was significant perhaps that the pigeons were bled mostly in late spring and early summer.

To date (December 15, 1965) 58 human cases of encephalitis were serologically confirmed (30 WE, 28 SLE). Forty-two equine cases

were confirmed for Western encephalitis. One WE isolate was obtained from a horse brain.

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

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

IN COLLABORATION WITH THE COLORADO STATE DEPARTMENT OF PUBLIC HEALTH AND THE TEXAS STATE DEPARTMENT OF HEALTH

The largest encephalitis outbreaks on record for the state of Colorado occurred during 1965. Following extensive flooding during mid-June in the eastern part of the State, mosquito population increased to unusually high levels. By early October 260 human cases had been reported, exclusive of cases which upon review of clinical history were thought to represent illnesses other than encephalitis. Based upon clinical history, at least 240 equine cases occurred. Serologic study of paired sera indicated that 77 human cases had WE, and 18 had SLE; 131 showed no serologic evidence of recent WE or SLE infection and 33 were either equivocal or insufficient sera or information prohibited an assignement to etiology. Assessment of the WE infection rate of Culex tarsalis on a weekly basis indicated the highest rates on record for the state. The period of virus activity in C. tarsalis was unusually prolonged, extending from the fourth week of June to the third week of September. A total of 134 WE isolations were obtained from C. tarsalis, along with isolations of SLE and Turlock viruses. A Cache Valley-like agent was isolated from Aedes dorsalis. Two WE isolations were obtained from A. vexans and one from A. dorsalis. Sentinel chickens' acquisition of antibody was indicative of the unusually high WE virus activity; by 21 July, 23 percent of the chickens in one flock had WE antibody and none had SLE antibody; by the end of the season 83 percent had WE antibody and 19 percent had SLE antibody. Details of the outbreak are being prepared for publication.

A smaller encephalitis outbreak occurred in our Hale County, Texas, study area during 1965. Serologic studies are not complete, but all of the patients with diagnostic antibody rises were shown to have had WE. While the number of suspect human cases (45) were small compared to past years and no equine cases were reported, study of the outbreak did afford some interesting observations. The C. tarsalis population was significantly delayed in reaching maximum levels, and these maximum levels were generally lower than levels attained during previous years. However, the weekly

C. tarsalis WE infection rates were the highest ever found in our studies. Using the method of Chiang and Reeves (1962) the infection rates varied from 11.5 per 1000 to 45 per 1000 during July and August. WE was isolated from 69 C. tarsalis mosquito pools along with Turlock and Hart Park. Nestling house sparrows had WE virus from mid-June through mid-August. Small mammals from the Hale County area also gave evidence of WE virus activity; 6 isolations of WE were obtained from jackrabbits in July and other as yet unidentified virus isolates were made from mammals. Sentinel chicken flocks had WE antibody rates of 85-96 percent by the end of the season; 30-78 percent had SLE antibody. This outbreak is still under study by CDC, Greeley, CDC, Kansas City, and the Texas State Department of Health.

REPORT FROM THE CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH AND THE ROCKEFELLER FOUNDATION ARBOVIRUS STUDY UNIT BERKELEY, CALIFORNIA

There were nine confirmed cases of WE encephalitis in man in California in 1965. The first case occurred in Shasta County, with onset August 11th. There were three cases in August, three in September and two in October. One of the cases that occurred in August sickened in San Diego County but it is presumed that this person was infected while visiting in Montana. The one case from Orange County was hospitalized in Fresno County, and it is presumed that this individual was infected in Fresno County. The other cases were from Sacramento, San Joaquin, Shasta, Sutter, Tehama, Tulare and Yolo Counties.

There were 59 confirmed cases of WE encephalitis in horses in California during 1965. A total of 181 clinical cases of horse encephalitis were positive for WEE. One case was from 32 miles east of Elko with onset September 20th, and the other was from 30 miles east of Battle Mountain with onset August 15th. The first case in California occurred July 21st in Tehama County. Among the confirmed cases where the date of onset was known, there were four cases in July, 16 in August, 15 in September and 1 in October. The disease was particularly active in Fresno, Kern, Sacramento and Shasta Counties.

WEE virus was isolated from three gray tree squirrels, Sciurus griseus. Two of these were from Butte County, one found sick on July 16th and the other on August 15th. One of the positive tree squirrels was from Shasta County. This animal was found sick on

September 5th. WEE virus was isolated from tree squirrels found sick in Butte County in 1953 and 1955 and from ground squirrels found sick in this county in 1955 and 1962.

Large flocks of blackbirds were seen feeding in irrigated pastures where clinical cases of encephalitis in horses were investigated in Sacramento and Fresno Counties. Most of these were Brewer's blackbirds. The number of Brewer's blackbirds increases in the late summer and fall in irrigated agricultural land and in grain fields, and presumably these birds do migrate in large numbers into California in the fall. These birds do not maintain a definite roosting place but move from place to place according to feeding conditions. They are usually seen in flocks of several hundred birds. There is a large influx of redwinged blackbirds and starlings into California in the fall. The estimated winter populations in known roosting areas is more than 30 million birds.

There was one case of encephalitis in Glenn County with onset August 1st. It is presumed that this person was infected with St. Louis virus. The first blood taken on September 16th had a CF titer of 1:32 for SLE virus. The second specimen taken on September 30th had a titer of 1:64.

There were eight cases of Colorado tick fever, three with onset in June, four in July and one in November. Two were exposed by tick bite in Nevada and two by tick bite in Wyoming and Idaho.

Hughes virus originally isolated from Ornithodoros ticks collected on Bush Key, Dry Tortugas, Florida, in 1962, by the staff of the Rocky Mountain Laboratory, Hamilton, Montana, was isolated from a pool of 20 Ornithodoros ticks (capensis group) collected on the Farallon Islands, just west of San Francisco, between July 5 and 9, 1965, from the nests of California gulls.

A field trial of an attenuated WEE live virus vaccine for horses was carried out in 1965. A total of 367 horses and one donkey were vaccinated. There were no illnesses attributable to the vaccine and the pregnant mares that were vaccinated foaled normally.

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

The following details are summarized from work done by Kaiser under the Guest Researcher Program in the Virology Section of the

Communicable Disease Center, Atlanta, and under an NIAID training grant at Emory University.

The life cycle of Argas (Persicargas) arboreus, Kaiser, Hoogstraal, and Kohls, reared at 27° C and 75% relative humidity requires approximately three months. Survival periods recorded for each developmental stage and instar when unfed suggest that should the bird hosts fail to return to their usual nests for one year, the existence of the tick deme would be severely jeopardized, and that larvae and first instar nymphs are unable to remain alive during the winter, when heron hosts are absent from nesting sites. The standardized laboratory model for rearing the argasid ticks developed for this study is useful for various biological research needs and for obtaining large number of uninfected ticks for microbiological investigations. Using these techniques, it was observed that oviposition is delayed, even under controlled laboratory conditions, in winter collections of female ticks from both Egypt and South Africa.

Laboratory chickens and pigeons appeared to develop resistence to bites of these ticks but in some instances to succumb to tick paralysis during feeding by the parasites. Bird hosts from which ticks were removed in the early stages of paralysis appeared to be immune to paralysis for at least a year following recovery.

A. (P.) arboreus ticks were collected monthly between March, 1963 and February, 1964 from a nesting site of Bubulcus i. ibis L. (cattle egret) in the Nile Barrage Park near Cairo, Egypt. A total of 2,500 of the ticks, tested in 500 pools of 5 each, yielded 42 strains of Quaranfil virus and 76 strains of Nyamanini virus. No virus strains were recovered from 2,400 specimens of A. (P.) persicus from domestic chickens in the same general area.

Natural infection indexes of both viruses showed an early spring rise and a less marked rise in the fall. The spring rise of Nyamanini was maintained through most of the summer months; that of Quaranfil dropped in midspring and remained at a low level until fall.

While no Nyamanini viremia resulted from experimental inoculation of newly hatched domestic chicks, Quaranfil viremia occurred regularly and death ensued on the fourth and fifth days. Nyamanini virus is postulated to be transovarially transmitted by A. (P.) arboreus.

The possibility of concurrent natural infection of single ticks with both viruses appears to be slight. The presence of residual blood in field-collected A. (P.) arboreus appeared to have little

or no diminishing effect on virus detection in the laboratory. Quaranfil and Nyamanini viruses were found to be antigenically unrelated to each other or to Chenuda virus and to differ in pathogenicity to experimental animals and in tissue culture.

It is concluded that Quaranfil and Nyamanin viruses are primarily bird viruses. A. (P.) arboreus, the tick vector which is sustained by and restricted to the avian host population, provides for the maintenance and dissemination of these viruses.

Experimental transmission of Quaranfil virus by A. (P.) arboreus indicated a high vector potential of this species. Widespread infection beyond the digestive tract of the tick usually occurred by and after 44 days of extrinsic incubation.

A. (P.) persicus, the fowl tick, could be experimentally infected with Quaranfil virus and retained the virus for up to 120 days. However, transmission was accomplished only by mass feeding, indicating that this species is a poor vector. Since the virus was detected in only one of 25 coxal fluid samples, poor spread of virus beyond the digestive tract is suggested. Mechanical transmission through regurgitation of partly digested blood retaining some active virus might have accounted for the transmissions noted.

Transstadial transmission of Quaranfil virus was obtained with both tick species. The failure to demonstrate transovarial transmission of Quaranfil virus in A. (P.) arboreus, the natural vector, indicates that a vertebrate host is necessary for perpetuation of this virus.

Genetically controlled differences in susceptibility to Quaranfil virus account for the high vector efficiency of A. (P.) arboreus and the low efficiency of the closely related A. (P.) persicus. Ecological factors appear to be of secondary importance in the virus-tick relationships. These studies demonstrate the need for meticulous taxonomic investigations to accompany research on vector efficiency, pathogen transmission, and arthropods in relation to epidemiology of disease.

Infection of A. (P.) arboreus with the virus of St. Louis encephalitis was attempted. Only a low proportion became infected and transmission by bite was not accomplished. Infection of nymphs and trasstadial transmission of the virus by nymphs was shown once in nine attempts. Trasovarial transmission of the virus was not demonstrated. Virus was retained in some infected females for at least 52 days, despite failure to transmit. The possibility that other bird-feeding ticks may serve as reservoirs of SLE virus should be considered.

(H. Hoogstraal and M.N. Kaiser)

REPORT FROM VIRUS DIAGNOSTIC LABORATORY SHIMOGA, MYSORE STATE, INDIA

The Kyasanur Forest Disease (KFD) epidemic started in 1965-1966 rather earlier than in any previous year. KFD virus isolation from a human case and monkey autopsied specimens were achieved in the month of November for the first time since the beginning of the investigation of KFD in 1957.

The disease has shown a spread in the south-west direction, involving new villages located towards south-west of Sagar Town in 1966.

Beginning from November 1965, KFD virus has been isolated from 26 out of the 37 autopsied monkeys and 69 out of 127 human cases suspected of KFD.

(Sridhar Upadhyaya)

REPORT FROM THE MEDICAL RESEARCH INSTITUTE COLOMBO, CEYLON

Arbovirus antibodies to Group B infections were first demonstrated to be present in Ceylon sera by the Virus Research Centre, Poona on a small sample of bloods from ? Virus encephalitis cases in the years 1957 and 1960-1962. Group B arbovirus antibody was also found in a random sample of Ceylon sera tested in Dr. C.E. Gordon Smith's Arbovirus Research Unit at the London School of Hygiene and Tropical Medicine.

On the basis of these findings, the writer, in 1962 obtained seed virus of the prevalent Group B arboviruses from Dr. Gordon Smith, and propagation of haemagglutinating antigens according to Casals and Brown was carried out.

Arbovirus serology was commenced by the Virus Laboratory, M.R.I. in 1963. Results of the arbovirus serology done in the Administration years 1963, 1964 and 1965 are summarised in Table 1. (1965 results also included Group A Chikungunya which will be explained later). Positive results were assessed on a four-fold or greater rise in titre on paired sera, and on titres of 1:80 or greater in single sera. Negative results were on sera showing $<$1:80.

Table 1.

Summary of arborvirus serology for Administration Years 1963, 1964, 1965

Year	Paired sera	Single sera	Positive Group B.	Positive Group A.	
Oct. 1962-Sept. 1963 (1963)	21	71	16	Not done	
Oct. 1963-Sept. 1964 (1964)	45	65	44	Not done	
Oct. 1964-Sept. 1965 (1965)	196	527	42	100	

1965 Epidemic caused by Chikungunya and possibly Dengue Viruses.

In February, 1965 clinicians reported seeing cases resembling dengue fever, but where the joint and muscular pains were severe and persisted for weeks after the acute illness. Most of the cases were in the paediatric wards of the Childrens' Hospital, some were reported from the medical wards of the General Hospital, and also cases were seen by private practitioners in their clinics. As the clinical picture closely resembled chikungunya fever, the seed virus was obtained, propagated under strict mosquito-proof conditions, and HA antigen was prepared (sucrose-acetone extracted).

In April 1965, chikungunya antigen was included in the HI serology of eight selected cases giving reliable histories suggestive of chikungunya fever. These eight sera were then sent to Prof. Lim Kok Ann of the Enterovirus Laboratory, Singapore for confirmation of our findings. Their results by CFT showed co-relation with ours obtained by HI and established that chikungunya virus was present in Ceylon in epidemic proportions. The serological results on these eight sera by HI and CFT are tabulated in Table 2.

Table 2

1965 Serological results of Cases tested by HI (Virus Lab. Colombo) & CF (Virus Lab. Singapore)

Name	Age/Sex	Date of collection	Day of illness	JE		D1		D2		Chik	
				HI	CF	HI	CF	HI	CF	HI	CF
1. Weerasinghe	40/F	9.4.65	3	160	16+	160	128+	160	128+	<10	128+
		30.4.65	24	160	ac	160	ac	160	ac	40	ac
2. Smith	29/F	11.4.65	5	20	Ins	20	Ins	20	Ins	<10	Ins
		12.5.65	36	80	8	80	32	160	16	160	128
3. Ratnatunge	18/F	21.4.65	10	160	ac	160	ac	320	ac	<10	ac
		27.5.65	46	160	16	160	128	320	64	40	64
4. Anura	6/12M	3.5.65	13	10	Ins	10	Ins	10	Ins	80	Ins
		19.5.65	29	10	<4	10	<4	10	<4	320	128
5. Kuganesan	26/M	12.5.65	4	40	8	40	16	80	32	<10	4
		27.5.65	19	160	8	40	32	80	64	320	128
6. Abeyratna	30/F	26.4.65	22	160	32	160	64	320	64	80	128
7. Bastiampillai	33/M	6.5.65	41	1280	128	640	256	1280	256	80	64
8. Shanmuganathan	30/M	6.5.65	45	40	4	20	8	20	4	40	64

+ Possible interchange of the acute and convalescent specimens.

Subsequent to the epidemic, there was an appreciable increase in the number of sera sent for arbovirus investigation. The peak months appeared to be June and July 1965. Table 3 gives the monthly statement of arbovirus serology for the year 1965, and the last three months of 1964 for comparison. The discrepancy between the number of sera received and examined is explained by the fact that many were haemolysed or decomposed and unfit for serology; also single sera were not examined unless the Lab was notified that the patient had expired before a second specimen was collected, or the history justified the examination of one sample only. From Table 3 it will be seen that of 347 sera tested for chickungunya antibody 112 (32%) were positive. Of the 112 positive, 102 or 91% showed a serological conversion from a titre of <1:10 in the acute specimen to more than a four-fold rise of titre in the convalescent specimen.

Virus isolations from cases of "dengue-like" fever in 1965.

Attempts were made from April, 1965 to isolate virus from blood or post-mortem specimens of cases clinically diagnosed as "dengue-like" fever. Eleven specimens were passaged in suckling mice, and mouse brain suspensions were checked by HA and identified by CFT. Rise of these attempts at virus isolation were failures due to various technical reasons. Improved technical conditions resulted in two positive virus isolations in August-September 1965.

Chikungunya virus isolation from two cases.

Case 1. An adult male gave a history of high fever and severe joint pains of one day's duration. Blood was collected within 48 hours of onset of illness by veni-puncture and transported in ice to the Virus Laboratory. The blood clot was ground in pH9 borate buffer, and this inoculum was injected both undiluted and in a 1:100 dilution into baby mice; three mouse passages were carried out and in all three the mice showed characteristic illness ie severe cramp-like convulsions followed by paralysis and death within 24 hours. From the three mouse-brain passages virus was tested for by HA and identified by CFT using specific antisera. Serology was also done on the acute and convalescent specimens of serum. Table 4 gives a summary of virus isolation procedure, and virus serology of Case 1.

Case 2. A female child aged 4-1/2 years, was admitted to the Childrens' Hospital in a semi-conscious state with a history of high fever and vomiting of one day's duration, and followed twelve hours after onset by a generalised purpuric rash over the whole body, but most marked over the arms and legs. Blood was collected by veni-puncture within 48 hours of onset of illness and transported to the Virus Lab in ice. The patient expired the next day,

but no post-mortem was allowed. The blood clot was treated as in Case 1 and similarly inoculated into baby mice. The mouse-brain passage (three) were tested by HA and CFT. Serology was also done on the acute specimen. Table 5 gives a summary of virus isolation procedure and serology of Case 2.

Virus isolations from mosquito pools.

From October to December, 1965 attempts were made to isolate virus from mosquito pools, using mosquito catches from homes of serologically positive chikungunya cases, and from areas in the city of Colombo where there appeared to be a preponderance of "dengue-like" cases. Seven pools were examined according to species, five Aedes aegypti, one Culex fatigans and one Armigeres obturbans (a species very prevalent in homes in the City). About 40 mosquitoes were included in a pool. Baby mice were inoculated and mouse-brain suspensions checked for virus. No isolations were identified by HA and CFT.

Arbovirus antibody survey.

In October-November 1965 an arbovirus survey was carried out using a random sample of sera collected 6-8 months before the above mentioned chikungunya epidemic. These sera were obtained from children up to 12 years attending the Outpatients' Department of the Children's Hospital, Colombo (collection made for a Polio antibody survey); sera from adult age groups were collected from Army volunteers and from the Venereal Diseases Clinic at Colombo.

Results obtained from Group A (chikungunya) and for Group B (JE, D1 and D2) are listed, and the % +ves calculated in Table 6. These results establish that Group B arboviruses (JE and Dengue) are present in Ceylon in endemic proportions, and perhaps have been here for a considerable time. With regard to Group A arboviruses, the results obtained from the Virus Research Centre, Poona and the Arbovirus Research Unit at the London School of Hygiene established that chikungunya and related Group A viruses were not present in Ceylon sera; however, these were small samples and mostly collected from children. The results of this survey on age groups from 6 months - 1 year, to those over 50 years show definitely that chikungunya or a closely related virus was present over ten years ago in Ceylon, and now appears again in epidemic proportions. A survey is now being done to assess chikungunya antibody levels of similar age groups in a similar sample of sera collected after the chikungunya epidemic.

(In December, 1965, Dr. J. Vesenjak-Hirjan, WHO Consultant in Virology, was appointed to the Virus Laboratory, Medical Research Institute to advise re the setting up of a new Virus Diagnostic Laboratory to meet the growing requirements of the Island).

(Yvette Hermon)

Table 3

1964 - 1965 Monthly Statement of Arbovirus serology by haemagglutination inhibition Test

Month	1964			1965											
	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Sera received	8	16	12	11	21	22	13	21	241	247	45	70	51	77	56
Sera examined	2	5	5	5	7	16	6	16	102	77	14	38	17	51	26
Positive Group A only	ND+	ND+	ND+	ND+	ND+	ND+	3	6	51	37	1	4	3	6	1
Positive Group B only	1	0	3	5	0	3	0	1	7	12	2	7	1	14	7
Positive Group A & B	-	-	-	-	-	-	1	1	22	10	2	2	0	2	6
Negative	1	5	1	0	7	13	2	8	22	19	9	12	13	29	11

+ ND - Not done.

Table 4.

Summary of virological procedure for Case 1.

Material used for mouse inoculation.	Day of illness in mice.	HA	CFT
1. Original (blood clot)	3rd day	0	Negative
2. Mouse brain p ool (Passage 1)	2nd day	0	+(Undiluted)
3. Mouse brain pool (Passage 2)	2nd- 3rd day.	crude suspension = 0 prot. sulph. purified suspension = 1:40	+(undiluted and 1:4 dilution)

Serology of Case 1.

	JE		D1		D2		CHIK	
	HI	CFT	HI	CFT	HI	CFT	HI	CFT
Ac. serum (2nd day)	320	4	80	4	80	4	<10	<4
Conv. serum (48th day)	320	4	80	4	80	4	>1280	16
Conv. serum	40	<4	20	<4	40	<4	20	4

Table 5

Summary of virological procedure for Case 2.

Material used for mouse inoculation.	Day of illness in mice.	HA	CFT
1. Original (blood clot)	4th day	0	Negative
2. Mouse brain pool passage 1)	2nd day	crude susp. = 0 prot. sulph. = 1:10	+(undiluted)
3. Mouse brain pool (Passage 2).	1-2 days	prot. sulph. = 1:40	+ undiluted and ±1:4 dilution.

Serology of case 2.

	JE		D1		D2		CHIK	
	HI	CFT	HI	CFT	HI	CFT	HI	CFT
Ac. serum	< 10	< 4	< 10	< 4	< 10	< 4	< 10	< 4

Pt. expired on the 2nd day of illness.

Table 6

(ARBORVIRUS ANTIBODY SURVEY on sera collected before the Chikungunya Epidemic of February, 1965

Age	No. of sera	Group A (Chik)		Group B (JE, D1 & D2)	
		+ve	% + ve	+ve	% + ve.
6/12-1 Yrs	8	0	-	7	87.5
1 - 2 "	15	0	-	6	40
2 - 3 "	15	0	-	5	33.3
3 - 4 "	15	0	-	7	46.6
4 - 5 "	15	0	-	10	66.6
5 - 6 "	15	0	-	12	80
6 - 7 "	15	0	-	9	60
7 - 8 "	18	0	-	11	61.1
8 - 9 "	15	0	-	7	46.6
9 - 10 "	15	0	-	8	53.3
10 - 11 "	15	0	-	11	73.3
11 - 20 "	28	4	14.2	22	78.5
20 - 30 "	18	0	0	11	73.3
30 - 40 "	15	1	6.6	9	60
40 - 50 "	24	14	58.3	24	100
50 "	18	15	27.7	16	88.8

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

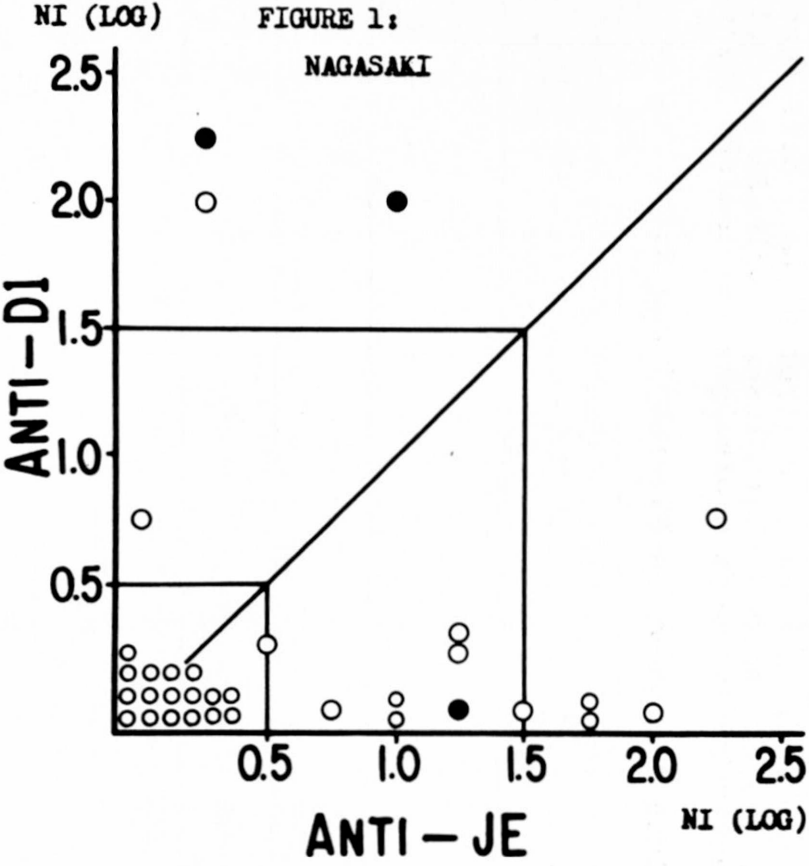
Survey of Anti-Dengue Antibodies in Sera of Residents of Japanese Mainlands.

Sera were collected during 1964 from residents of Nagasaki (where large epidemics of dengue occurred from 1942 through to 1944) and of Shiga (where no dengue epidemic has been recorded). All tested individuals were born before 1942, none of whom entered dengue-endemic areas after 1945. The sera received incubation at 56 C for 30 minutes immediately prior to test. No accessory substance was added.

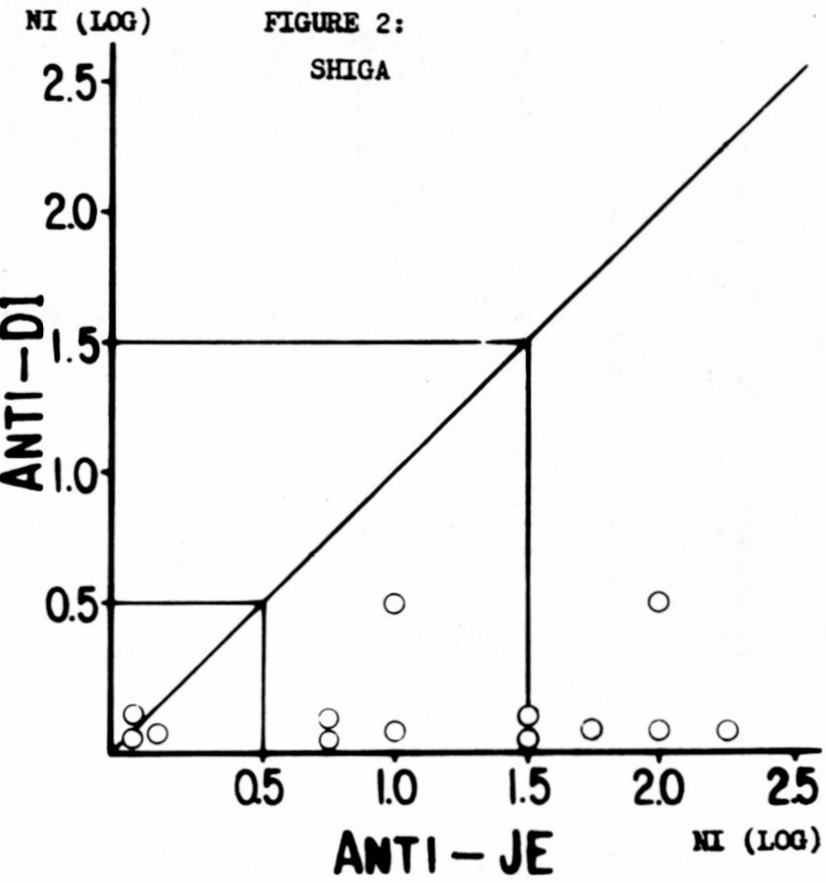
Mouse-passaged Mochizuki strain type 1 dengue (D1) and G1 strain Japanese encephalitis (JE) viruses were employed.

Constant amounts of serum (diluted two-fold in Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate) and serial dilutions of virus (infected mouse brain homogenates in the above diluent) were mixed in equal volume and kept at 37 C for 1 hour and at 4 C for an additional hour; 0.02 ml of the mixture was then inoculated into two-to three-week-old mice intracerebrally. Difco bovine serum which had been proved to have no anti-dengue neutralizing activity, served as control serum. Differences in mouse-LD_{50} of the tested and control sera, calculated applying the Reed-Muench formula, were neutralization indices (NI's).

Results obtained are shown in Figures 1 and 2.



Black dots indicate cases with confirmed history of dengue during the 1942-44 Nagasaki epidemics.


NI (LOG)
FIGURE 2:
SHIGA
2.5
2.0
1.5
1.0
0.5
ANTI-D1
0.5
1.0
1.5
2.0
2.5
ANTI-JE
NI (LOG)

Some of the sera from Nagasaki possessed anti-Dl neutralizing (NT) antibodies of significant titers, whereas all of those from Shiga did not. Anti-JE NT antibodies, on the other hand, were present in many of the tested sera from Nagasaki and Shiga as well. In both instances, there is apparently no correlation between anti-Dl and anti-JE NT antibodies, at least under the conditions adopted in the present investigations.

Human Reactivity of a Dengue Virus Strain Cultivated in Tissue Culture.

Type 1 Mochizuki strain virus, passed through more than ten serial subcultures in primary Japanese monkey (Macacus fuscatus) kidney cell cultures, was used. "Live virus" was harvested in infected culture medium. "Formalinized virus" was prepared by mixing the infected fluid with formalin (35% aqueous solution of formaldehyde) at a concentration of 0.2% and holding the mixture at 4 C for two weeks. A control inoculum consisted of non-infected culture medium.

Four adults who had had no opportunity for contacting dengue, were inoculated intracutaneously with the above inocula: Two with "live virus", one with "formalinized virus", and one with "control fluid". Doses injected were 0.4 ml, at four sites with 0.1 ml each. At intervals sera were separated and NI's measured. Results obtained are summarized in Table 1.

Table 1: Anti-dengue NT antibodies in sera of human volunteers inoculated with tissue-cultured dengue virus (type 1)

Volunteer		NI at time indicated								
Inoculated with	No.	Before inoculation	After first inoculation# (in weeks)				After second inoculation (in weeks)			
			1	2	4	6	1	2	4	6
Live virus	1	0*	0	6	0	0	32	18	>32	>1,000
	2	0	0	3	18	0	10	>56	18	10
Formalinized virus	3	0	0	0	0	0	0	0	0	0
Non-infected fluid	4	0	0	0	0	0	0	0	0	0

Second inoculation was made 19 weeks after the first inoculation.

* NI= <3 - 0.3

In the individuals receiving a single injection of "live virus", anti-dengue NT antibodies were produced, which rose rapidly after the second injection. In those inoculated with either "formalinized virus" or control fluid, no such immunogenic phenomena were evident.

All of the volunteers showed no abnormal signs following the first inoculation. After the second inoculation, however, those inoculed with "live virus" exhibited red patches, 1.0 to 1.5 cm in diameter, at the sites of inoculation, which became most distinct about 48 hours after the inoculation and lasted for 4 to 5 days. No such reaction was visible in the persons inoculated with "formalinized virus" or control fluid. Whether the "red patch reaction" is of allergic nature, or whether there is a correlation between this reaction and the skin reaction observed in dengue patients during the 1944 Japanese epidemic (Hotta, 1952), deserves further considerations.

(N. Fujita, S. Hotta, S. Sakakibara, Y. Shimazu, M. Tokuchi, Y. Yasui)

REPORT FROM LABORATOIRE DE MICROBIOLOGIE GÉNÉRALE ET MÉDICALE UNIVERSITÉ DE LIÈGE, BELGIUM

The biological properties related to the outer layers of Semliki Forest virus were studied.

We noticed, as did Westaway (1965), that fresh normal guinea pig serum inhibits the neutralization of infectivity by high concentrations of antiserum. This phenomenon is not related to the animal species of the antiserum donor. A detailed account of these experiments will be published in the Journal of Hygiene (Cambridge).

The efficiency of the adsorption of the virus to chick embryo cells monolayers was measured. Carried out at room temperature (25°), in Gey's balanced salt solution buffered with Tris (pH 7.6) containing 0.1% W/V bovine plasma albumin, the adsorption rate was + 75% of the theoretical rate calculated from the formulas of Valentine and Allison (1959).

We gathered evidence that the haemagglutinating activity is not necessary for infectivity, and that the infectivity can be destroyed without impairing nor liberating the viral RNA.

Electron micrographs showed that Semliki Forest virus has an outer envelope covered with thin projections (as reported in the 1963 annual report of the virus laboratory of the Rockefeller Foundation) and that this envelope contains a "core" which contains the RNA. Similar findings were reported for Sindbis virus (Mussgay and Rott, 1964) and recently for Venezuelan Equine Encephalitis virus (Klimenko et al., 1965). We did not observe the filament like structures described by the latter authors even when a large percentage of the particles were severely damaged. By means of enzyme treatment the projections could be removed without impairing the infectivity of the virus, but the infectivity was destroyed when the envelope was degraded.

Ultracentrifugation experiments gave a provisional value for the sedimentation constant of Semliki Forest virus $S_{w20} = 303$ which is in fair agreement with the value (345) reported in the 1961 annual report of the virus laboratory of the Rockefeller Foundation.

(Paul M. Osterreith)

REPORT FROM THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH LONDON N.W.7, ENGLAND

Phlebotomus (Sandfly) Fever Viruses in Tissue Culture.

This study is concerned with the behavior of these viruses in tissue culture and the possibility of applying tissue culture for reliable and quick assays. The question of the application of tissue culture for field and clinical material will also be probed. Several tissue cultures have been tried without success; neither CPE nor plaques were produced.

A continuous cell line of baby hamster kidney (BHK21/clone 13) was also examined; no clear-cut CPE was produced under fluid media and no plaques were formed under agar overlay and the cells degenerated after five days. Then, the following technique was used; cells, in a small volume of Eagle's medium e.g. 0.2 ml, were infected in suspension and gently shaken at 37° C for 30 minutes, 5 ml of medium were then added and the cell-virus suspension was poured into a petri dish and incubated at 37° C in an atmosphere of 5% CO_2. After five hours incubation, the medium was removed and the cells were overlaid with an overlay medium containing Eagle's medium, calf serum and the sodium salt of carboxymethylcellulose in a final concentration of 0.75%. This overlay

allowed the cells to form a monolayer, in which plaques eventually appeared after incubation as before for 4-5 days. The overlay was removed and the cells stained with Giemsa or Crystal violet stains.

Plaques of 1-2 mm were produced by a Sicilian type of sandfly fever virus: the average titer was 7×10^7 PFU/ml as compared to 7.4×10^8 LD_{50} in suckling mice.

Attempts to produce plaques with the Naples type have so far proved unsuccessful.

(A.R. Salim)

EDITORIAL NOTE

Considerable delay in preparation of this issue of the Information Exchange has resulted from having to re-type tables. We have previously requested that tabular material and figures be carefully prepared and submitted in the original on white bond or art paper ready for photographic reproduction. More than half of the tables appearing in this issue were received as carbon copies of other reports or as grey stained xerox copies of originals.

The deadline for contributions to Issue Number 14 of the Arthropod-borne Virus Information Exchange will be July 1, 1966.