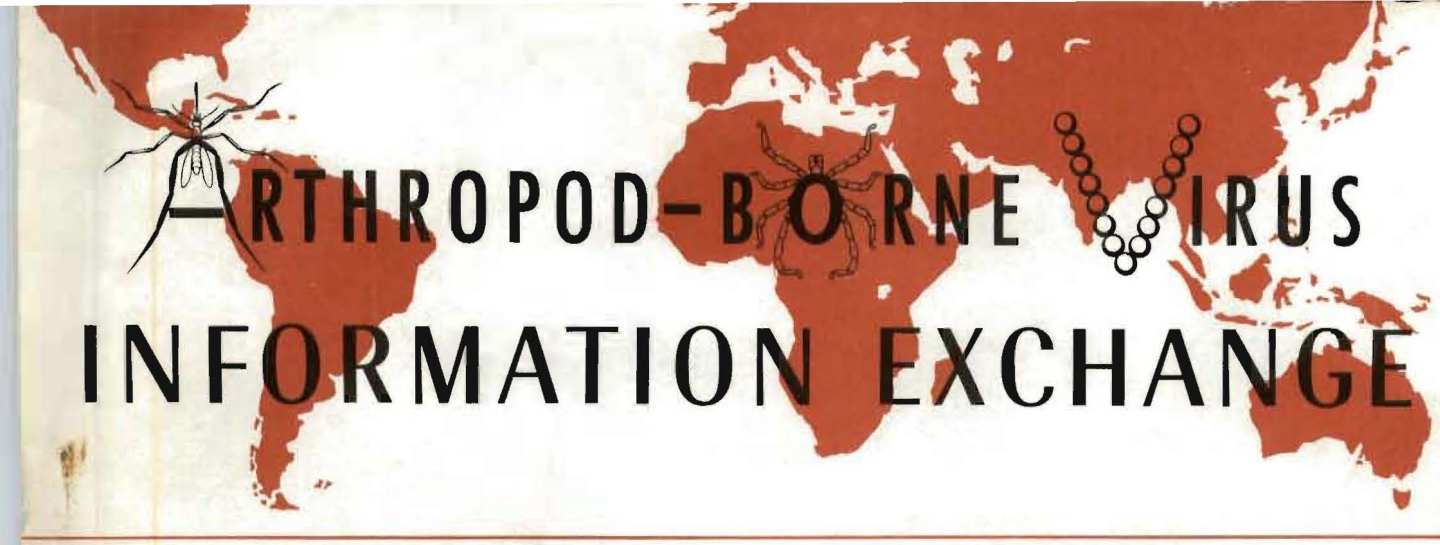

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

NUMBER 22

ARBOVIRAL DISEASE SECTION
ECOLOGICAL INVESTIGATIONS PROGRAM
P. O. BOX 551
FORT COLLINS, COLORADO 80521

Number Twenty-two June 1972

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MESSAGE FROM THE OUTGOING EDITOR

With Issue Number 21, the Arthropod-borne Virus Information Exchange came of age. Over a period of twelve years it grew to be one of the most successful instruments yet evolved for global exchange of information among scientists. To get it started, over the skepticism of many peers, took the interest and contributions of an increasing number of participants.

As it grew, so did the effort to produce it. Transcription of the contents from a variety of manuscripts and illustrations to a readable and clear presentation of increasing volume required the sequential training of six different secretaries in The Rockefeller Foundation, Center for Disease Control, and the University of California at Los Angeles. The operation has finally exceeded the resources here to properly produce it. To train a seventh key transcriptionist is beyond present possibility.

So your editor must bow to the passage of time and the excesses of the Infoexchange's success and hand it over to Dr. Roy W. Chamberlain at the Center for Disease Control, Atlanta, Georgia where the resources which produced it from 1961-1966 are presently available, and where, in Dr. Chamberlain, there is editorial maturity and extensive knowledge that will continue to serve the best scientific requirements of the participants.

We have all witnessed changes in extent and areas of emphasis in our favorite field of arbovirology. That there are so many fundamental areas of investigation yet to solve the biological, medical and public health problems, current and imminent, requires that this means of communication be further enhanced. This requires devotion of more time and resources that are available and invited at the Center for Disease Control.

Many thanks are due to many people who have contributed to the success of the Infoexchange in the past. As I have, Dr. Chamberlain will grow to appreciate that it is a human effort and accomplishment of many. Thank you for your continued participation.

Telford H. Work, M.D.
Professor of Infectious
and Tropical Diseases
University of California
at Los Angeles

REPORTS FROM S.I.R.A.C.A.
OF THE
AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

Meeting of October 22, 1970

The Subcommittee on Immunological Relationships Among Catalogued Arboviruses (S.I.R.A.C.A.) met at the Walter Reed Army Institute of Research, Washington, D.C. to examine the registration cards of the phlebotomus fever viruses.

1. SIRACA examined the cards of the following registered viruses:

   Anhanga (#143), Arumowot (#245), Bujaru (#144), Candiru (#127), Chagres (#39), Icoaraci (#129), Itaporanga (#44), Karimabad-I-58 (#159), Punta Toro (#216), Salehabad-I-81 (#160), Sandfly fever Naples (#105), and Sandfly fever Sicilian (#106).

2. The available evidence, in the cards, published and unpublished, indicates that by one or more of the used serological methods -- complement-fixation, hemagglutination-inhibition and to a lesser extent by agar gel precipitation -- these 12 viruses show crossreactions, i.e., are related.

3. The available evidence is to the effect that no two registered viruses of this group are identical.

4. Further, the available evidence is inadequate to permit or justify an attempt to formulate subset relationships among these viruses, as have been proposed by this subcommittee for other groups.

5. The members of SIRACA noted the singular lack of data by neutralization test with these viruses; they urge that this lack be remedied by interested workers.

Submitted December 7, 1970 by: E.L. Buescher
C.H. Calisher
J. Casals, Chairman
G.E. Sather
R.E. Shope, Consultant
W.F. Scherer, absent

Meeting of March 18, 1971. SIRACA met at Silver Spring, Maryland, to examine the registered viruses in Bunyamwera group.

1. The cards of the following registered viruses were examined; the registration number and date of first isolation are given:

Batai (#48, 12-28-55)
Bunyamwera (#7, 9-20-43)

Cache Valley (#107, 4-1-57)
Calovo (#184, 8-6-60)

Germiston (#13, 4-3-58)
Guaroa (#125, 5-13-56)

Ilesha (#86, 4-26-57)
Kairi (#27, 8-10-55)

Lokern (#220, 8-1-62)
Maguari (#151, 6-7-57)

Main Drain (#219, 3-10-65)
Sororoca (#133, 6-12-61)

Tensaw (#137, 1-23-61)
Tlacotalpan (#192, 5-25-62)

Wyeomyia (#83, 8-11-40)

2. Not only were the data on the cards considered but, as in previous occasions, other evidence was also studied in the form of available literature and annual reports from various sources.

3. It was apparent to the members of the Subcommittee that a great deal of work is needed, particularly in the area of the neutralization test, in order that an attempt can be made to determine the precise or even approximate cross-relationship between many of the registered viruses.

4. The available evidence allowed easily to separate 5 sets or individual viruses among which only moderate or little cross reactivity was reported, as follows:

   a) Bunyamwera (and associated viruses, see Table)

   b) Cache Valley (and associated viruses)

   c) Guaroa

   d) Kairi

   e) Wyeomyia (and associated viruses)

5. The evidence available showed such close serological proximity among several viruses, in each of two sets, that each set can be considered as representing varieties of the same type, as follows:

   a) Cache Valley - Maguari - Tensaw - Tlacotalpan

   b) Wyeomyia - Taiassui - Tucundubu (the latter two not registered but mentioned on the Wyeomyia card).

6. An attempt was made to place these registered viruses in sets or levels - as done with previously examined groups. The best the Subcommittee could do is shown in the Table.

There was no hesitation about the placements under columns 1, 2 and 5 and Kairi and Guaroa under column 3. The rest of the entries have been deliberately placed in the Table half-way between columns 3 and 4 to indicate the opinion of the Subcommittee that placement of the agents in question either under type or subtype is not warranted by the available evidence.

This conclusion is not to the effect that the viruses in-between columns 3 and 4 are indistinguishable; they are distinct viruses to be used accordingly.

Submitted March 29, 1971 by: E.L. Buescher
C.H. Calisher
J. Casals, Chairman
G.E. Sather
W.F. Scherer

1 Group	2 Complex	3 Virus (or type)	4 Subtype	5 Variety
Bunyamwera	Bunyamwera		Bunyamwera	
			Ilesha	
			Germiston	
	Cache Valley		Cache Valley	Cache Valley
				Maguari
				Tensaw
				Tlacotalpan
			Batai-Calovo	
			Lokern	
			Main Drain	
	Wyeomyia		Wyeomyia	Wyeomyia
				Taiassui
				Tucundubu
			Sororoca	
	Kairi	Kairi		
	Guaroa	Guaroa		

Meeting of March 15, 1972. SIRACA met again at Silver Spring, Maryland, to examine the registered viruses of group Simbu.

1. The following cards were examined:

Aino (#228)	Sango (#232)
Akabane (#121)	Sathuperi (#42)
Buttonwillow (#140)	Shamonda (#233)
Ingwavuma (#126)	Shuni (#234)
Manzanilla (#31)	Simbu (#2)
Mermet (#227)	Thimiri (#258)
Oropouche (#24)	
Sabo (#231)	

2. The sources of information in addition to the cards were the YARU annual reports, particularly 1968; and a thesis on "Utinga" virus by a candidate to the M.D. degree at Yale Medical School (Igor Zachary).

3. It was noted that for some of the registered viruses the available information is so scant as to render the classification of the viruses difficult and tentative; there is no direct evidence given that Thimiri belongs in Simbu group; and the question was raised whether Aino virus may in fact be a strain of Akabane.

4. The Chairman was directed to look into the results obtained by the certification program at YARU (certification of serological reagents) to see whether additional information pertinent to this group was available. None was found.

5. SIRACA expressed the desire that the responsible persons would soon register Yaba 7 and Utinga viruses.

6. The table and footnotes appeared to SIRACA to represent the present position of the Simbu group viruses.

7. As always SIRACA welcomes criticism, feedback and additional information.

Submitted May 19, 1972 by: E.L. Buescher
C.H. Calisher
J. Casals, Chairman
G.E. Sather
W.F. Scherer

Simbu Group

1 Group	2 Complex	3 Virus (or type)	4 Subtype	5 Variety
Simbu	Simbu	Akabane Sabo Sango Sathuperi Shamonda Shuni Simbu		
	Manzanilla	Manzanilla	Ingwavuma Manzanilla Mermet	
		Buttonwillow		
	Oropouche	Oropouche		
	Aino	Aino		
	Thimiri (?)			

Thimiri: stated in the registration form to be a member of group Simbu; there is no evidence given on the card, nor elsewhere, that it is so.

Buttonwillow: tentatively placed in Manzanilla complex; it may, in fact, head a complex and belong in column 2.

Aino: classification based on limited evidence. CF only; it may belong in the Simbu complex and be close to Akabane.

Two additional viruses, Yaba 7 and Utinga, known to be part of Simbu group are unregistered, therefore not entered in the table.

LISTING OF AVAILABLE ARBOVIRUS REFERENCE REAGENTS, RESEARCH RESOURCES BRANCH, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

The Research Resources Branch of the National Institute of Allergy and Infectious Diseases, National Institutes of Health has previously announced the availability of arbovirus reagents. The list below is an updating of certified seed and ascitic fluids available as of June 11, 1971. Reagents can be obtained by completing and submitting Form NIH-381-2 to the Research Resources Branch and can usually be provided within 7 to 10 days after the request is received.

For further information on the availability of arbovirus reference reagents write to the Chief, Research Resources Branch, National Institute of Allergy and Infectious Diseases, Building 31, Room 7A-23, Bethesda, Maryland 20014.

1. Arbovirus Seed Virus

Anopheles
Bimiti
Bluetongue
Buttonwillow
Bwamba
California Encephalitis
Catu
Chagres
Changuinola
Chikungunya
Colorado Tick Fever
EEE
EHD
Guama
Guaroa
Hart Park
Hughes
Ilheus
Kern Canyon
Manzanilla
Mayaro
MML
Modoc
Naples SFF
Nepuyo
Oriboca
Oropouche
Patois
Powassan
Rio Bravo
Ross River
Sawgrass
Semliki Forest
Sicilian SFF
Silverwater
Sindbis
SLE
Tensaw
VEE
VSV (Indiana)
VSV (New Jersey)
WEE
West Nile
Wyeomyia
Yellow Fever (17D)
Zegla

2. Arbovirus Immune Ascitic Fluids

Anopheles A
Anopheles B
Buttonwillow
Bwamba
California Encephalitis
Chagres
Colorado Tick Fever
EEE
Guama
Guaroa
Hart Park
Ilheus
Kern Canyon
Manzanilla
Mayaro
Melao
MML
Modoc
Naples SFF
Oropouche
Patois
Rio Bravo
Sicilian SFF
Silverwater
Turlock
VSV (Indiana)
VSV (New Jersey)
Wyeomyia
Yellow Fever

3. Arbovirus Grouping Ascitic Fluids

Group A
Group B
Group C
Group Bunyamwera
Group Capim
Group Guama
Group Quaranfil
Group Simbu
Group Tacaribe
Group VSV

(R.M. Pennington)

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

West Nile (WN) and Sindbis (SIN) viruses in an arid environment

During the past two years field studies have been in progress along the Orange River with the object of ascertaining the current prevalence there of arboviruses so that a comparison will be possible in later years after the environment will have been altered as a result of the Orange River Irrigation Scheme.

The study area is semi-arid with Karoo-type xerophytic vegetation and an annual rainfall of 439 mm which falls as localized thunder storms during the summer. Most streams cease running during the dry season and, apart from the Orange River, permanent ground water is sparsely distributed. This is found mainly either in cement dams, supplied from undergound water, or in small dams in the beds of some of the streams. The later contain aquatic vegetation and their permanency provides a suitable habitat for the breeding of Culex mosquitoes, while the associated trees, shrubs and reeds support large numbers of a varied avifauna. The adults of aedine species are usually only found in numbers for a limited period following heavy rain showers. Day temperatures during the summer are extremely high although winter temperatures are rather low.

The field investigations have revealed quite a high prevalence of WN and SIN viruses despite low populations of Culex univittatus and both viruses were active during the two summers in which observations were made. During the 1968/69 season 4 strains of WN and 2 of SIN were isolated from mosquitoes and 6 of WN and 1 of SIN were isolated in the following season. Nine of these isolations came from Culex univittatus which seems to be the maintenance vector of both viruses here as elsewhere on the inland plateau region of South Africa.

Sentinel pigeons have been exposed in the study area and infections by both viruses were recorded during each of the two summer seasons studied. A total of 27 WN and 4 SIN infections were detected.

There seems to be little doubt that the two viruses are well adapted to survival in the existing arid environment. That this occurs under conditions of low densities of Culex univittatus is of particular interest.

More questionable, is the significance of the single isolation of Wesselsbron virus from a Desmodillus rodent collected in the area. The virus had been isolated from aedine mosquitoes and sheep during an epizootic in sheep in 1957 but this was presumably the result of incidental transmission cycles and might well have followed the introduction of virus. For lack of firm evidence no conclusion can be made at this stage with regard to the persistence of this virus in the area but the possibility certainly warrants bearing in mind.

Rift Valley fever

After a period of 13 years during which no activity on the part of RVF virus was observed an extensive epizootic in cattle occurred on the plateau region of Rhodesia during the 1968/69 summer. The virus apparently overwintered successfully on the plateau as widespread but rather localized outbreaks reappeared on the plateau in South Africa and Rhodesia during the 1969/70 summer. So far, no epizootics have come to our knowledge this summer. Hence it seems that the course of events will follow the pattern observed during the epizootics in the 1950's when the virus eventually disappeared after a few seasons and it seems likely that the epizootics on the plateau grassland and savanna represent incidental cycles and follow the introduction of virus from elsewhere.

Observations in East Africa have also revealed RVF to be elusive and sporadic in appearance but have suggested that forest may be its natural habitat. The isolation of this virus during February 1971 from Eretmapodites quinquevittatus, collected in forest in the coastal regions of southern Natal, in circumstances unassociated with any epizootic in domestic animals, has also pointed to the possible maintenance of this virus in forest in South Africa. Significantly, the early isolations of RVF virus from mosquitoes in Uganda also implicated an Eretmapodites species as a vector.

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

Several isolates mentioned in the last report of this laboratory (see No. 18 of the Information Exchange) have since been identified. At the same time virus isolation attempts from mosquitoes have been continued. To these have been added isolation attempts from serum of patients suffering from fever. Several new isolates have been obtained, some of which have been identified.

The identifications are shown on the following table.

Apart from Bunyamwera virus which was reported earlier all are first isolates in Kenya.

From all the areas in the table there is serological evidence of past circulation of yellow fever virus. So far this virus has not been isolated.

All identifications were done at the East African Virus Research Institute at Entebbe. Several more isolates are being examined.

(D. Metselaar)

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE ENTEBBE, UGANDA

Humans:

One strain of Nairobi Sheep Disease (SG 17407) was isolated from a 16 year old male patient attending at the Institute clinic during January 1970. All human cases reported previously have been due to laboratory infections. This is the first natural human infection to be reported from East Africa.

Place of origin	Identification: Group	Identification: Virus	Isolated: From	Isolated: Number
Coast:				
North of Mombasa	Bunyamwera	Bunyamwera ∠	Ae. (S.) pembaensis	1
	Ungrouped	new	Eretm. subsimplicipes	1
	A	Middelburg	Ae. (Aed.) cumminsi group	1
South of Mombasa	Simbu	Under invest.	human serum	1
	Bunyamwera	Bunyamwera	"	1
Malindi	Simbu	Sango	M. (M.) uniformis	1
	Ungrouped	AR1169/64*	"	1
	Bunyamwera	Bunyamwera	"	1
	Bwamba	Pongola	C. (C.) antennatus	1
North:				
Marsabit	Bwamba	Pongola	M. (M.) africana	3
			C. (C.) zombaensis	1
			Ae. (Aed.) dentatus	9
	A	Semliki Forest	An. (C.) funestus	1
Central plateau:				
near Nairobi	A	Semliki Forest	Ae. (Aed.) dentatus	1

∠ different from the one reported earlier
* AR1169/64 was first isolated in the Sudan from M. (M.) uniformis by Dr. Jack Schmidt (Pers. comm.)

Mosquitoes: Zika Epizootic in Zika Forest:

A total of 33, 291 mosquitoes were processed for virus isolation in 1970. The majority were from Zika forest from which 7 strains of Zika virus were isolated. The total number of isolates now confirmed as strains of Zika virus is 15. These span a 13-month period, with a gap of 6 months from October 1969 to April 1970 when no isolations were made.

Small Mammals:

One strain of virus isolated during 1969 from the brain of a rodent (L. flavopunctatus) collected from Lunyo forest, Entebbe, has been identified as Banzi virus (Z 13517). Two other isolates made during 1969 from Culex mosquitoes taken from the same forest have been identified as strains of Germiston virus and Witwatersrand virus respectively. Between 1968 and 1969, a total of five different viruses (RVF, Germiston, Usutu, Witwatersrand and Banzi) have been isolated from rodents and mosquitoes collected from Lunyo Forest.

Ticks:

A total of 719 ticks collected in Western Uganda from domestic animals mostly cattle, were processed and one strain of Congo virus was isolated from A. variegatrum (A-MP 10358). Since 1956 all isolations of Congo virus made at this Institute were from humans, and virus isolation attempts from ticks had been negative. This would appear to establish for the first time, the local vector of Congo virus in Uganda.

Referred isolates:

9 virus isolates were received from Dr. Metselaar of the Medical Research Centre, Nairobi, Kenya, and 5 were identified as follows:

Middelburg virus - 3 strains all from mosquitoes.
Bunyamwera virus - 1 strain from organs of a baboon.
Simbu virus - 1 strain from serum of a febrile patient.

The other 4 are still unidentified.

Yellow fever Survey in Bwamba, Western Uganda:

Yellow fever human immunity surveys carried out during 1968 by Henderson et al (EAVRI Report No. 18, 37) indicated an absence of immunity in

children in Bwamba, Uganda, as opposed to a rate of 4.5% found by Hughes et al. (Trans. Roy. Soc. Trop. Med. Hyg., 40, 1946). The immunity in the adults was also very low. The finding prompted a similar survey to be carried out in Bwamba forest monkeys. 68 forest monkey sera were obtained and tested for yellow fever neutralizing antibody in mice giving a standardized yellow fever immunity rate of 64.7%. This result is similar to the 1942-47 surveys in the same area. It is concluded from the above findings that yellow fever immunity rates in Bwamba forest monkeys, is fairly stable representing a balanced enzootic condition. The risks of a human population with such low immunity rates to yellow fever living adjacent to such forests is being further defined.

Arbovirus Transmission Experiments:

Aedes (Stegomyia) woodi was initially shown to be refractory to yellow fever (strain Ethiopia 19) and later a high titred virus initiated infection but no transmission to mice was achieved. Chikungunya virus replicated in Mansonia fuscopennata and was transmitted to newborn mice when the mosquitoes fed on them.

Birds:

424 birds of some 112 species were mist netted in Entebbe, Karamoja, Acholi, Bunyoro and Ankole Districts of Uganda. No palaearctic migrants were captured. Sera were obtained from 343 of them from which no virus was isolated. Haemagglutination inhibition tests gave 44% positives to West Nile virus; 19% to Chikungunya virus and 18% to Ntaya virus.

(P.M. Tukei and G.W. Kafuko)

REPORT FROM THE ARBOVIRUS LABORATORY INSTITUT PASTEUR AND O.R.S.T.O.M. BANGUI - REPUBLIC OF CENTRAL AFRICA

I. Strains isolated from human specimens

A: Seven strains were isolated in sera from patients showing clinical symptoms of an arbovirus infection.

HB 320 ILESHA: Eruptive fever, with headache, myalgia, arthralgia. Confirmed by serology: the neutralization index of the late serum is 2.

HB 364 BWAMBA: Eruptive fever with headache and stiff-neck. Confirmed by serology: the neutralization index of the late serum is 2.8.

HB 403 ILESHA: Eruptive fever with a mild evolution. Confirmed by serology: the neutralization index of the late serum is 2.3.

HB 413 ILESHA: Macular rash without fever. Confirmed by serology: the neutralizing index of the late serum is 2.6.

HB 688 BWAMBA: Eruptive fever with headache. Confirmed by serology: the neutralizing index of the late serum is 2.

HB 754 (BANGUI): A temporary new virus. Exanthema with headache, little fever. Confirmed by serology: the neutralizing index of the late serum is 2.2.

HB 789 BWAMBA: Eruptive fever with myalgia, headache, acute lumbar pains. Confirmed by serology: the neutralizing index of the serum is 2.5.

B - one strain was isolated from spinal fluid.

HB 251 DUGBE: meningitic syndrome.

II. Strains isolated from mosquitoes

1 TATAGUINE from Anopheles gambiae
1 ILESHA from Anopheles gambiae
1 (BAMBIO) temporarily new virus from Anopheles paludis

5 MOSSURIL from Culex pruina and Culex telesilla
1 (GOMOKA) temporarily new virus from Culex perfuscus
1 (NOLA) temporarily new virus from Culex perfuscus
1 SEMLIKI FOREST from Aedes belonging to group palpalis
1 SINDBIS (Subtype Y 251) from Culex telesilla
1 ARB 3241 from Culex pruina (under process of identification)

III. Strains isolated from birds

Twenty three strains were isolated from birds. They were particularly interesting since they allowed us to know one of the wild hosts for a certain number of viruses.

Andropadus virens for Mossuril
Bycanistes sharpii for Usutu
Halcyon senegalensis for Semliki Forest
Saxicola rubetra for Uganda S
Euplectes oryx for Ingwavuma

Eighteen strains are either new or in the process of identification.

IV. Strains isolated from rodents

Four strains were isolated. They are under the process of identification, but two of them seem to be new.

(J. C. Jacobi)

REPORT FROM THE VIRUS RESEARCH LABORATORY UNIVERSITY OF IBADAN, NIGERIA

During the past several months a number of agents have been recognized for the first time in Nigeria. These are listed in the table.

Isolation of African Horse-Sickness in Nigeria

Sporadic cases of African horse-sickness (A.H.S.) have occurred in Nigeria for many years. During 1951, deaths of animals presumably due to A.H.S. were reported from Maidugeri in North Eastern Nigeria. During 1966 veterinarians in Kaduna diagnosed A.H.S. Again in 1967 an ill horse which had recently arrived in Jos from the Sudan was considered on clinical grounds to be suffering from A.H.S. In both 1966 and 1967 sera taken from horses in the areas where the sporadic cases occurred were shown to contain neutralizing antibody when examined at Onderstepoort.

To our knowledge there is no record to suggest that A.H.S. has occurred in epizootic form in Nigeria in spite of devastating outbreaks in nearby African countries, (Howell 1963) nor can we find records of the virus having been previously isolated in Nigeria.

On October 8, 1970, a horse was seen at Zaria by clinicians from the School of Veterinary Medicine, Ahmadu Bello University. This animal was diagnosed clinically as having A.H.S. The horse had been ill for three days when first seen and died on October 9, 1970. Tissues from this dead horse and blood from another suspected case of A.H.S. were kept frozen at -20°C until October 19, 1970, when placed in liquid nitrogen for delivery to the Virus Research Laboratory, University of Ibadan on October 20, 1971.

Virus was isolated from liver, lung and spleen of the dead horse but not from several different specimens of blood from the live suspect case. The isolates from spleen, liver and lung all reacted with the A.H.S. immune serum (supplied by Onderstepoort) at titres of greater than 1 . 32. There was no reaction with normal brain suspension.

A.H.S. virus was not present in the Ibadan laboratory until the reported isolations were made.

We believe these to be the first isolations of A.H.S. in Nigeria.

Reference:

Howell, P.G., (1963): African horse-sickness, In Emerging Diseases of Animals, F.A.O. agricultural Studies No. 61, Rome 1963, p.p. 71-108, 206-229

Isolation of Ixodid tick agents from small mammals

Dugbe Virus: During the years 1964-1969 a total of 744 strains of Dugbe was isolated. Domestic animals yielded 187 strains, ticks 549, mosquitoes 1, culicoides 3, and human 4. This virus was not isolated from any of the several thousands of bird or small mammal tissues examined.

Bhanja Virus: This virus has been regularly isolated during each of the years 1964-1969 but in lesser numbers than Dugbe virus. Domestic animals have yielded 15 strains and ticks have yielded 105 strains. As with Dugbe no wild animal had been found through 1969 which yielded this agent.

Congo Virus: Isolations of this agent have also been made each year 1964-1969; from domestic livestock 5 strains, ticks 36 strains, and culicoides 1 strain. One isolate of Congo virus was made from a liver spleen pool of a wild caught animal, Atelerix albiventris, in June 1967.

Recent isolations from small mammals: During the course of investigations aimed towards elucidation of a possible wild life reservoir of Lassa virus, small mammals were trapped at Bassa, a small village near Jos, in December, 1970. Tissues and sera were taken from about 500 animals intended for examination at C.D.C. for virus isolation and for the detection of antibodies to Lassa virus. It was decided to inoculate serum only, into infant mice prior to sending the tissues and sera to America.

A number of isolates have been obtained from these serum specimens. Those identified by C.F. to date have included (a) Two strains of Bhanja, one each from Xerus erythropus and Atelerix albiventris (b) one strain of Congo virus from Atelerix albiventris (c) one strain of Dugbe virus from Critcetomys gambianus.

A new member of the Rhabdovirus group

IB AR23380, isolated from a pool of Culicoides spp. collected in Ibadan in December, 1967, has been shown to be related in complement fixation tests to IB AN27377, a rabies related agent.

MAF

Antigen	23380	27377
23380	64+/16+	64+/4
27377	(4/4)	64+/16+

No relation was detected by complement fixation tests with Rabies (Flury strain) or Chandipura. In one way complement fixation tests, IB AR23380 antigen did not react with antiserum to Lagos Bat or to Vesicular Stomatitis virus (New Jersey).

A Nigerian virus related to the Palyam group

IB AR22388 is a chloroform resistent agent first isolated from Culicoides spp. in Ibadan in 1968. Since then there have been 45 additional isolates, one from Aedes fowleri and the remainder from Culicoides, all from Ibadan. This agent has been shown to react in C. F. T. with antiserum to Vellore and Palyam viruses from India.

IMMUNE FLUID

Antigen	IB AR22388	Vellore	Palyam
IB AR22388	32/64	128/64	16/4
Vellore*	NT	256/?	NT
Palyam*	NT	NT	64/?

*Neither Vellore nor Palyam antigens were available at Ibadan. Serum titers reported for these agents were received with the sera from Vellore.

RECENT ISOLATES OF VIRUSES NOT PREVIOUSLY REPORTED FROM NIGERIA

IDENTIFICATION	PROTOTYPE			SUBSEQUENT ISOLATES			
	Source	Location	Date	Source	Location	Date	Number
Semliki Forest	*Atelerix albiventris*	Kware	29-V-70	*Quelea erythrops*	Kware	May 1970	1
				Sentinal Mice	Ibadan	June, July 1970	5
Sindbis	Sentinal Mice	Ibadan	22-IX-69	Sentinal Mice	Ibadan	1969, 1970	3
African Horse Sickness	Sick Horse	Zaria	19-X-70				
Nyando group	*Anopheles gambiae*	Vom	27-X-69	*Anopheles gambiae*	Ibadan	Feb., 1970	1
Quaranfil	*Argas persicus*	Potiskum	17-X-70	*A.persicus*	Northern Nigeria	Nov., 1970	9
Nyamanini	"	"	"	" "	"	" "	4
Rift Valley Fever	*Culicoides spp.*	Ibadan	10-XII-70	*Culicoides spp.*	Ibadan	Dec., 1970	1
				Culex antennatus	"	" "	1

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

During the period July 1, 1970 through December 31, 1970, surveillance for arboviruses was continued at Bandia and Saboya field Stations. This surveillance involved also study of tick samples collected at the slaughter house in Dakar.

The year 1970 saw a marked decrease compared with the previous years in the average rainfall in Senegal. The small number of isolations in 1970 rainy season was no doubt related to this very low density of precipitation.

In order to enlarge our field yellow fever survey, we have planned a preliminary serological survey of the South Eastern part of Senegal, a Northern Guinea Savannah vegetation zone. School children sera and wild vertebrate samples were collected in this country and an entomological program was oriented towards mosquito capture for identification and inoculation.

I. Virological Studies

Human blood samples

Of 128 human blood samples processed in the laboratory 124 come from febrile children seen at Bandia dispensary: they yielded one isolate killing 1 day old suckling mice with AST of about 10 days, not yet identified.

Wild vertebrates samples

Most of the material was collected at the Bandia and Saboya field stations and around the city of Kedougou during the Senegal South East trip.

1. Bandia : Two strains of Bandia virus have been isolated: one from Mastomys, one from Xerus erythropus.

One strain of virus identified as DAK An D 5314 (Keuraliba) from Taterillus.

2. Saboya: One strain of virus identified as DAK An D 3150 (Fika) from Taterillus. Two strains of virus identified as DAK An D 4611 (Touré) from Tatera kempi.

3. Kedougou: No virus have been yet isolated from materials collected in Kedougou.

Arthropods

463 pools of arthropods were processed for virus isolation. One pool of Aedes luteocephalus from Bandia yielded one isolate killing 1 day old suckling mice with AST of 10 days, not yet identified.

II. Serological Studies

A. Human sera

1. Dakar: Serological studies of paired sera from 62 patients showed in four case extensive cross reactions among the group B viruses in HI tests, but it was impossible to give the specific diagnosis even by neutralization test.

2. Bandia: 113 sera collected from febrile children seen at the dispensary were tested for HI antibody to the following viruses: CHIK, ONN, YF, UGS, DAK, WN, ZIK, BUN.

28 sera were positive with chikungunya: 22 of them giving a titer superior to 1/640. 99 were positive with group B viruses, 55 of them with an high titer; 13 sera were positive with Bunyamwera.

3. Kedougou: Sera of 513 school children have been studied by HI in order to find out which viruses had been active in that country.

The results show a high rate of chikungunya activity in this area: 360 of 460 sera tested had antibody to chikungunya 38 of them showing a titer above 1/640.

The second striking feature emerging from these results is that, while yellow fever antibody rate is closely parallel with Uganda S and Dakar Bat, the antibody rate against Zika is significantly higher than the rate found for West-Nile, as it is always found in a forest country, West-Nile antibody being prevalent in the Savannah.

B. Wild vertebrate specimens

950 wild vertebrate have been bled and the sera have been tested (477 from Bandia, 375 from Saboya, 88 from Kedougou) for HI antibodies to the

following antigens: CHIK, YF, UGS, DAK, WN, ZIK, BUK, WESS.

Results are summarized in the following table:

Origin of the specimens	No. of sera tested	No. of positive sera ($\geq$ 1/10)							
		CHIK	YF	UGS	DAK	WN	ZIK	BUK	WESS
Bandia	477	4	12	6	8	10	10	7	12
Saboya	375	3	27	24	22	29	22	9	27
Kedougou	88	10	22	19	20	22	24	10	22

(G. Le Gonidec and Y. Robin, Institute Pasteur de Dakar; R. Taufflieb, M. Cornet and J.L. Camicas, O.R.S.T.O.M. - Dakar)

REPORT FROM THE ARBOVIRUS LABORATORY DEPARTMENT OF BACTERIOLOGY AND VIROLOGY NATIONAL SCHOOL OF PUBLIC HEALTH AND TROPICAL MEDICINE LISBON, PORTUGAL

During the year of 1970 the studies concerning arboviruses that took place in this laboratory were the following:

1. Grouping and sorting several pools of mosquitoes captured during the summer of 1969 and 1970 in areas where we had previously obtained data concerning the activities of some arboviruses namely the viruses WEST NILE, TAHYNA and CALOVO or viruses antigenically very similar to these (Ann. Esc. Nac. Saúde Públ. Med. Trop. 1(1/4) 197:204, 1967; Amer. J. Trop. Med. Hyg. 18(3): 423:426, 1969).

At this stage the pools of mosquitoes already sorted and studied show that the species of mosquitoes captured were the following: Anopheles maculipennis;

Aedes caspius; Aedes detritus; Culex pipiens; Culex theileri.

From the captured mosquitoes it was possible to isolate 2 infectious agents. One was studied and identified as the virus WEST NILE, which strain we call ROXO. It was isolated from a pool of mosquitoes Anopheles maculipennis, captured by the Roxo dam, 20 Km. south of the city Beja, in the southern part of Portugal.

The second agent was isolated from a pool of mosquitoes Aedes caspius and Culex theileri. These mosquitoes were captured in a dovecot about 70 Km. northeast of Lisbon. This agent has not been yet thoroughly studied.

2. Working together with several groups of wild birds markers headed by Professor Santos Junior of the Porto University, we captured wild birds with the purpose of marking them with identification rings for migratory studies. We captured 400 wild birds from 38 different species, in 11 different places spread from the north to the south of the country. We took the blood by the filter paper disc method, making the puncture in the wing's axilar vein. The serological survey was made using a haemagglutination-inhibition test for 14 antigens: 2 from the Group A (Sindbis and Chikungunya) 8 from the Group B (West Nile, Wesselsbron, Ntaya, Zika, T.B.E., Yellow Fever, Dengue 1 and Dengue 2) 2 from the Phlebotomus Group (Sicily and Naples) and still the Tayhna and Calovo virus. The results obtained showed that 13 of the birds had antibodies against arboviruses of Group B and 1 was positive to a virus of Group A - Sindbis - (titer 1:20 with 8 haemagglutinating units). Attending at the amount of serum eluted from the filter paper discs - 0.05 ml - and also the fact that this material had been treated by acetone, it is likely that the results obtained after serological examination represents an inferior value to what it really means as titer of antibodies against arboviruses circulating in nature. We would like to point out that the swallow - Hirundo rustica - is the species of bird we think has more interest for future study in this country because it is the migratory bird par excellence and in 25 captured birds 4 presented antibodies for virus of the Group B. Our work concerning birds scheduled for the current year will fall upon the study of this species of birds.

3. During the year of 1970 we also worked together with a French team, formed of parasitologists of the Rennes University and from the Arbovirus Laboratory of the Pasteur Institute of Paris. They came to Portugal to study, in collaboration with the National School of Public Health and Tropical Medicine of Lisbon, the rodents of Portugal. A total of 360 wild rodents were captured belonging to the following species: Arvicola amphibius; Mus musculus; Apodemus sylvaticus; Crocidura s.p.p.; Pitymys s.p.p.;

Rattus rattus.

From this material and with interest for the arboviruses study, blood was taken by the disc method for serological examination, as well as fragments of organs to attempt the isolation of the virus. The study of the discs for the serological tests will take place in the Arbovirus Laboratory of the Pasteur Institute of Paris. The isolation of the virus starting from the organ fragments will be the responsibility of our laboratory. However this material has not yet been studied.

4. To verify if there had been any alteration in the immunological state of Portuguese Guinean population after several outbreaks of yellow fever epidemics in the West Coast of Africa and after the vaccination campaigns against yellow fever held in that zone in 1964/65 (M. R. Pinto, Bull. Wld. Hlth. Org., 1967, 37, 101:108) a total of 544 sera proceeding from the interior of that Portuguese province and taken during 1968 were examined. Only the immunological state against the virus of Group B was considered, and the sera were examined by the haemagglutination-inhibition test with the following antigens: Yellow Fever West Nile, Wesselsbron, Zika, Ntaya and Banzi. It was stated that the immunological condition of the population did not suffer any alteration since the study held in 1964/65.

5. We started a serological survey on the immunity state of the human population of Portugal against the arboviruses. A total of 635 samples of sera for study were already obtained.

(Dr. A. R. Filipe)

REPORT FROM THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH
MILL HILL, LONDON N.W.7, 1AA

The M29 line of Aedes aegypti cells derived by Varma, M.G.R. and Pudney, M. (1969, J. Med. Entomol. 6, 432-439) has been studied as a host cell for arbovirus replication. The parent culture was heterogeneous with respect to cell type, and 14 clones were derived by the ring cup method. Two clones grew poorly, but 12 clones were established in serial culture and were infected with West Nile virus, the culture fluids being tested for haemagglutinin at intervals for up to one month. Seven clones yielded a specific West Nile haemagglutinin in low titre on one day or more, and five clones failed to yield a haemagglutinin on any occasion. One positive clone and one negative clone were selected for more detailed study of yield of infectious virus. The titre of virus in culture fluids from the haemagglutinin negative fluid never rose about 10^2 p.f.u. per ml, and virus was detectable in trace amounts only on some days and was undetectable on others. The haemagglutinin positive clone yielded 10^6 p.f.u. per ml on day 4, after which the level fell to 10^3 p.f.u. per ml and remained at this level for 30 days.

No cytopathic effect was seen in the uncloned cells following infection with West Nile virus, but the sensitive clone showed a cytopathic effect with high doses of virus, and plaques formed with West Nile virus in these cells, although the sensitivity was about one thousand-fold less than that of pig kidney cell cultures (PS cells).

(J.S. Porterfield and A.T. de Madrid)

REPORT FROM THE FEDERAL INSTITUTE
FOR ANIMAL VIRUS DISEASES
TÜBINGEN, WEST GERMANY

Persistence of Sindbis virus in BHK 21 cells

After infection of BHK 21 monolayer cultures with Sindbis virus individual surviving cells can be observed. These cells are capable of multiplication, thereby forming small colonies in which the virus persists; it causes periodical cell destruction confined to the colony centers. Infectivity titers in the supernatant rise during the phases of cell destruction and fall during the phases of recovery. In the course of several weeks the slowly growing colonies may form confluent cell sheets which can be trypsinized and grown in continuous passages. Periodical spontaneous cell destructions are no longer visible and an equilibrium between the BHK 21 cell and Sindbis virus has been established. About 6% of the cells are virus producers, as evidenced by infective center tests. The supernatant contains about 10^6 to 10^7 PFU/ml. The behaviour of the cells after elimination of the persistent infection is changed with respect to plaque formation by Sindbis virus although cell morphology is unaltered. Virus from persistently infected cells also differs from the original virus; it produces smaller plaques in BHK 21 and chick embryo cells.

(W. Schwöbel, and R. Ahl)

Induction of an "interferon-like" substance in insect cells infected with Sindbis virus

After infection of an Aedes albopictus cell line (SINGH) with Sindbis virus an antiviral substance is produced. Cultures containing this substance synthesize 1.2×10^6 PFU within 24 hours after reinfection whereas control cultures of same cell age and number produce 1.3×10^7 PFU. The substance can be demonstrated in the tissue culture fluid; it is acid-resistant (pH 1.8). Cultures which had been infected after 16 hours of incubation with this substance were found to produce 6.0×10^5 PFU during the following 24 hours, the controls having titres of 5.4×10^6 PFU. Based on this data the substance was designated as "interferon-like".

(H. J. Enzmann)

REPORT FROM THE ARBOVIRUS UNIT
DEPARTMENT OF VIROLOGY
ISTITUTO SUPERIORE DI SANITA
ROME, ITALY

Sentinel goats experiment in a Bhanja virus focus zone

The present study has been done in the southeast region (Calabria) of Italian peninsula. Goats previously surveyed in Calabria showed remarkably high HI titers against Bhanja virus with positive percentages ranging from 30% found in goats pasturing at low altitudes near the sea to 71% in those grazing in the hilly or mountaineous pastures. Differences in the frequency of positivity may be explained by different location of pastures, corresponding to different biotopes. Similar results (72% of positives) were obtained by HI test in the sera of goats grazing in the focus in Central Italy, where Bhanja virus was isolated.

An elementary focus, where a flock was 100% serologically positive, was chosen in the mountains near Longobucco (Cosenza province) to start an experiment on sentinel goats. Nine goats serologically negative for Bhanja virus and one incidentally positive were introduced in the area in the period May 1969-January 1970 (Table 1). The sentinel animals, 1-3 years old, were grazing together with the resident flock. At monthly intervals (during the month of October at weekly intervals) blood samples were taken. Two goats showed seroconversion to positivity in September, two in October and one in November. Incidentally, one goat died in December and three were still negative at the last bleeding.

The high HI antibody titers found in sera of goats grazing in the focus-area, were probably due to reinfection. Evidence for this could be the case of sentinel goat n° 305, the only one which was positive when the animals were introduced into the focus. After a decrease of HI antibodies from May till middle of October, a sharp increase of HI titer, which could be considered as the result of a reinfection, was observed.

Bhanja virus was not isolated from the blood samples collected at weekly intervals in October and November from the sentinel goats. However, in two young goats, experimentally infected with Bhanja virus, viremia was not detected presumably because it was short lasting and limited, although antibodies to the virus were present starting from the 12th p.i. day. Goats, therefore, do not probably share in the cycle of Bhanja virus in the focus.

Table 1 – Antibody response in sentinel goats grazing in Rhanja virus focus zone (Longobucco).

Goat No.	Date of Bleeding										
	May, 1969		July	Aug.	Sept.	October				Nov.	Jan. 1970
	(0)	(21)	(54)	(102)	(121)	(145)	(152)	(160)	(173)	(204)	(246)
301	0+	0	0	0	0	0	0	0	0	0	0
302	0	0	0	0	0	0	0	–	0	160++	160
303	0	0	0	0	0	0	0	0	0	0	0
304	0	0	0	0	0	0	0	0	0	0	dead+++
305	1280	640	320	320	160	160	80	640	320	160	80
306	0	0	0	0	0	0	0	0	320	1280	40
307	0	0	0	0	320	160	320	–	640	320	160
308	0	0	0	0	0	640	640	640	1280	640	320
309	0	0	0	0	640	640	320	–	160	160	160
310	0	0	0	0	0	0	0	0	0	0	0

Note : In parenthesis days counted from the beginning of the experiment.

+ negative at 1:20 dilution.

++ recirrocal of the serum titer.

+++ incidental death.

The period, when sentinel goats started to have seroconversion to Bhanja virus, corresponds to the seasonal occurrence of the virus in the focus zone in Central Italy, where it was isolated. In fact Bhanja virus was isolated from Haemaphysalis ticks collected in September, October and November 1967, but no virus was recovered from ticks collected between December 1967 and April 1968.

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

REPORT FROM THE INSTITUTE OF HYGIENE OF THE UNIVERSITY OF PALERMO PALERMO, ITALY

Studies on viruses isolated from ticks in Western Sicily

As reported in the 21st issue of the Arthropod-borne virus information exchange, six viral agents were isolated from ticks, Rhipicephalus bursa species collected part on cattle, sheep and goats and part on the ground in a mountainous area of Western Sicily. Isolates seem to belong to three different serotypes. Preliminary results of the studies on some characteristics of isolates are summarized in table 1. By using complement-fixation test, it appeared that Ar 88 is similar to Ar 93 and Ar 129 to Ar 137; the antigenic characteristics of Ar 125 and Ar 126 were investigated by the hemagglutination-inhibition test: they appeared to be identical. Ar 125/Ar 126 hemagglutinating antigens were extracted from suckling mice serum and hamster serum and liver and not from the brain. These viruses kill adult mice and hamsters in 2-3 days; they also produce cytopathic effect and plaques in VERO cell cultures. Moreover, as reported in table 2, preliminary results of hemagglutination-inhibition tests carried out on sera of domestic animals living in the area where tick specimens were collected have shown high titers of antibodies against Ar 125/Ar 126 viruses.

The identification of isolates is in progress as well as further studies on their biological characteristics.

TABLE 1

Isolate	(suckling mice) incubation period	titer (logs) per ml	pathogenic for adult mice	adult hamsters	HA activity	cytopathic effect in tissue culture (VERO)	$CHCl_3$ test	SDC test	nucleic acid
Ar 88 *	-	-	antibody	antibody	no	no	ND	ND	ND
Ar 93	3 days	10^{-5}	" "	" "	no	no	ND	ND	ND
Ar 125	2-3 "	10^{-8}	death	death	yes**	yes	ND	ND	ND
Ar 126	2-3 "	10^{-8}	death	death	yes**	yes	sensitive	ND	RNA
Ar 129	5 "	10^{-5}	antibody	antibody	no	no	ND	ND	ND
Ar 137	4-5 "	$10^{-4,5}$	" "	" "	no	no	ND	sensitive	ND

* = Ar 88 has not presented a constant incubation period

** = hemagglutinating antigens extracted from suckling mice serum and hamster serum and liver

TABLE 2

sera	Tested	Positive ≥1:20	Positive ≥1:160
Bovine	132	16	7
Ovine	46	0	7

Dr. Sunthorn Srihongse of the Gorgas Memorial Laboratory, Panama, has guided the research during his stay in this Institute from August through October 1970.

(M. Albanese and G. Di Cuonzo)

REPORT FROM THE VIRUS LABORATORY INSTITUT OF MICROBIOLOGY MEDICAL FACULTY UNIVERSITY, LJUBLJANA

For a number of years we have been testing various substances for their activity against arboviruses. Some derivatives of thiazolidine acetic acid were found to be prophylactic drugs against Semliki forest virus encephalitis in adult CBA mice.

Of some interest is the activity of 5-carboxymethyl-3-p-tolyl-thiazolidine-2, 4-dione -2-acetophenonhydrazone. Adult CBA 6-8 week old mice were given i.p. daily 1 ml of 2% solution of the drug in Hanks' balanced salt solution for five successive days. On the sixth day mice were infected in groups of six selected at random with Semliki forest virus in dilutions from 10^{-1} to 10^{-7}. The titer of the strain used was $10^{-5,8}$. On the day of the infection and on days 7 and 8 mice were given additional doses of the drug.

In this experiment all the mice survived Semliki forest virus infection. If the experiment is done with a smaller number of doses of the drug the protective effect is reduced.

It is understood that in these experiments all the necessary controls were included.

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

The role of the lymphatic system during experimental infection of mice with arboviruses Ťahyňa and Semliki Forest viruses was studied. Beside the conventional titration method, a cannulation of thoracic lymph duct of mice was performed in order to follow spreading of virus from the peripheral site of inoculation (hind footpad) to the central lymphatic channel and from it to blood.

Footpad inoculation of adult mice with large doses of Ťahyňa virus (10^7 LD_{50}) did not result in apparent disease although the presence of neutralizing antibodies in sera of these mice indicated inapparent infection. Infectious virus was detected in draining popliteal lymph nodes within 30 minutes after inoculation. The titre was higher than $10^{3,5}$ LD_{50}/0,03 ml of 10% suspension. In iliac lymph nodes the titre was $10^{1,6}$ LD_{50}/0,03 ml.

Similar results were obtained during intervals of 1 and 2 hours and 1 day after inoculation. No virus was found in these lymph nodes at the later stages of infection nor in other groups of lymph nodes (cranial mesenteric, axillary) and lymphatic organs (thymus and spleen). No significant amount of infectious virus was detected in the thoracic lymph duct. These results indicate that lymph nodes draining the peripheral site of viral infection react as effective filters of Ťahyňa virus and prevent the spreading of virus to the central lymphatic channel at the early stages of infection. The lymphatic system appeared not to support the replication of Ťahyňa virus.

In contrast to Ťahyňa virus, peripheral - footpad - inoculation of Semliki Forest virus (10^5 PFU) leads inevitable to the generalized fatal infection of adult mice. The lymphatic organs serve as foci for viral multiplication. From the lymph collected within the first 30 minutes, virus was recovered in amounts of 100 - 600 PFU per ml of lymph. Similar amounts were present for 4 hours, until at 7 hours the amount increased reaching a maximum of 10^6 - 10^7 PFU per ml of lymph at 24 and 48 hours. These results show that for Semliki Forest virus lymphatic channels act as the effective route of quick spread of virus in the body of experimental animals, and that lymphatic organs have no effective filtering capacity.

(Z. Wallnerová)

During the summer of 1970, serologic and virus isolation studies were continued to further determine if Tahyňa virus infections had been associated with clinical illness. In an area of the natural focus of Tahyňa virus in South Moravia paired blood samples from 97 patients with a variety of acute febrile illnesses were collected. Six of these patients had significant increases in Tahyňa virus neutralizing antibodies between their acute and convalescent phase sera and were classified as serologically confirmed cases of acute Tahyňa virus infections. In one case a severe abacterial meningitis was diagnosed. In five patients there was a mild course of illness, characterized by fever with pharyngitis. Tahyňa virus isolation experiments were conducted on these patients with negative results.

(A. Šimková and F. Sluka)

REPORT FROM THE WHO REGIONAL REFERENCE LABORATORY FOR ARBOVIRUSES, INSTITUTE OF VIROLOGY, SLOVAK ACADEMY OF SCIENCES, BRATISLAVA, CZECHOSLOVAKIA

Transmission of tick-borne encephalitis (TBE) virus with Dermacentor marginatus and D. reticulatus ticks

Laboratory bred D. marginatus and D. reticulatus larvae, in special capsules, were allowed to feed on white mice. The mice weighing 6-8 g were infected intraperitoneally with 0.1 ml of 10% brain suspension of the TBE virus, from the 8th mouse passage. The virus in the 10% suspension was $10^{8.0}$ mouse intracerebral LD_{50} per 0.03 ml. The larvae of D. marginatus and D. reticulatus fed on simultaneously infected white mice for 2 or 3 days, respectively. The larvae of both species were infected in the middle of June. They were kept in a thermostatically controlled chamber at +22° - +24°C and 90%-95% relative humidity. The engorged larvae were periodically exposed to light.

The premoulting period in D. marginatus lasted 10 days; in D. reticulatus, 14 days. The prefeeding period in D. marginatus lasted 38 days; in D. reticulatus, 34 days. Virophoric period lasted in D. marginatus and D. reticulatus 50 or 51 days, respectively.

Positive results in interstadial transmission were obtained in 5 cases in D. marginatus and in 3 cases in D. reticulatus nymphs. Incubation period in mice of 10-14 days was observed in each species. Titer of suspensions from adults of both tick species after metamorphosis was in threshold value to $10^{2.0}$ mouse intracerebral LD_{50} per 0.03 ml. Identification of virus was carried out by neutralization test against hyperimmune goat serum (NI 10,000).

In comparison, the infection and transmission rates are a little lower in the Dermacentor species than in species of the genera Ixodes and Haemaphysalis.

(O. Kožuch., J. Nosek, - Acta virol. in press).

REPORT FROM THE WHO VIRUS DISEASES UNIT, GENEVA, SWITZERLAND

Arbovirus infections in human beings, 1969-1970

The meeting of Directors of WHO Reference Centres for Arboviruses expressed in 1969 the necessity to concentrate research activities upon arboviruses of public health importance. It was agreed that Reference Centres would develop closer contact with laboratories in their area to channel data on human diseases to WHO.

The following 4 tables are the result of this effort. Data on arbovirus infections in human beings have been collected from quarterly reports received in WHO from Regional Reference Centres and Collaborating Laboratories in 1969 and 1970. Tables 1 and 3 present isolated or sporadic cases diagnosed by virus isolation or a 4-fold rise in serological tests in paired sera. In tables 2 and 4 are presented the results of surveys motivated by outbreaks or routine surveillance. Cases in these surveys are diagnosed either by clinical examination, serological findings most frequently found in one single serum, or virus isolation.

It is evident that only a small part of arbovirus infections in human beings is recognized and that even less are diagnosed in laboratories. It is also evident that only a few confirmed arbovirus infections appear in these tables. It is felt that

HUMAN ARBOVIRUS INFECTIONS REPORTED 1969/1970

ABBREVIATIONS

ATL Virology Section, Center for Disease Control, Atlanta, Georgia, USA (Regional Reference Centre). Human Arbovirus infections have been reported to the Reference Centre by the following laboratories:

- California Department of Public Health, Bureau of Communicable Disease Control, Berkeley, California
- Arboviral Disease Section, Center for Disease Control, Fort Collins, Colorado
- Florida State Department of Health & Rehabilitation Services, Division of Health, Jacksonville, Florida
- New York Department of Health, Division of Laboratories and Research, Albany, New York
- Texas State Department of Health, Austin, Texas
- Zoonoses Research Unit, Department of Preventive Medicine, University of Wisconsin Medical Center, Madison, Wisconsin

BAD Perusahaan Negara "Bio Farma" (Pasteur Institute), Bandung, Indonesia
BAG Virus Research Institute, Bangkok, Thailand
BAN Institut Pasteur, Bangui, Central African Republic
BEL Virus Laboratory, Instituto Evandro Chagas, Belem, Brazil
BRI Queensland Institute of Medical Research, Brisbane, Australia (Regional Reference Centre)
BUD Department of Virology, National Institute of Public Health, Budapest, Hungary
DAK Institut Pasteur, Dakar, Senegal (Regional Reference Centre)
ENT East African Virus Research Institute, Entebbe, Uganda (Regional Reference Centre)
FRG Federal Republic of Germany
GUA Instituto Nacional de Higiene, "L Izquieta P", Guyaguil, Ecuador
HON Virus Unit, Medical and Health Department, Hong Kong
KIN Microbiology Department, University of West Indies, Kingston, Jamaica
MAR Instituto de Investigacion clinical, Maracaibo, Venezuela
OTH Other laboratory, i.e. laboratory from outside the country
PAN Gorgas Memorial Laboratory, Panama
PAR Institut Pasteur, Paris, France (Regional Reference Centre)
SOF Virus Department, Research Institute of Epidemiology and Microbiology, Sofia, Bulgaria
TOK Department of Virology and Rickettsiology, National Institute of Health, Tokyo, Japan
TRI Trinidad Regional Virus Laboratory, University of the West Indies, Trinidad and Tobago
WAR Arbovirus Unit, Department of Virology, State Institute of Hygiene, Warsaw, Poland
YAO Institut Pasteur, Yaounde, Cameroon

Table 1. Human arbovirus infections reported in 1969 by laboratory: sporadic cases (see notes)

Virus		North America	Central America	South America	Africa			Europe	Australia
		ATL	PAN	BEL	BAN	DAK	ENT	WAR	BRI
Group A									
Chikungunya	(i)				1				
Eastern equine encephalitis	(i)	2							
Venezuelan equine encephalitis	(i)		1						
Western equine encephalitis	(s)	2							
Group B									
Murray valley encephalitis	(i)								1
St. Louis encephalitis	(i)	1							
" " "	(s)	1							
Tick-borne encephalitis	(s)							26	
West Nile	(i)				1		1		
Yellow fever	(i)			1					
Group Bunyamwera									
Ilesha	(i)					1			
Group Simbu									
Oropouche	(i)			1					
Group California									
California encephalitis	(s)	56							
Group Vesicular stomatitis									
Piry	(i)			1					
Ungrouped									
Tataguine	(i)					1			

Notes on Table 1: Cases of human arbovirus infections shown in this table have been diagnosed by isolation of the virus (i) or serological examination of paired sera (s) in 1969 on material mostly collected in 1969. Some results correspond to samples collected in 1968. Confidential detailed information may be found in arbovirus quarterly reports which are available from WHO, Virus Diseases unit, upon justified request. For yellow fever cases, please refer to the Weekly Epidemiological Record.

Eastern equine encephalitis:	2 strains isolated in Florida, USA
Western equine encephalitis:	2 strains isolated in Texas, USA
St. Louis encephalitis:	1 isolation and 1 positive serology in California, USA
Oropouche:	one infection case
California encephalitis:	33 cases in Ohio, 21 in Wisconsin and 2 cases in Albany, New York
Piry:	infection case

Table 2. Human arbovirus infections reported in 1969 by laboratory: outbreak surveys or surveillance activities

Virus	Region	Source of information	Clinical obs. only	Serology*	Isolation	Remarks
Chikungunya or O'nyong-nyong	Lango District, Uganda	ENT		140		March 1969
Ross River	Queensland Australia	BRI		28		Epidemic polyarthritis
Venezuelan equine encephalitis	Hosp. Aislamiente, Playas, El Limon Ecuador	GUA	880	320	29	
" "	El Salvador, Guatemalo	ATL Ministry of Health	12	15		
" "	Goajira, Zulia State Venezuela	MAR Ministry of Health	993	20 2	3 59	4th quarter 1968
" "	Mara, Venezuela	MAR Regional Health Centre	1015		1	Oct-Nov. 1969
Dengue, type 1	Bandung, Djakarta, West Java, Indonesia	BAD		10 23		Surveillance 1968 Surveillance Jan-March 1969
Dengue, type 2-like	Puerto Rico	ATL Department of Health	10 033	31	37	

Table 2 continued

Virus	Region	Source of information	Clinical obs. only	Serology*	Isolation	Remarks
Dengue, type 1, 2	Martinique and Guadeloupe	PAR		37		2nd quarter 1968
Dengue, untyped	Jamaica	KIN		47		Jan-Sept. 1969
Dengue, untyped and/ or chikungunya	Thailand	BAG		DEN: 273 CHIK: 18 Double: 7		Surveillance of haemorrhagic fever 1968
" "	"	"		DEN: 267 CHIK: 8 Double: 23		Surveillance of haemorrhagic fever 1969
Japanese encephalitis	Japan	TOK		C: 270 D: 234		Surveillance 1968
" "	"	TOK		C: 1449(353) D: 448(254)		Surveillance up to Sept. 1969
" "	Chieng Mai Prov. Thailand	Comm. Dis. Control. Div. Dept. of Health Bangkok	185 C 45 D	21	1	May-Aug. 1969
Tahyna	France	PAR		5		1968-69

C = Cases D = Deaths

*Notes: Cases of human arbovirus infections shown in this table have been diagnosed by serological examination of paired or single sera. Only reports of outbreaks of regular surveillance are included. For details see arbovirus quarterly reports.

Dengue untyped: (Indonesia) most of examined paired sera presented a simultaneous increase of JE, HI antibodies
Japanese encephalitis: Figures given are suspected cases, clinically diagnosed cases are indicated in brackets.

Table 3. Human arbovirus infections reported in 1970 by laboratory: sporadic cases (see notes)

Virus		N. Amer.	Cent. Amer.			S. Amer.		Africa				Europe					Asia	Australia
		ATL	KIN	PAN	OTH	BEL	TRI	BAN	DAK	YAO	ENT	BUD	FRG	PAR	SOF	WAR	HON	BRI
Group A																		
EEE	(i)	1					3											
	(s)						1											
MAY	(i)					1												
RR	(i)																	4
VEE	(i)			1														
WEE	(s)	2																
Group B																		
Dengue-2	(i)				2		5											
	(s)				17													
Dengue untyped	(i)		16		2													
JE	(i)																1	
TBE	(i)											7		1				
	(s)												9			30		
SPO	(i)									1								
SLE	(s)	2																
WN	(i)								1									
Zika	(i)								2									
Group C																		
CAR	(i)					1												
Group Bunyamwera																		
Untyped	(i)										1							
ILE	(i)							2	6	1								
Group Bwamba																		
BWA	(i)							2										
Group California																		
CE	(s)	33																

Table 3 continued

Virus		N. Amer.	Cent. Amer.			S. Amer.		Africa				Europe					Asia	Australia
		ATL	KIN	PAN	OTH	BEL	TRI	BAN	DAK	YAO	ENT	BUD	FRG	PAR	SOF	WAR	HON	BRI
Group Nyando																		
NDO	(i)							1										
Group Phlebotomus																		
CHG	(i)			1														
Group Simbu																		
Untyped	(i)										1							
Group Ganjam																		
DUG	(i)							1										
Ungrouped																		
CTF	(i)	5																
	(s)	1																
Crimean HF	(i)														14			
NSD	(i)										1							

Notes: Cases of human arbovirus infections shown in this table have been diagnosed by isolation of the virus (i) or serological examination of paired sera (s) in 1970 on material mostly collected in 1970. Some results correspond to samples collected in 1969. Confidential detailed information may be found in arbovirus quarterly reports which are available from WHO, Virus Diseases unit, upon justified request. For yellow fever cases please refer to the Weekly Epidemiological Record.

Eastern equine encephalitis: the case in N. America was reported in Florida by Jacksonville laboratory
Western equine encephalitis: one case in Georgia and one case in Texas.
Dengue, type 2: two isolations and 17 sero-conversions reported in Haiti (in column OTH) between May 1969 and March 1970 by Miami School of Medicine
Dengue, untyped: two isolations from Haiti.
Tick-borne encephalitis: seven cases in Freiburg reported as Spring-Summer encephalitis and one case in Köln with one in Essen reported as Central European encephalitis (in column FGR)
California encephalitis: thirty-one cases reported in Wisconsin and 2 in Albany, New York
Colorado tick fever: four isolations in California and one by the CDC, Atlanta

Table 4. Human Arbovirus infections reported in 1970 by laboratory: outbreak surveys or surveillance activities (see notes)

Virus	Region	Source of information	Clinical obs. only	Serology*	Isolation	Remarks
Ross River	South Queensland Australia	BRI		36		Epidemic of polyarthritis 1969-70
Venezuelan equine encephalitis	Mara, Paez, Venezuela	VEN Regional Health Centre	1640	49	2	Nov-Dec. 1969
Dengue, type 2	San Juan Puerto Rico	ATL		36	32	July-Sept. 1969
Dengue, untyped	Djakarta, Bandung Indonesia	BAD		248		Surveillance 1970
Japanese encephalitis	Japan	TOK		C: 79 (9) D: 25 (8)		Surveillance up to August 1970
Crimean haemorrhagic fever	Bulgaria	SOF		12 24 8		Jan-Dec 1968 Jan-Dec 1969 Jan-June 1970

C = Cases D = Deaths

*Notes: Cases of human arbovirus infections shown in this table have been diagnosed by serological examination of paired or single sera. Only reports of outbreaks or regular surveillance surveys are included. For details see arbovirus quarterly reports.

Dengue type 2: (Puerto Rico) information given by the Walter Reed Army Institute of Research
Dengue - untyped: (Indonesia) most of examined paired sera presented a simultaneous increase of JE, HI antibodies
Japanese encephalitis: Figures given are suspected cases, clinically diagnosed cases are indicated in brackets.

the value of such information is dependent upon the number of laboratories participating in the reporting scheme and it would be necessary that many more contribute to this task. Those willing to do so should make contact with Reference Laboratories for their regions or the WHO Virus Diseases Unit, Geneva.

(P. Bres)

REPORT FROM THE NATIONAL INSTITUTE OF PUBLIC HEALTH, BUDAPEST, HUNGARY

In 3 districts of Hungary, each situated far from the other, ticks and mosquitoes have been collected for virus isolation experiments in suckling mice and blood samples from the residents, from domestic and wild-living animals for examination of their sero-immune state by haemagglutination inhibition and by neutralization tests. We resulted in isolating TBE strains in each of the examined districts. A Tahyňa virus strain and an UUkuniemi virus strain were isolated too, the first strain from mosquitoes, the other from ticks collected in one of the investigated districts. A mosquito-borne and a tick-borne arbovirus strain are not yet identified. Results of the serological survey, performed in collaboration with the Institute of Virology, SAS, Bratislava (M. Gresikova), show that 5-18% of the examined human sera proved to be positive against TBE, 4-10% against WN, 5-36% against TAH, 3-14% against WEE, 0-4% against Calovo, and 5-14% against UUK antigens, respectively; of the investigated cow sera 2-38% proved to be positive against TBE, 1-8% against WN, 6-38% against TAH, 0-2% against WEE, 37-47% against CLV, and 0-1% against UUK, respectively. Of the wild-living small mammals, 5-10% were found to be positive against TBE virus.

(E. Molnár)

REPORT FROM THE NATIONAL INSTITUTE OF HEALTH,
TOKYO, JAPAN

Duration of antibody in man following inapparent infection with Japanese encephalitis virus

In 1937 it was reported by Mitamura, Kitaoka et al that neutralizing (Nt) antibody for Japanese encephalitis (JE) virus could be detected in people who moved from JE endemic areas to a non-endemic area (Hokkaido) in a ratio 36.8% (14/38) for one to 5 years, 15.0% (6/40) for 6 to 10 years, and 14.3% (2/14) for 11 to 15 years but 0% (0/34) for 15-50 years after they left the endemic areas. Considering approximately 80% of the inhabitants positive due to inapparent infection at the time they left the endemic area, these data show that Nt antibody might persist for up to 15 years, although it does decrease rapidly once exposure to infectious mosquitoes ceases. Similar rates of antibody decline have been described in Americans who returned to their home country after residence in JE endemic areas of Japan.

The serological survey of JE in man and horses in Hokkaido (Miura and Kitaoka, 1955) revealed that the northern part of Hokkaido was free of JE virus but the southern part was slightly endemic of JE. In order to estimate more accurately the duration of antibody in man following inapparent infection with JE virus, long-term sentenced prisoners of the Abashiri prison which was located in the northern part of Hokkaido were tested in 1957. The volunteers, 63 in number who lived once before at the endemic area, were selected for bleeding and testing by CF and HI tests and by Nt tests in mice against JE antigen (Nakayama strain).

Three of the 63 tested (4.7%) were found to be CF antibody-positive at a 1:20 titer at the same year, 3 years and 10 years after imprisonment. HI antibody was found in 16 of 63 tested (25.4%), 4 (2 being 1:20, each one 1:40 and 1:10 in titer) in the same year, 2 (each one 1:40 and 1:640) in one year, 5 (3 being 1:20 and 2 1:40) in 2 years, 2 (each 1:20) in 3 years, one (1:20) in 7 years, and 2 (one being 1:20 and the other 1:40) in 10 years after imprisonment. As for Nt antibody, 48 of 62 tested (77.4%) were positive, 15 in the same year, 10 in one year, 11 in 2 years, 5 in 3 years, one in 5 years, 3 in 7 years and 3 in 10 years after imprisonment.

From the foregoing it is seen that the antibody to be detected in man following inapparent infection with JE virus decreased in titer year after year after moving from a JE endemic area to a non-endemic area such as the northern part of Hokkaido, but the different types of antibody (CF, HI and Nt) decreased at different rates. The CF antibody is first to go, then the HI antibody, and lastly the Nt antibody, which might be detected up to 15 years after infection. It must be kept in mind, however, that some individuals still give CF or HI positive reactions 10 years after infection.

REFERENCES

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(Masami Kitaoka and Takayuki Ogata)

Serological survey on Japanese encephalitis virus in inhabitants and animals on the Ogasawara Islands in 1968

The Izu Seven Islands are known to be endemic for JE virus by the incidence of JE cases and the results of serological surveys for JE antibody in inhabitants. HI tests on school children aged 6-11 years, 57 in number on Aogashima Island, 350 Km south of Tokyo (see Map), in July 1963 revealed 5.2% (3/57) positive against Nakayama antigen, 56.1% against JaGAr-01 antigen (a current strain of JE virus) and only one positive against Sagiyama antigen (1:10-20 for Nakayama antigen and 1:10-160 for JaGAr-01 antigen) (Table 1). From the foregoing it can be said that Aogashima Island is endemic for JE, as are the Izu Seven Islands.

The Ogasawara Islands are located 1,050 Km south of Tokyo in the Pacific. The inhabitants, 7,711 in all, had to be evacuated to the main islands of Japan due to the operation of the war in February 1944. Since the end of World War II the islands were placed under the sovereignty of U.S.A. and the former inhabitants who were not really Japanese, 129 in number, were allowed to go back to the islands in October 1946. They had no commuication with Japanese until 1968 when the islands were returned to the Japanese Government and to the Tokyo Prefecture as before.

It was important from the viewpoint of public health to carry out surveys for diseases and vectors on the islands comprising the main islands of Japan.

An attempt was made to determine whether Japanese encephalitis (JE) virus is distributed on the Ogasawara Islands in December 1968, under the support of the Health Bureau of Tokyo Prefectural Government.

Materials and methods:

In December, 1968, blood samples were collected from almost all Chichijima Island inhabitants, 84 in number (the age of 3 of them is unknown), and animals including 10 Rattus norvegicus, 17 dogs, a cat, 2 pigs, 2 oxen, a goat and 15 chickens.

HI tests against JaGAr-01 antigen were carried out according to Casals and Clark's method and Nt tests were done by plaque reduction technique against JaGAr-01 strain.

Results:

Table 2 indicates that HI antibody tests were all negative on sera collected from 24 males aged 13 to 25 and from 18 females aged from 12 to 35, and Nt antibody also was all negative on sera from 20 males aged 13 to 20 and from 18 females aged 12 to 35. However, HI antibody was detected in 88.8% (22/25) of males over 26 years of age and in 73.3% (11/15) of females over 36 years of age. Nt antibody tests were positive in 14 of 29 males (48.3%) over 21 years of age and in 8 of 14 females (57.1%) over 36 years of age. In other words both males and females who were born during the period under the sovereignty of U.S.A. were HI and Nt antibody negative for JE virus while more than half of inhabitants who were born during the period of the prewar were found still positive. From the foregoing it is likely that JE virus was not distributed on the Ogasawara Islands at least for 23 years covering from 1945 to 1968, and it is noteworthy that both HI and Nt antibodies could be still detected in more than half of inhabitants who were born during the prewar period. They had the risk to be bitten by the infectious mosquitoes on the main island of Japan during each of three summers, 1944 to 46. The HI antibody survey on sera collected from animals and birds above-mentioned on the same day of bleeding of inhabitants in December 1968 reveals that all of them were negative for JaGAr-01 antigen except for one pig which was transported from Tokyo to the island in October 1968.

Discussion:

In the consideration of the age of most of the domestic animals tested, such as the dogs, oxen, goat, chickens, and their being HI-antibody negative against JaGAr-01 antigen, it is very likely that no JE virus was distributed on the Chichijima island for the past few years. The result is coincident with lack of HI and Nt antibody in inhabitants who were born during the postwar period and never left the island. Thus it can be concluded that the island was not contaminated with JE virus for the past few years. The question arose, however, as to whether the antibody detected in more than half of the inhabitants was due to the inapparent infection acquired during their stay on the main islands of Japan from 1944 to 1946, or whether Chichijima was endemic for JE virus once before like Guam Island in 1944. The final answer will be given by further investigation.

On the other hand the entomological survey performed by Dr. Sasa and his colleagues in November and December 1968 reveals that Culex tritaeniorhynchus, vector of JE virus, is distributed on the island. The menace of an outbreak of JE cannot be denied if infectious mosquitoes or viremic-domestic animals such as pigs are transported from the main islands of Japan during the JE epidemic season. JE vaccination is recommended for inhabitants born on the island since 1946.

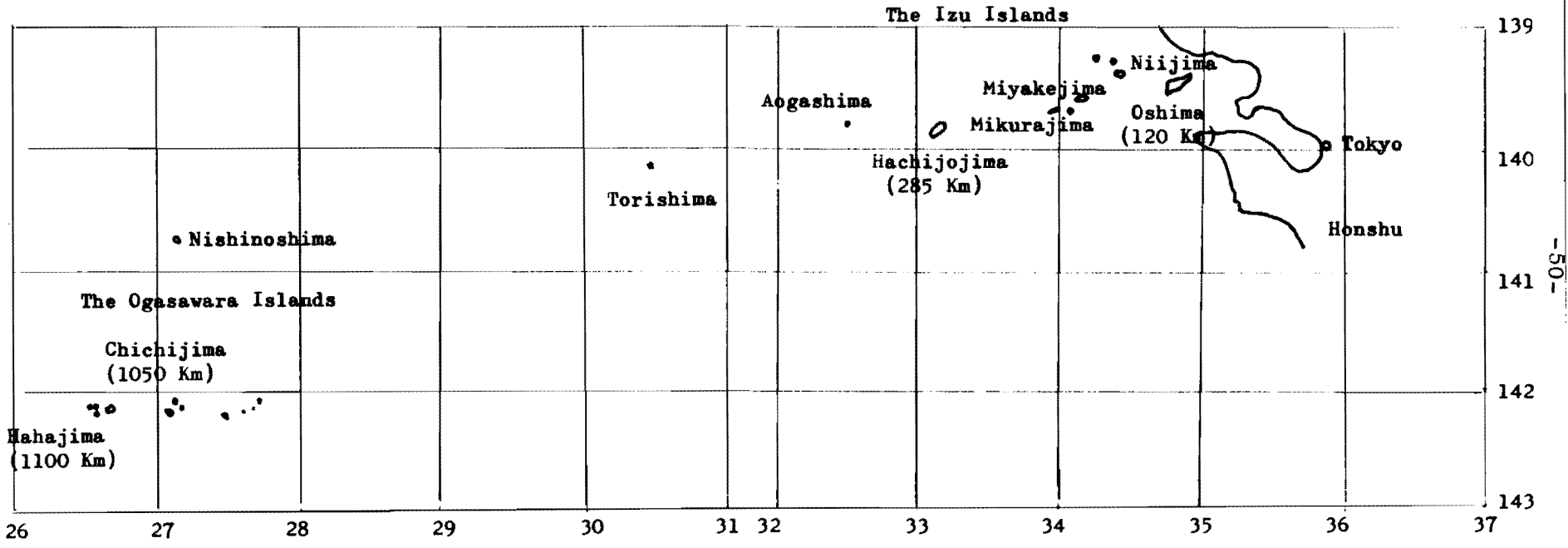
Conclusion:

Both HI and Nt antibodies were positive on sera collected from more than half of inhabitants in December 1968 who were evacuated from the island and spent 3 summer seasons of 1944 to 1946 in the main islands of Japan while all the inhabitants born on the island since 1946 were found antibody negative. No HI antibody for JE was found in domestic animals and birds on the island in December 1968 except for one pig transported from Tokyo in October 1968. It can be concluded that the island is nonendemic for JE virus.

(Masami Kitaoka, Takayuki Ogata and Akira Oya)

Map

The Izu Seven Islands and the Ogasawara Islands



(Km).....Distance from Tokyo

Table 1

HI antibody survey for Japanese encephalitis virus on sera of school children on Aogashima Island in 1963

Age	Children tested	Antigens used		
		Nakayama	JaGAr-01	Sagiyama
6	10	0	4	0
7	11	1	9	0
8	10	0	4	1
9	8	0	3	0
10	13	1	9	0
11	5	1	3	0
Total (%)	57	3 (5.2%)	32 (56.1%)	1 (1.7%)

HI titer	Antigens used		
	Nakayama	JaGAr-01	Sagiyama
1:10	2	15	1($\geq$1:10)
1:20	1	7	0
1:40	0	2	0
1:80	0	5	0
$\geq$ 1:160	0	3	0

Table 2

Serological survey on Japanese encephalitis
in all inhabitants on Chichijima in 1968

Age	Male		Female		Total	
	HI	NT	HI	NT	HI	NT
12			0/5	0/5	0/5	0/5
13	0/3*	0/3	0/3	0/3	0/6	0/6
14	0/2	0/2	0/1	0/1	0/3	0/3
15	0/2	0/2	0/6	0/6	0/8	0/8
16	0/5	0/5	0/1	0/1	0/6	0/6
17	0/2	0/2	0/0	0/0	0/2	0/2
18	0/3	0/3	0/1	0/1	0/4	0/4
19	0/1	0/1	0/0	0/0	0/1	0/1
20	0/2	0/2	0/0	0/0	0/2	0/2
21 ~ 25	0/4	1/4	0/0	0/0	0/4	1/4
26 ~ 30	1/2	1/2	0/0	0/0	1/2	1/2
31 ~ 35	1/2	0/2	0/1	0/1	1/3	0/3
36 ~ 40	3/4	3/4	2/2	2/2	5/6	5/6
41 ~ 45	6/6	2/6	4/7	3/7	10/13	5/13
46 ~ 50	2/2	2/2	2/2	1/2	4/4	3/4
51 ~ 55	3/3	2/3	1/1	1/1	4/4	3/4
56 ~ 60	2/2	1/2	0/0	0/0	2/2	1/2
61 ~ 65	1/1	1/1	1/1	0/1	2/2	1/2
66 ~ 70	2/2	0/2	1/1	1/1	3/3	1/3
over 71	1/1	1/1	0/0	0/0	1/1	1/1
Total	22/49	14/49	11/32	8/32	33/81	22/81

HI: Hemagglutination inhibiting antibody

NT: Neutralizing antibody

*Numerator: Positive number, Denominator: Number of people tested

REPORT FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY
INSTITUTE OF PUBLIC HEALTH
UNIVERSITY OF THE PHILIPPINES

Human arbovirus infections

Detection of arbovirus infection among cases admitted to the measles ward of an infection disease hospital was determined by HI test. 63 of 278 of paired serum samples tested or approximately 23% showed a significant increase in antibody titer to group B and 5 of the 278 (approximately 1.8%) showed a significant increase of antibody titer to group A. Antigens used were sucrose acetone extracts of dengue 2, JE, WN and Chikungunya.

Arbovirus antibodies in birds

A survey of birds of different species caught in the northern and central parts of Luzon using HI test showed that 119 out of 142 or 84% had antibodies to group B viruses represented by dengue 2, JE and WN and 9 of 138 or 0.6% had antibodies to Group A represented by Chikungunya. Ninety-nine of the 119 samples had antibodies to all 3 viruses of group B used in the test; 1 had antibodies to D_2 alone, 5 to WN alone and 4 to JE alone. Ten had antibodies to JE and WN but not to D_2 and 6 had antibodies for D_2 and WN but not for JE. The initial serum dilution used was 1:20.

Immunoglobulin studies

The possible diagnostic use of 19S Ig in late single serum samples as an indicator of current illness is being studied with the dengue virus complex. Serums are fractionated by sucrose density gradient, and antibodies are tested by HI. The presence of 19S and 7S Ig is confirmed by treatment with mercaptoethanol. Preliminary results out of 25 serums seem to suggest that the pattern of the 7S Ig rather than the presence of 19S Ig may prove more meaningful in the detection of infection in single serum samples obtained around the 10th day after onset of illness.

(Lourdes E. Campos)

REPORT FROM THE ARBOVIRUS RESEARCH UNIT HOOPER FOUNDATION AND UNIVERSITY OF CALIFORNIA ICMRT, SAN FRANCISCO AND THE UNIVERSITY OF MALAYA, KUALA LUMPUR

Last year, we reported the apparent introduction of Aedes aegypti mosquitoes into the labor lines area of the Carev Island study site and its replacement of most of the previously dominant A. albopictus population. Additional surveys of the area in 1970 showed the continued but slow spread of A. aegypti several additional yards beyond the housing perimeter and the failure of A. albopictus to compete favorably.

Through June 1970, we continued to provide a laboratory diagnostic service for arbovirus infections for the entire Federation of Malaysia through the kind cooperation of the Virus Research Division of the Institute for Medical Research. From July 1970, the service was returned to the Institute, except for material submitted by the University Hospital. During 1970, 968 specimens were received from 606 patients suspected of having arbovirus infections in Malaysia. Dengue was diagnosed in 88 cases (264 in 1969) and Japanese encephalitis was diagnosed in 21 cases (84 in 1969).

A detailed clinical and epidemiological review of all the laboratory confirmed cases from 1965 to 1970 is being initiated in collaboration with Malaysian physicians.

An apparent Sindbis infection occurred in an 11-year-old Indian male from Penang. The child was suffering from encephalitis for over one month. A presumptive positive laboratory diagnosis of Japanese encephalitis virus infection was made serologically on late specimens. From the 32nd to 42nd days of illness, however, an 8-fold rise in titer was demonstrated for Sindbis HI antibody, indicating that a Sindbis (or closely-related virus) infection had occurred before the patient had recovered from the initial Japanese encephalitis infection. Further studies are planned in an attempt to verify the serology. Illness due to Sindbis virus has not been reported yet in Malaysia, although human infection is believed to occur on the basis of normal serological surveys.

Twenty-six agents, isolated from a series of 84 pig sera collected in the Manila, Philippines slaughterhouse, do not appear to be arboviruses. All 26 agents are similar and produced illness and death in suckling mice with an incubation period of 2-3 days. One agent, representative of the group, was studied in greater detail and has the following characteristics:

1) it does not produce death in weanling mice, 2) it is resistant to treatment with sodium desoxycholate and chloroform, 3) it measures less than 50 mu in size as determined by filtration with millipore filters, 4) it produced no significant reaction in guinea pigs, chick embryos, HeLa cells, L-cells, and LLC-MK-2 cells, and 5) it caused cell degeneration in PK-15 cultures in 2-3 days but titered low.

Recent difficulties in mouse isolation and identification of new virus isolates were traced to the presence of reovirus type 3 in our source mouse colony in Malaysia. The presence of reovirus 3 may have masked other viruses present in the suspensions and, in at least one case, was isolated in place of the original agent detected (that virus was subsequently re-isolated from the original material). A total of 10 strains of reovirus 3 have been identified and another possible 19 isolates are suspected to be reovirus 3, but the identifications have not yet been confirmed. Infected mice often showed typical reovirus 3 symptoms, the most striking being steatorrheic diarrhea. Adaptation to central nervous system tissue on brain passage with development of fatal encephalitis in mice developed readily after a few passages. Improvement of general sanitation and breeding practices in the source colony appears to be solving the problem.

A virus isolated from the blood of a wild Macaca irus monkey trapped in mangrove swamp forest has been identified as a strain of Bakau by complement-fixation, mouse neutralization, and plaque reduction neutralization tests. Comparisons were made with Bakau strains AMM-2325 and E-210, both from mosquitoes. This represents the first isolation of Bakau from a vertebrate. Neutralizing antibody was demonstrated in wild monkeys by screen neutralization tests in mice and confirmed by plaque reduction neutralization tests in Vero cells. Of 29 monkey sera tested, 8 from mangrove swamp forest were positive.

Further tests with the Wad Medani virus strains continue to show that the Singapore strain (SM-214) and the Malaysian strain (P5-127) differ significantly from the Indian (IG-673) and the original Egyptian strains. The name Seletar has been proposed for our strains.

Two isolates from Argas ticks taken from Scotophilus bats in Malaysia and one from the blood of the same bat species are strains of the same virus and appear to be unrelated to other known arboviruses tested by us and by Dr. Jordi Casals at YARU. This virus will, therefore, be registered in the Catalogue of Arthropod-Borne Viruses and will be named Keterah for the locality where it was recovered.

Several additional agents, including a number of dengue strains, are under study. Of particular interest are five isolates from bat salivary glands (2 from Macroglossus lagochilus and 3 from Cynopterus brachyotis).

A new jungle dengue study area in isolated forest reserve in Kedah has been initiated recently. The area is relatively undisturbed and has large populations of wild primates. Several methods of primate capture are being tried with the aid of aborigine trappers. The methods include the use of aboriginal blowpipes and an attempt is being made to use syringe-darts (with anesthetic) adapted to the blowpipe.

(A. Rudnick, N.J. Marchette, and R.W. Dewey)

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

Outbreak of dengue in Asansol in West Bengal

Asansol, a railway town about 160 Km west of Calcutta, has a population of over 100,000 of which the population of railway colony constitutes about 20 percent. During the months of July through October 1967, Asansol experienced an epidemic of febrile illness clinically resembling dengue fever. Exact data about the total number of people affected by fever are not available, but roughly it can be said that there were about 2000 cases of fever during these months in the railway colony. Of course not all of them clinically simulated dengue fever, and some of them were likely to be influenza, common cold, etc.

A team from this department proceeded to Asansol for investigation. Altogether, 77 patients with dengue-like symptoms could be virologically studied. Samples of blood from the patients were dispatched on wet ice to Calcutta through a courier by train to Calcutta. It took 12 to 20 hours before the samples were preserved at -70°C.

Out of 77 samples of sera inoculated into mice, viral agents, subsequently identified as dengue, were isolated from the sera of 10 cases. Seven of the isolated strains were dengue type 2 and three were dengue type 4. Two patients had haemorrhagic manifestation, and no virus could be isolated from their sera.

One of the cases, a 12 year old male child showed high HI & CF antibodies against chikungunya in the acute sample of serum although dengue virus was isolated from the same. He had 4-5 days of fever followed by 4-5 days of afebrile period; then he had another bout of fever when on the 2nd day, his blood was collected and it yielded the dengue virus. Results of HI & CF tests of the paired sera of this case:

Serum	Day of illness	Chik		Dengue		JE		WN	
		HI	CF	HI	CF	HI	CF	HI	CF
Acute	2 (11 from the first bout of fever)	640	32	<20	<4	<20	<4	<20	<4
Convalescent	53	320	16	1280	32	640	16	640	16

There is possibility in this case of the first bout of fever to be of chikungunya origin, while the second bout was caused by dengue infection.

(J.K. Sarkar, J.M. Ghosh, S.N. Chatterjee, and S.K. Chakravarty)

REPORT FROM THE MYSORE VIRUS DIAGNOSTIC LABORATORY, SHIMOGA MYSORE STATE, INDIA

As usual, the surveillance for the detection and follow-up of Kyasanur Forest Disease (K.F.D.) amongst human beings and of monkey mortality was continued during the year 1969-70 also.

HUMAN

The human incidence of K.F.D. during the current epidemic season was restricted to the period from January 1970 to June 1970, with a peak incidence during the month of February 1970. However, the detection of a solitary virus positive case during the first

week of August 1970 from a new theatre adjoining an established area and long after the subsidence of the K.F.D. epidemic, was an exceptional feature seen during the present epidemic.

A total of 255 human cases were encountered and investigated during the above period and blood samples had been obtained from every case so investigated. Of these, blood samples obtained from 77 cases yielded the K.F.D. virus, giving an isolation rate of 30.2%. Of these virus positive cases, one was an accidental laboratory infection involving an animal attendant of the laboratory at Shimoga.

Of these 77 virus positive cases, 85.7% occurred during the months of January, February and March 1970 alone.

The epidemic occurred in 30 villages of which 15 were the old and established theatres. The rest of the areas were quite new where the disease had never been reported during any of the previous years.

Three cases ended fatally giving a case fatality rate of 3.89%. These cases were either untreated or hospitalized in advanced states of the disease.

In addition to the existing facilities, the State Government has now provided improved additional facilities in 8 medical institutions located and functioning in the K.F.D. area. These additional facilities include additional number of hospital beds, additional accomodation, additional supply of drugs and equipments required to hospitalize and treat cases of Kyasanur Forest Disease.

MONKEY MORTALITY

The season of virus positive monkey mortality closely preceded that of the human epidemic. The season started during the second week of December 1969 and lasted till the second week of June 1970. A total of 75 monkey deaths were reported of which 13 dead monkeys were fit for autopsy. Autopsy materials collected and processed from 7 dead monkeys yielded the K.F.D. virus.

PROGRESS OF CONTROL AND PREVENTIVE MEASURES

In order to further assess the value of the method of "area spraying" of B.H.C. (Lindane Quality) with a view to devise comprehensive methods for the control of ticks as a means for the control of K.F.D. infections in man, further large scale experiments had been undertaken by the Department under the technical guidance of the Virus Research Centre, Poona. The experiments lasted from January 1970 to May 1970. The results of these experiments have been identical to those of the earlier experiments carried out in this behalf. Spraying of the forest floor with Lindane at the rate of 1/2 to 1 lb per acre holds out a promise of the reduction of the vector density for a period of 4-6 weeks.

As a further measure of boosting the immunity of the residents inhabiting the K.F.D. area, consideration is now given to the administration of a specific vaccine against the Kyasanur Forest Disease. The concept of the vaccine administration against Kyasanur Forest Disease is new and for which a preliminary field trial of the vaccine is necessary before the same could be administered on a large scale. A formalin killed K.F.D. vaccine has been prepared and supplied to the State Health Services Department by the Virus Research Centre, Poona for being subjected to a field trial. The State Government has entrusted this task of field trial studies of the K.F.D. vaccine to an independent officer who has started the work. The vaccine trial studies are progressing, under the technical guidance of the Virus Research Centre, Poona.

(R. Rama Rao)

REPORT FROM THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE, AUSTRALIA

The following is extracted from the annual report of the Institute for the year 1970. Copies of the complete report can be made available to anyone interested.

A number of viruses were isolated and found distinct from any known in Australia (and, in some cases, in collaborative studies with the WHO International Reference Laboratory, from any known elsewhere), increasing to 27 the number of arboviruses isolated in Australia. Five viruses have now been isolated from midges and one from sandflies, confirming that these insects may indeed be of importance in virus transmission in Australia.

Other investigations extended our knowledge of where and when arboviruses cause infection and in what animal hosts, by laboratory tests to detect antibodies, proteins basic to immunity which persist in the blood for long periods after infection. Most arboviruses depend for their survival in nature on birds or mammals other than man, and in this year several recently isolated viruses were found to infect cattle, sheep, horses, kangaroos and wallabies. Such tests may also be used to show the extent of human infection, and tests this year showed several viruses isolated originally in Queensland to have caused widespread infection in the Northern Territory.

As part of this research programme the Institute investigated patients suspected to have illnesses caused by arbovirus infection, and in the year tested over 1,000 serum samples. These tests proved infection with Ross River virus in 52 patients with epidemic polyarthritis in Queensland in November 1969-March 1970, and gave some support to that diagnosis in 121 others from whom less adequate specimens were received. Epidemic polyarthritis is an illness with mild fever, painful joints and sometimes a rash, usually benign but sometimes producing arthritis severe enough to cause disability for some months. Analysis of the 52 cases in this epidemic and 63 cases diagnosed in the period 1959-1969 showed that the disease has occurred especially in young adults, rarely in children, over a wide area of eastern Australia in the summer and autumn months, most commonly in the period January to May.

A major part of this study of insect-borne disease concerns the insects themselves, as sources from which viruses are isolated, as important parts of the cycle of transmission and as possible targets in control measures. In this report the entomology section describes a wide range of mosquitoes, midges, sandflies and other insects collected in field work at Mitchell River, Charleville and Brisbane, and discusses some of the factors influencing insect populations and behaviour, which in turn determine what species can transmit viruses effectively. It also reports trials of methods of vector control that might be used in future epidemics of arbovirus infection. Aerial spraying offers possibilities of major advance, but with some problems that, as is pointed out, need careful scrutiny if the method is to be effective and safe.

One Institute unit has over the years described and classified many species of blood-sucking mites as a necessary preliminary to consideration of their medical importance, and has also investigated several disease problems in which mites are known or suspected to be involved. The present report describes basic studies of mites which parasitize bats in Australia and more applied work which failed to detect Leptotrombidium deliense, the vector of scrub typhus, in south Queensland, and found no relation in Brisbane between seasonal peaks of asthma and populations of the mite Dermatophagoides pteronyssinus, suspected to be a source of allergen in house-dust.

Some Institute scientists have been concerned with more basic laboratory research on the viruses isolated from insects and other hosts. This is important from several points of view - to provide better ways of handling the viruses, better diagnostic tests and possibly better vaccines (if they should prove desirable) and also to contribute to the world-wide efforts of virologists to classify viruses in a rational way based on their structure and composition. This year the investigation is described of five viruses isolated from midges or mosquitoes but with physical and chemical properties sharply different from those of most arboviruses, although similar to the agents of two important veterinary diseases not known in Australia. In other work, largely unsuccessful attempts were made to develop a simple laboratory test to survey cattle serum for antibody to ephemeral fever virus, and small but reproducible differences were demonstrated between a strain of Murray Valley encephalitis virus isolated by the Department of Health in 1969 and other available strains of that virus.

(R.L. Doherty, J.G. Carley, R. Domrow, H.A. Standfast (to June 1970), B.M. Gorman, E. Judith Barrett, B.H. Kay, and Patricia Goss)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF OTAGO
DUNEDIN, NEW ZEALAND AND THE
MEDICAL RESEARCH COUNCIL OF NEW ZEALAND'S VIRUS RESEARCH UNIT

Arboviruses in Fiji

Ten years of arbovirus survey work in the Fiji group of islands have now been completed. During this time, over 200,000 mosquitoes, 9,000 ticks and 575 serum samples were tested for viruses, but no arboviruses were isolated. Eight thousand human and 1,117 bird, bat and animal sera were tested for haemagglutination-inhibiting arbovirus antibody using a variety of Group A, Group B and Bunyamwera Group antigens. Only a small number of low-level reactions were found among the non-human sera, but 14% of all human sera were found to contain Group B antibody. The antibody prevalence increased with increasing age, from less than 1% for persons born since 1950, to 70% for persons born before 1900. From the age differences in prevalence it was possible to estimate the time and size of previous epidemics. These occurred in 1905 (with 17% of susceptible persons becoming infected over a five year period), 1930 (14% over five years) and 1943 (23% over five years). Between these epidemics, virus infections still occurred. For example, between the years 1907 and 1927 when there were no known epidemics, the infection rate was still 1.3-1.4% of susceptibles per year. Differences were found in antibody prevalences between the sexes, between ethnic groups and between persons from different regions. These differences could be explained in terms of climate, location and custom.

Historical and serological evidence both suggest that all the antibody detected was due to past exposure to dengue virus. The very high proportion of the population with no dengue antibody (86%) makes Fiji a high risk area for a further epidemic. Dengue virus is known to be active at the present time in the Pacific and South East Asian region.

Experimental infection of wild birds with Whataroa virus

Field studies have indicated that various species of wild birds act as vertebrate hosts of Whataroa virus in an area where the virus is enzootic. In order to increase our knowledge of the response of normal birds to a simulated natural infection, wild birds, caught in an area where Whataroa virus is not known to occur and shown to be free of specific serum neutralizing antibody, were injected with small doses of Whataroa virus by a

shallow intramuscular route. Blood samples were taken at intervals for virus and antibody titration. Virus in blood was titrated by counting plaques produced on duck embryo cells maintained under agar, and sera were titrated for plaque-neutralizing antibody.

A total of 65 birds of six species were infected. The majority of the birds responded with a viraemia which began on the first or second day after injection and reached a maximum level on the third or fourth day. At its peak level the viraemia in individual birds ranged from $10^{7.1}$ to $10^{8.2}$ plaque-forming units of virus per ml. of blood. The disappearance of virus from the blood between the fifth and seventh day was associated with the appearance of serum neutralizing antibodies. Antibody titres reached their highest levels during the first two weeks after infection. Some birds have been tested for as long as 10 months after infection and although the antibody titres have dropped, in no case has antibody disappeared.

A feature of the plaque-neutralizing antibody titrations was the presence of a prozone, particularly in sera collected immediately after the end of viraemia. Attempts to identify the component responsible for the prozone by separation of serum proteins have not yet been entirely successful as the component appears to be rather labile.

(F.J. Austin)

REPORT FROM THE
DEPARTMENT OF TROPICAL MEDICINE AND MEDICAL MICROBIOLOGY
UNIVERSITY OF HAWAII SCHOOL OF MEDICINE
HONOLULU, HAWAII

Studies on dengue virus isolation methods from tissue samples

Dengue virus has proven to be very difficult to isolate from human autopsy material of fatal dengue hemorrhagic fever cases. The reason for this difficulty is not known, but preliminary experiments in dengue susceptible rhesus monkeys have clearly shown that many tissues contain potent dengue virus inhibitors when triturated. There is suggestive evidence that dengue virus

inhibitors are also released when infected tissues are stored for even short periods at -70°C prior to trituration or explant culture. These "inhibitors" are particularly effective in preventing multiplication of dengue virus in the tissue culture plaque assay system, less so in infant mouse brain.

Since studies on the pathogenesis of dengue virus in rhesus monkeys required a sensitive assay system free from the effect of normal viral inhibitors, it was necessary to investigate new techniques of virus recovery from tissues. An assay system involving the co-cultivation of trypsinized, washed cells from infected monkey tissues on LLC-MK2 cell monolayers showed some promise. This technique resulted in a higher rate of recovery of virus from monkey tissues than the inoculation of triturates onto susceptible LLC-MK2 monolayers. However, the method was rather laborious, requiring many hours to process tissues and resulted in a considerable loss of cell mass.

Consequently the Tissue Fragment Culture Technique was devised. In this method small samples of tissue are minced with scissors and placed in a 1 oz. prescription bottle containing tissue culture growth medium (BME - Earles with 10% calf serum, 1% glutamine and 200 units each of penicillin and streptomycin per ml and 3 ml of 7.5% $NaHCO_3$ per 100 ml). Minced tissues are cultured at 37°C for up to 13 days with periodic changes of growth medium. The fluid overlay is tested for dengue virus using the LLC-MK2 plaque method. The metabolic activity of tissue fragments is readily monitored by observing pH changes in the culture medium. The optimum interval between medium changes has not been established. In the system currently employed, the original growth medium is discarded after 3 days incubation at 37°C; two to three additional medium changes are made at 3 day intervals and the removed medium assayed for dengue virus.

The tissue fragment culture method has been used for detection of dengue virus in the tissues of rhesus monkeys experimentally infected with low tissue culture passage dengue types 1, 2, 3 and 4. The results vary with the virus type used and the time of sacrifice after inoculation. Preliminary results suggest that after subcutaneous inoculation, dengue virus replicates in skin at the site of inoculation then in the regional lymph nodes and eventually spreads to other lymphatic tissue and the digestive tract and skin.

The tissue fragment culture technique so far has been used mostly for dengue virus isolation from tissues of experimentally infected monkeys taken immediately after death, whereas tissue samples from fatal cases of human dengue hemorrhagic fever usually cannot be obtained until 24-48 hours or longer after death. It is not known how long dengue virus can remain viable in tissues of fatal hemorrhagic cases, but rapid loss of viability of virus particles after host death could explain the difficulty of recovering dengue from human autopsy material. Experiments with tissues from experimentally infected monkeys, however, suggest that dengue virus may remain viable in tissue samples for long periods. Virus was readily recovered by culture of tissue fragments stored at 4°C for 1, 2, 3 and 4 days. Periods longer than 4 days were not tested.

On the other hand, dengue virus could not be recovered from tissue samples stored for 2 weeks at -70°C even though virus was readily recovered from duplicate samples of the same tissue when tested fresh or after 1 to 4 days storage at 4°C.

(Scott B. Halstead)

REPORT FROM THE INSTITUTO DE VIROLOGIA, CORDOBA, ARGENTINA

LCM virus in the endemic area of Argentinian hemorrhagic fever

In March, 1969, a virus strain closely related to LCM virus was isolated from the brain and blood of one Mus musculus rodent, captured in the field of the endemic area of AHF in the province of Cordoba, Argentina. The brains of two Calomys musculinus rodents caught simultaneously with this M. musculus yielded Junin virus.

The reisolation attempts from the brain and blood of the M. musculus were again positive. In addition, serological evidence of LCM activity was obtained in blood from Mus also caught in the same area.

These results meet the requirement for the validity of the isolation. Furthermore, it was also shown that the colony of laboratory mice used for the isolation attempt was free of infection with LCM virus.

This is the first time that two different members of the recently proposed "Arenovirus" family are demonstrated in the same geographic area. This is not unexpected because the host of one of them is a cosmopolite species but it presents an interesting situation in the ecological investigations.

These studies have been carried out with the participation of Dr. J. Barrera Oro and Dr. J. Maiztegui.

(M.S. Sabattini)

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

Following the widespread 1967-68 epizoodemic of Venezuelan equine encephalomyelitis (VEE) in areas of low and moderate seasonal rainfall in Colombia, a strain of VEE virus was sought in the high-rainfall region of the Pacific lowlands where previous serologic studies had shown past activity of VEE virus in humans without indication of overt clinical illness in epidemic form. A total of 20 sentinel hamsters, in two groups, were exposed for two 2-week periods in July and August-September 1969 along the margins of a grass-overgrown freshwater swampy area 50 km inland from the port of Tumaco near the Ecuadorian border. One hamster yielded VEE virus and two others yielded Eastern equine encephalomyelitis (EEE) virus. Virus was not isolated from 7,199 mosquitoes captured in the vicinity of the exposed hamsters. This isolation of VEE virus confirms the suspected presence of the agent in the Pacific lowlands of Colombia and also the utility of sentinel hamsters for detecting endemic VEE virus activity in the American tropics. The isolations of EEE virus are the first from Colombia and the first from the west coast of South America.

Early in September 1970 word was received from Dr. Kenneth S. Preston of the University of Nebraska Mission in Bogota of horses dying with signs of encephalitis in the rural area of Suaza, Departamento del Huila. Hemagglutination-inhibition tests of horse sera collected there by Dr. Preston indicated recent activity of the VEE virus and prompted a field reconnaisance with the objective of isolating a virus strain from the outbreak. A field trip was made from 23 to 28 September when clinical cases of equine encephalitis were seen in the rural areas of Suaza, Pitalito, Tarqui and Guadalupe. VEE virus was recovered from the brain of a horse which had died near Suaza. It appeared that the equine cases there were following a pattern quite different from that observed during the 1967-1968 epizootic in areas where mosquitoes were the main vectors, i.e., Carmelo, the Atlantic coastal plain and areas which lie in the floor of the upper Magdalena River valley. Now in Huila, sickness and deaths of horses seemed to be sporadic and of spotty distribution over a relatively large area of both hilly and flat country varying between 1,000 to 1,300 meters of altitude. The situation resembled to some extent that observed in 1967-1968 at Atuncela and La Maria (described in previous contribution) where Simulium black flies seemed to be associated with the VEE epizootic. The distinctive feature in Huila was that in some instances only one or a few of several horses pastured together got infected, as evidenced by clinical observation and serological tests, showing practically no secondary cases within some horse groups and implying poor vector conditions for the VEE virus. Human and bovine sera collected during this trip were essentially negative for VEE antibodies.

To get basic information on the haematophagous arthropod fauna of the Huila area a preliminary entomological survey was done between October 14 and 24 in the region of Suaza where sick horses were currently being seen. For the collection of volant haematophagous arthropods, horse-baited stable-traps, captures on free horses, hamster-baited Trinidad No. 10 traps and CDC miniature light-traps were employed.

Table 1 shows the generic composition of the mosquitoes captured from dusk to dawn in horse-baited traps. Only 104 specimens were collected, representing a mean of 9 mosquitoes per trap-night. These striking low figures would indicate a low density of the mosquitoes attacking horses at night in Suaza. The high proportion of Psorophora seems to be the reflection of the soggy terrain of San Calixto-San Isidro where the traps were operated. The mosquitoes collected in the stable-traps were processed in 32 pools for the presence of viruses, after being kept alive for no less than 48 hours for the digestion of ingested blood. No viruses were isolated from these specimens.

Table 1

Generic Distribution of Mosquitoes Captured in Horse-Baited Stable-Traps in Suaza, Huila, October, 1970

Total of Mosquitoes	104
No. of Trap-Nights	11
Mean per Trap-night	9
Genera	%
Anopheles	16.3
Psorophora	56.7
Aedes	8.7
Culex	18.3
Total	100.0

Table 2

Generic Distribution of Mosquitoes Collected Off-Horses in Suaza, Huila, October, 1970

	Daylight		Evening (18:00 to 20:00 hours)		Total	
	Mosquitoes	Simuliids	Mosquitoes	Simuliids	Mosquitoes	Simuliids
Total	240	6430	244	41	484	6471
Mean per man-hour	8	198	24	4	11	152
Mosquito genera	%		%		%	
Anopheles	19.6		13.5		16.5	
Wyeomyia	42.9		30.7		36.8	
Mansonia	5.8		3.7		4.8	
Psorophora	13.8		17.2		15.5	
Aedes	12.5		6.2		9.3	
Culex	5.0		28.7		16.9	
Total	99.6		100.0		99.8	

Table 3

Generic Distribution of Haematophagous Arthropods
Collected in CDC Miniature Light Traps
Suaza, Huila. October, 1970

Total	511
No. of Trap-Nights	44
Mean per Trap-Night	12
Genera	%
Anopheles	4.5
Mansonia	4.9
Uranotaenia	12.1
Psorophora	6.5
Aedes	2.0
Culex	42.1
Total Culicidae	72.1
Simulium	1.2
Culicoides	26.7
Grand Total	100.0

Captures of volant haematophagous arthropods attacking free horses during the day and evening are presented in Table 2. Here the obvious finding is the large number of Simulium black flies taken during daylight hours. The evening collections did not extend beyond 20:00 hours, and represent therefore the entomological fauna active in the early evening. It should be explained that 45 of the 47 anopheline representing about 20% of the daylight captures and all the simuliids of the evening collections were obtained at dusk. In the case of Wyeomyia (a mosquito reputedly of diurnal habits) of the 75 specimens, representing nearly 31% of the evening captures, 46 were obtained close to 8 o'clock; a potent portable fluorescent light which was used during the evening collections off horses could account for this nocturnal activity of Wyeomyia; members of this genus, on the other hand, were not present in the stable-traps captures.

Hamster-baited Trinidad No. 10 traps were entirely unproductive during 32 trap-days and 32 trap-nights. These negative results could represent different host availabilities and/or feeding preferences of the local entomological fauna.

The yield of the light-traps is summarized in Table 3. Culex now represents 42%; Uranotaenia (a genus whose feeding habits are not well known) makes 12% of the collections. The relatively high proportion of Culicoides is again an expression of the ground characteristics.

(Pablo Barreto, Clara Lesmes, Ronald B. Mackenzie, Carlos Sanmartin, and Harold Trapido)

REPORT FROM THE MIDDLE AMERICA RESEARCH UNIT, BALBOA HEIGHTS, PANAMA CANAL ZONE

Venezuelan equine encephalitis in Costa Rica, 1970

The Central American epizootic of Venezuelan equine encephalitis, which began in El Salvador in 1969 and by early 1970 had spread into Mexico to the north and Nicaragua to the south, made its first appearance in Costa Rica in August 1970. Guanacaste, the northwestern-most state in Costa Rica, is a cattle raising region with a relatively dense equine population, which had been shown by an earlier serum survey to be largely susceptible to VEE infection.

The first cases were reported from a large coastal ranch near the Nicaraguan border on August 8 and 9, 1970. The initial isolate was made in our laboratory from a blood sample taken on August 11 from an asymptomatic horse belonging to the herd containing the index cases. This isolate was shown by the kinetic hemagglutination-inhibition test to be a strain of VEE identical antigenically to the strain isolated on multiple occasions from equines farther north in the 1969 Central American epizootic.

A team from our laboratory was in the field soon thereafter and was joined by a group from the CDC Arbovirology Unit who carried out investigations of mosquito vectors. Our group selected a small village near the place where the original outbreak had occurred for epidemiological studies of the equine and human population. The study site was located in a nearly sea level, flat valley, about 2 kilometers from the Pacific shore. There were approximately 200 human inhabitants and an equine population of roughly 150.

Data on horses were collected by questioning the ranchers carefully as to numbers of animals owned, their ages and sexes, as well as the number of cases and deaths which occurred among them. All horses were bled on September 12, when they were brought together to be vaccinated. The information we have represents mortality rates up to the time vaccination took effect, estimated to be September 21 to 23. Table 1 reflects the fact that mortality rate bore no relationship to age or sex except possibly in animals over 11 years of age. The overall mortality rate of 38% is consistent with that estimated from surveys in Guatemala and Colombian epizootics. Virus was isolated from nine equines bled on September 12. All were asymptomatic at the time. Eight developed symptoms from one to seven days afterwards and four died. One mule did not develop symptoms. Eleven horses had neutralizing antibody against VEE at the time of this bleeding. A detailed serological study of a large horse herd near this area, bled just before the epizootic's onset, indicates that VEE virus has been in this area previously, though probably more than four years ago. Therefore, we cannot assume that the presence of antibody reflects recent exposure. Work is presently in progress which may enable us to identify the specific VEE variant with which an animal has been infected and it may then be possible to calculate an accurate attack and case mortality rate for the horses studied.

One hundred and sixteen of the human inhabitants of the village were bled between August 29 and September 2, and followed clinically at three to four day intervals during the subsequent two weeks when the epizootic was at its height, and then rebled on October 10. There were no positives on the first bleeding. Thirteen persons developed significant neutralizing antibody titers during this period for an attack rate of 11%. Six individuals who converted gave histories of a severe, incapacitating febrile illness lasting three to four days, followed by marked malaise for up to one week. Three of these patients were seen by us one day after onset of symptoms. All had fevers ranging from 101°F to 103°F, and essentially no other findings, except mild photophobia. A fourth was seen on day three of her illness with a temperature of 100°F, lassitude and mild photophobia. All noted sudden onset of illness, severe headache, myalgia and vomiting. There were no respiratory symptoms. Blood samples from these individuals yielded a virus shown by HI test to be VEE. Two others gave similar histories but were not available for bleeding during the acute phase of their illness. Three individuals who converted gave histories of non-specific, mild, flu-like symptoms during the five-week period but were not sick at the time this history was given and no blood was taken for isolation attempts. The remaining four manifested no symptoms of illness whatsoever. It is well recognized that VEE virus causes encephalitis infrequently in humans and this is supported by our data. There are little data concerning the occurrence of subclinical infections among humans. Data from laboratories where infections have occurred as a result of working with the virus suggest that this occurs infrequently, if at all. In our study, four out of thirteen or 30% of individuals infected with virus highly virulent for equines manifested no signs of illness.

There was no significant difference between males and females in conversion rates (9/62 and 4/54 respectively). All converters were between eight and fifty years of age. Twenty-two children less than 8 years of age and six adults greater than fifty did not convert. The number of older adults is too small to demonstrate any differences, but the lack of conversions among a larger group of younger children suggests a difference between this population and that of all older individuals. Surveys of populations involved with epizootics in Colombia and Venezuela indicate at least an equal, if not higher, attack rate among young children than in the older population. Studies are presently underway to test the significance of this difference and to examine its possible causes. It is felt that the difference probably hinges on the type of mosquito vectors principally involved in this epizootic. If the principal vector were a non-domestic nocturnal biter, it might explain why young children, who are kept indoors after sundown, show a significantly lower attack rate.

TABLE 1

Age-specific mortality in equines on 11 ranches having at least one death during VEE epizootic. Cuajiniquil, Costa Rica, 9 August-21 September 1970

Sex	Number deaths/Number equines aged:					
	<0.5 yrs.	0.5-2	3-4	5-10	>10	Total
Male	1/5	4/10	5/6	10/31	3/3	23/55
Female	2/3	2/7	3/13	13/38	3/5	23/66
Both	3/8	6/17	8/19	23/69	6/8	46/121

The question as to how the virus made its way into this area cannot be answered with the data presently available. We are carrying out investigations of the possibility that it may have continued to spread southward down the Nicaraguan Pacific coast through the "immune barrier" created amongst equines by an extensive vaccination campaign in November 1969, utilizing other mammals as the intermediate host until it reached Costa Rica where the presence of susceptible equines caused it to surface again clinically. It is more probable, however, that an infected animal, either domestic or wild, brought the virus into the area. If this was the case, it is unlikely to be proven in retrospect.

(David H. Martin, Gerald A. Eddy, William C. Reeves, and Karl M. Johnson)

REPORT FROM THE DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES, SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION, SAN ANTONIO, TEXAS

As stated in the last issue of the exchange, a collaborative program with the Center for Disease Control in Atlanta, Georgia, was initiated attempting to infect baboons with selected California group viruses. The viruses selected were LaCrosse, Tahyna, Trivittatus and Keystone. The first experiment consisted of injecting subcutaneously two juvenile baboons each with 10,000 mouse LD_{50} of the above mentioned four viruses. Two controls were used, for a total of 10 baboons. No HI, CF or neutralizing antibodies were detected. The experiment was repeated using infant baboons. In this case one infant had HI and CF antibodies to Keystone virus. The neutralization test has not been done as of this date.

A serological survey using only LaCrosse virus on 514 selected nonhuman primate sera revealed the presence of 34 with HI antibodies of 1:10 and 13 with 1:20 or greater. These nonhuman primates were Old and New World species including baboons, chimpanzees, patas, and a number of others. LaCrosse virus is only one of 13 arboviruses being used in our survey. The others will be reported when more information is available. The hemagglutinating antigen was prepared in BHK-21 cells by the method of Chappell, et al. (Applied Microbiology 18:433-437, 1969).

This, according to Chappell, is a much more sensitive antigen and they reported 100% correlation between their HI test and neutralization tests. More sera are to be tested, possibly using other California group viruses.

(S.S. Kalter)

REPORT FROM THE DIVISION OF PUBLIC HEALTH SCIENCES, GULF SOUTH RESEARCH INSTITUTE NEW ORLEANS, LOUISIANA

Multiple infections due to antigenically related but different arboviruses frequently elicit a broad serologic response difficult to differentiate or interpret by the conventional laboratory techniques. Table 1 illustrates this dilemma.

The CF results of Puerto Rican (PR) and Jamaican (J) human sera obtained during epidemics of dengue in the Caribbean area reveal only a 2 to 4-fold difference in serum titers among the four dengue serotype antigens. Only in a few exceptions were titers to SLE any lower. These infections did not appear to have been due to a YF virus. Numerous other human sera from these two islands gave essentially the same broad reactive pattern.

These sera were also tested in a complement-fixation kinetics test (CFKT) in an attempt to determine if one could reveal serologically the identity of the infecting viruses. The assumption made in the CFKT is that the homologous virus type demonstrates the greatest degree of affinity (avidity) for its own antibody with the concurrent fixation of complement in the shortest interval of time. Thus, the homologous or homotypic antigen-antibody system should reveal the earliest CF titers during the course of the kinetics with a progressive increase in homologous titer. In the case of closely related viruses, cross reactions should not appear until much later in the course of the kinetics. The aforementioned assumption was tested.

TABLE 1 CF Results of Puerto Rican and Jamaican Human Sera

Serum Designation	CF Antigen					
	1 HAW	2 TR	3 H-87	4 H-241	Group B SLE	YF
PR-33003	256	256	512	512	0	0
32851	256	128	256	256	0	0
32173	512	512	1024	2048	8	16
J-4	16	32	64	32	32	0
38	16	32	64	16	64	0
43	16	16	32	8	32	0

0= <1:8

TABLE 2 CFKT With DEN (TR-1751) Monkey Serum At Twice An Optimal Dilution of Indicated Antigens [a]

Time (Hr.)	Dengue CF Antigen					
	1 HAW	2 NGC	2 TR	2 TH36	3 H-87	4 H-241
0	0	0	0	0	0	0
1	0	8	8	8	0	0
2	0	8	16	8	0	0
4	0	32	32	16	8	0
6	8	32	32	32	8	8
18	32	128	128	128	(64)	(64)

[a]Monkey received two injections (35 days apart) of mouse-adapted Den-2 TR 1751 virus.

( ) <4-fold difference

Antisera to each serotype as well as to various dengue strains within the type were prepared in mice and monkeys. Some human sera were available for testing. In each serum preparation homologous or homotypic titers appeared first in the course of the kinetics test in spite of the fact that many of the antisera tested were indistinguishable from at least one other dengue type after 18 hours incubation. One such CFKT result is presented in Table 2. At 1 and 2 hours, Den-2 Trinidad 1751 monkey serum was type 2 specific. At 4 hours a cross reaction with Den-3 appeared; and at 18 hours incubation time the type 2 serum was indistinguishable from the dengue types 3 and 4.

Table 3 presents the CFKT results of a serum drawn from a 35 year old Puerto Rican female during the 1969 dengue type 2 epidemic. Type 3 dengue was responsible for the epidemic in Puerto Rico in 1963. The CFKT revealed an initial Den-2 and 3 reaction. Titers to dengue type 2 Trinidad 1751 strain appeared 2 hours sooner than did the prototype New Guinea "C" virus. By 18 hours, CF titers to each antigen type did not differ by more than two-fold. Yet, at four hours, high titers to Den-2 Trinidad and Den-3 were the only reactions evident. No SLE or YF antibodies were detected at any time period. Thus, this person appears to have been infected with both dengue types 2 & 3. Unfortunately, one cannot determine from these results the sequence of infection. Other Puerto Rican sera obtained during the 1969 epidemic revealed essentially the same pattern.

Sera from Jamaican indigenes revealed a Den-2 and Den-3 pattern in combination with SLE. In Table 4 are shown the kinetics results with sera obtained from 15 (J-4) and 14 (J-43) year old boys during the 1969 dengue epidemic in Jamaica. The results indicate a history of dengue types 2 and 3, and Den-3 and SLE infections, respectively. The latter three viruses have been isolated from man and/or birds in Jamaica.

The CFKT technique appears to be a useful epidemiologic tool in revealing the history of multiple dengue and related Group B viruses in the Caribbean Area. This technique is especially applicable for single sera where paired sera are not available.

(John N. Hatgi and B.H. Sweet)

TABLE 3 CF Kinetics With Puerto Rican Serum

Serum Specimen	Time (hr.)	CF Antigen						
		1	2		3	4	Group B	
		HAW	NGC	TR	H-87	H-241	SLE	YF
PR-33003	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
Den-2,3	4	8	0	128	256	0	0	0
	6	8	32	128	128	64	0	0
	18	256	256	256	512	512	0	0

0= <1:8

TABLE 4 CF Kinetics With Jamaican Sera

Serum Specimen	Time (Hr.)	CF Antigen						
		1	2		3	4	Group B	
		HAW	NGC	TR	H-87	H-241	SLE	YF
J-4	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
(Den-2,3)	4	0	0	8	16	0	0	0
	6	0	0	16	16	0	0	0
	18	16	64	32	64	32	32	0
J-43	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
(Den-3,SLE)	4	0	0	0	8	0	16	0
	6	0	0	0	16	0	32	0
	18	16	16	16	32	8	64	0

0= <1:8

REPORT FROM THE DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH,
SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI, FLORIDA

To date 3574 human blood specimens have been collected from 12 towns in Haiti.

Of 1454 sera tested 51% (740/1454) were HI positive to Dengue-2 antigens. Two hundred thirty-six sera tested against Dengue-2, Dengue-3 and St. Louis encephalitis antigens produced respectively 70%, 56%, and 48% HI reactions to these antigens. The coastal towns and those on the main highways (37% to 66%) were found to have a greater prevalence of HI Dengue-2 antibodies than more remote inland towns (5% to 25%).

Four Dengue-2 isolations have been made from Port-au-Prince and La Boule. Two were recovered in August 1969 and two were isolated in October 1970. Eighteen sero conversions were found in 235 paired sera tested in HI tests. The first conversions were observed in October 1969 and the latest ones were detected in February 1971. Five other isolates were recovered during an outbreak of dengue-like illness in Port-au-Prince during October 1970 to January 1971. These agents are currently being identified.

Dengue-like disease and serological evidence of Group B arbovirus infections together with dengue virus isolations indicate the continuing activity of dengue in Hispañola.

(A.K. Ventura and N. Joel Ehrenkranz)

REPORT FROM THE ENTOMOLOGICAL RESEARCH CENTER,
FLORIDA DIVISION OF HEALTH,
VERO BEACH, FLORIDA

Host preference (?) in *Culex nigripalpus*

In SCIENCE (1968, v. 161:67), we reported a seasonal shift in the bird:mammal ratio of feeding for the vector of SLE in Florida. We now have 5 complete years demonstrating this phenomenon, which we have shown to be unrelated to host-density changes. Data from other researches indicate a definite seasonal shift in the activity pattern of *C. nigripalpus* which could explain the seasonally-related host associations we reported. Periods when *nigripalpus* feeds mainly on birds (mostly wading, perching, and shore birds--in that order), correspond with periods when its activity is restricted mainly to dense woods, where most birds roost. Conversely, periods with the highest feeding on mammals (mostly bovine, rabbit, and armadillo -- in that order) correspond with periods when its activity pattern takes it into open areas, where most mammals occur. If documented, this will explain the seasonal shift in feeding but it will raise a new question concerning the cause of the seasonal shift in *nigripalpus* activity between woods and open areas. Present evidence suggests a relationship to moisture. Months with total rainfall near or below 100 mm correspond closely (see chart) with months when feeding on mammals was near or below 40%. This was particularly evident in 1969 and 1970 when early summer rains failed to materialize and *nigripalpus* failed to switch to mainly mammalian hosts as it did in June of the preceding three years, when the summer rains came on schedule.

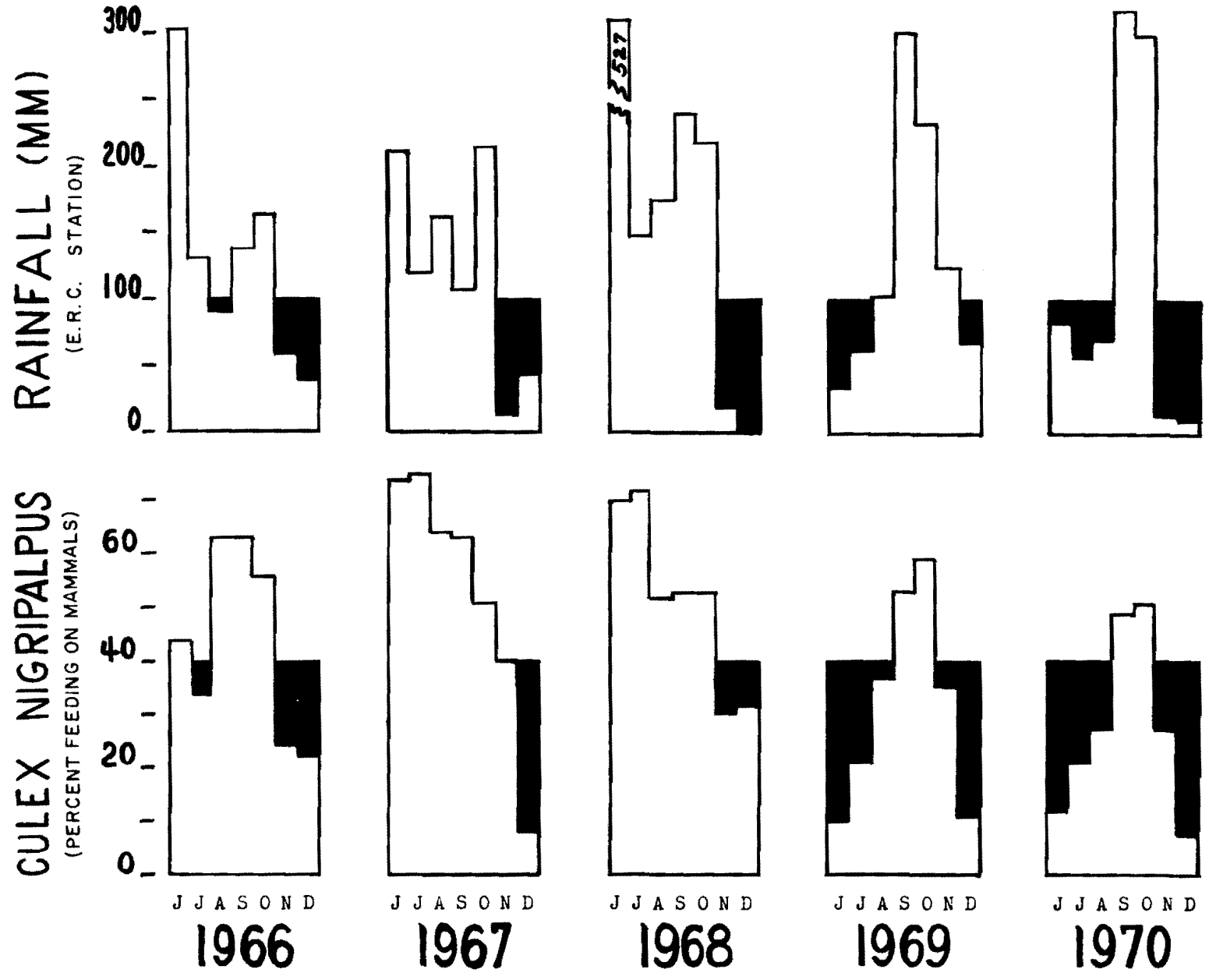
In the next issue of the ANN. ENT. SOC. AMER. (1971, in press) we will report our studies of overnight feeding rates of *nigripalpus* on 7 local species of wading birds while at roost in large screen cages. These were high (60%+) on black-crowned night heron and green heron, and low (<25%) on little blue heron, Louisiana heron, white ibis, snowy egret, and cattle egret. *Nigripalpus* fed equally on all 7 host species, however, when these were restrained, indicating no olfactory or visual preference for any species. A follow-up study (MS submitted for publication) involving the counting, on event recorders, of 15 anti-mosquito habits in these birds emphatically demonstrated that the defensive behavior of the 5 species with low *nigripalpus* feeding rates successfully prevented attracted mosquitoes from feeding and in fact was also responsible for a substantial mosquito mortality and a higher incidence of incomplete blood meals.

More recent experiments (MS in preparation) verified that the density of attacking mosquitoes affects the defensive behavior of birds and, in turn, the feeding success of the mosquitoes. Although feeding success, as a percentage, decreased with increasing densities of mosquitoes from 100 to 1200, each bird had its threshold of tolerance. Thus when the cattle egret was exposed to 400 mosquitoes, fewer (18) succeeded in feeding than when the same bird was exposed to only 100 mosquitoes (25 fed).

Although dietary spectra of mosquitoes have generally been ascribed to "host preferences", studies under way here demonstrate that host behavior and meteorology may determine feeding successes on potential hosts rather than preference per se. Thus the high antibody rates to JE, WE, and EE viruses often reported in night herons, when compared to other herons, can be explained without invoking preference. The role of the vector in the epidemiology of mosquito-borne diseases, often so illusive, could achieve earlier understanding if less reliance were placed on such gratuitous assumptions as "host preference", "flight range", etc. which more often reflect environmental, meteorological and host-behavioral factors than characteristics inherent in vector species. Olfactometers and flight mills reveal bona fide olfactory preference and flight potentials and are indispensable research tools. But the circumstances in nature are so complex that laboratory findings must be interpreted in an equally complex context.

(M.W. Provost)

Comparison of Culex nigripalpus feeding and rainfall, by month, 1966-70



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

St. Louis Encephalitis (SLE) virus reappeared in Polk and Dade counties in 1969. Extensive biological, entomological, and epidemiological surveillance for evidence of SLE activity was therefore carried on by the Florida Division of Health in 1970.

A total of 1,393 sera from patients with central nervous system involvement and 12,138 bird and animal sera were examined by the hemagglutination-inhibition (HI) technique for arbovirus antibody. Essentially negative SLE results were obtained. One serologically positive SLE-Group B virus finding in a 21-year-old male was determined to be due to Japanese B Encephalitis virus infection recently acquired in Viet Nam.

During the course of the surveillance, two human eastern equine encephalitis cases were discovered. Diagnostic rises in serum HI, CF, and neutralization antibody titers were demonstrable in both patients. The first case, a 45-year-old male whose illness occurred in July, was followed by a complete recovery. The second case, a 12-year-old female with onset in August, was left with marked brain damage.

A total of 5,903 pools of mosquitoes were tested for virus. No SLE virus was detected in any of the mosquitoes tested. However, other virus isolations from various mosquito species were made:

8 EEE:	Culiseta melanura	(2)
	Culex nigripalpus	(4)
	Mansonia perturbans	(2)
2 WEE:	Culiseta melanura	
14 California Group:	Aedes infirmatus	(6)
	Aedes atlanticus	(6)
	Culex nigripalpus	(2)

Arbovirus surveillance for the coming season will be concentrated around the metropolitan areas of the state, predominately about the periphery of the Everglades. Human surveillance will constitute the bulk of the continuing efforts in the state at large, but chicken sentinels, pauperized doves, and mosquito surveillance will continue in the central and South Florida areas about Disney World, Miami, and St. Petersburg.

(N.J. Schneider)

REPORT FROM THE ARBOVIROLOGY UNIT, VIROLOGY SECTION, CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

Dengue in Puerto Rico: 1969-1970

Dengue was first reported in Puerto Rico in 1915; dengue-like illnesses were again reported in 1918 and 1945. The first laboratory-confirmed cases on the island occurred in 1963, with isolations of dengue type 3 virus from Puerto Ricans in an epidemic that swept the Caribbean. More than 27,000 cases were officially recorded by the Puerto Rico Department of Health at that time. Recognition of another epidemic of dengue in the Caribbean area in 1968-1969 and in Puerto Rico in early summer of 1969 provided an opportunity to study the epidemiology of dengue in a large number of individuals who had experienced the infection previously. In 1969, 16,348 cases of dengue occurred and strains of dengue type 2 virus were isolated. Scattered cases were still occurring as late as December 1970. The 1969 epidemic in Puerto Rico probably began on the north coast and progressed slowly south and west. Reported attack rates per 100,000 ranged from 89 in the south to 1,873 in the north.

By age, sex, and location, clinical illnesses were similar: mild, with acute onset of fever, headache, muscle pains, and, in many cases, rash. No hemorrhagic fever manifestations were seen. There was no difference in severity of symptoms between individuals with serologic evidence of previous group B (probably dengue type 3) arbovirus infection and those with primary responses. It was interesting that 55% of individuals with illnesses which were categorized as not compatible with classic

dengue were, nonetheless, shown by serologic means to have had recent dengue infections. With the cooperation of other interested laboratories, surveillance of the island is continuing. The persistence of Aedes aegypti mosquitoes in the Caribbean, inter-island travel, travel from southeast Asia to the Caribbean, and a burgeoning population suggest that dengue epidemics may continue to occur, perhaps with more severe manifestations in the future. Moreover, the fact that epidemic dengue can occur there indicates the potential of a yellow fever epidemic, should the virus in some way be introduced.

Venezuelan equine encephalitis (VEE) virus in south Florida

Attempts were made to better define the ecologic characteristics of VEE virus in south Florida. Vertebrates were surveyed in the Big Cypress and Everglades habitats and tested for antibody by hemagglutination-inhibition (HI) and neutralization tests. Of 911 mammals captured from 1965 to 1968, 205 had HI antibody to VEE virus. However, 199 of 420 captured in the Everglades were positive as contrasted with 6 of 491 from Big Cypress. Ecologic considerations led to the conclusion that some factor other than frequent population turn-over of wild vertebrates is necessary to explain the maintenance of endemic VEE virus in focal habitats of south Florida.

Preliminary viremia studies in indigenous species (cotton mouse, cotton rat and opossum) indicate nothing unusual in duration or maximum titer reached. However, opossums react erratically and some may be partially resistant to 7000 SM icLD_{50} (subcutaneously given). In a study now being repeated, two opossums died 43 and 72 days after inoculation with VEE virus (FE3-7C strain) and the virus was recovered from heart tissue, salivary gland, and spleen of one of them. In the repeat studies, opossums are to be re-inoculated and examined for evidence of virus recirculation, with or without the presence of antibody.

(C.H. Calisher)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY,
U.S. NAVAL MEDICAL RESEARCH INSTITUTE,
NATIONAL NAVAL MEDICAL CENTER,
BETHESDA, MARYLAND

The premature and tragic death of Dr. Earl Suitor in July, 1969 cut short his excellent research on arthropod cell cultures and their ability to support virus growth. A symposium honoring Dr. Suitor, "Arthropod cell cultures and their application to the study of viruses" was held at the Naval Medical Research Institute on March 18-19, 1970. Publication of the proceedings by Springer-Verlag is expected this year (1971).

The research initiated by Dr. Suitor is being continued and extended in investigations on the mechanisms of replication of selected Group B arboviruses in vertebrate and insect cells.

Initial studies in our laboratory are concerned with the development of procedures for the elucidation of the sequence of ribonucleic acid (RNA) of Japanese encephalitis virus (JEV) in a porcine kidney cell line. The cell line, designated as PS(Y-15), was established by Inoue and Yamada in 1964 specifically for studies on JEV. PS(Y-15) cells support good growth and plaque formation by JEV. Final virus titers of 10^8 pfu/ml are attained within 3 days at 35 C.

Early experiments to study the RNA synthesis of JEV revealed that actinomycin D, a drug used to inhibit cellular RNA synthesis, also strongly inhibited the growth of this virus. Actinomycin D at 1 μg/ml added at time of infection or one day later reduced 3-day virus titers by 90%; however, when the drug was added on the second day post-infection virus growth was not reduced significantly.

The level of JEV RNA synthesis as measured by incorporation of radioactive uridine into viral RNA products was so low that it was totally obscured by the much greater activity of the cellular RNA synthesis. It was therefore necessary to devise methods for reducing cellular RNA formation to a point where viral RNA synthesis could be observed. Treatment of PS(Y-15) cells with actinomycin D for 30 minutes followed by removal of the drug resulted in an inhibition of total cellular RNA synthesis by 50% and ribosomal RNA synthesis by 90%; however, JEV growth in these cells was almost equal to that observed in untreated cells.

It is well established that the sites of arbovirus synthesis within the host cell are located on the membranes of the endoplasmic reticulum. In order to further reduce the cellular RNA background, the microsomal fraction of the cell was separated from other cellular components. This cell fraction consists of the internal cellular membranes as well as other cell structures such as mitochondria and ribosomes. The microsomes derived from infected cells also include the sites responsible for virus synthesis. The isolation of this cell fraction would increase the possibility of detecting viral RNA products.

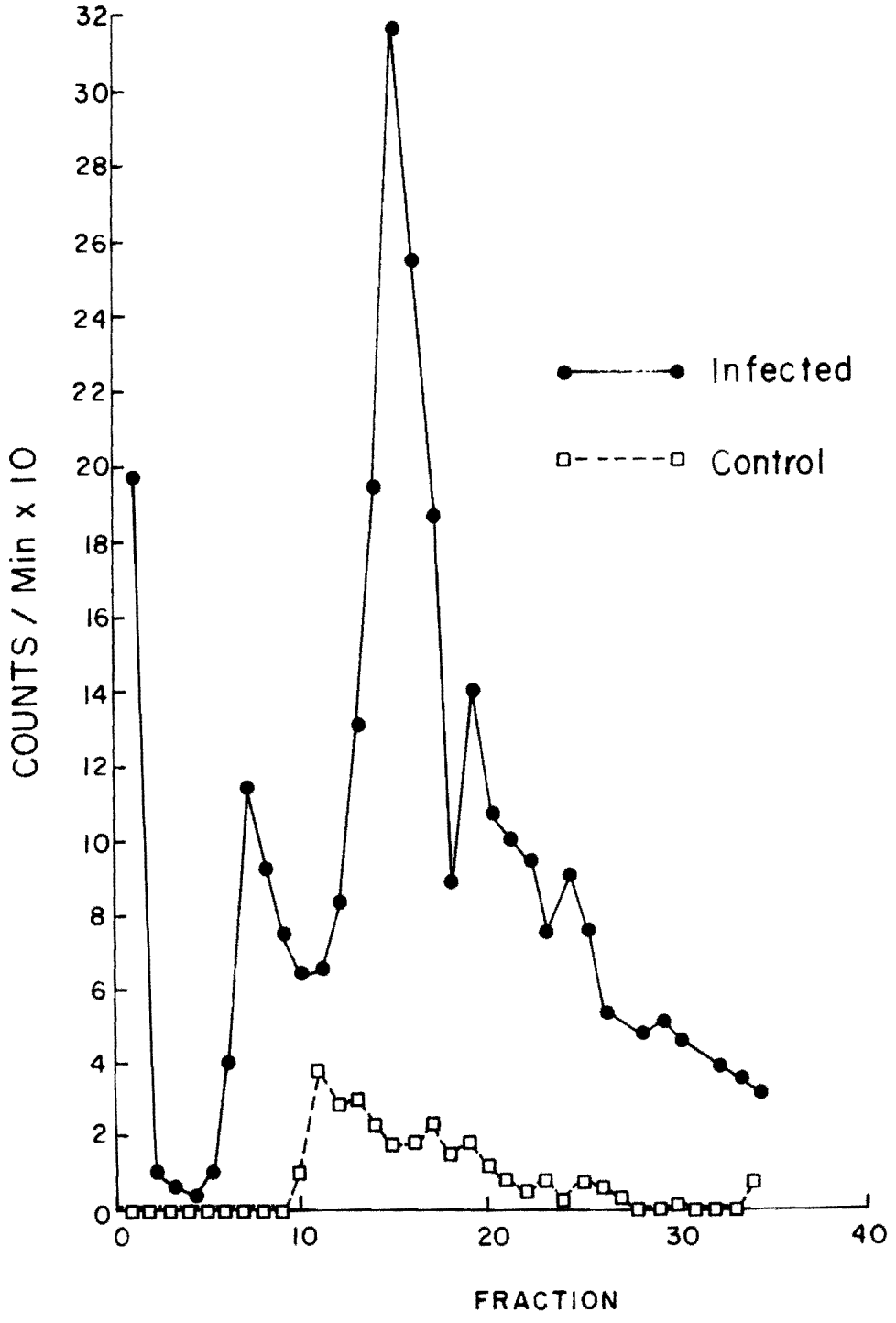
The combination of actinomycin D treatment of the cells prior to infection and the isolation of the microsomal fraction two days post-infection was successful in reducing the level of cellular RNA so that RNA products induced in JEV in PS(Y-15) cells could be detected.

In a typical experiment, actinomycin treated infected and control cells were given a 2 hour pulse with H^3-uridine on the second day after infection and then processed to isolate the microsomal fraction. RNA was extracted from the microsomes with cold phenol with sodium dodecyl sulfate and analyzed by polyacrylamide gel electrophoresis. Figure 1 shows the electrophoretic pattern of RNA obtained from infected and normal cells. Three or more radioactive RNA peaks are observed in infected cells that are not present in uninfected cells. It is believed that these RNA peaks represent different species of RNA induced in cells by JEV.

These experiments are being continued in an effort to identify and characterize the viral RNA species made by JEV and to extend these studies to insect cell lines and other Group B arboviruses.

(Eugene Zebovitz)

Polyacrylamide Gel Electrophoresis of RNA Extracted From the Microsomal Fraction of PS(Y-15) CELLS Infected With Japanese Encephalitis Virus



REPORT FROM THE DEPARTMENT OF VIRUS DISEASES,
WALTER REED ARMY INSTITUTE OF RESEARCH,
WASHINGTON, D.C.

Comparison of dengue-2 and dengue-3 strains: Identification of a dengue-3 subtype

The occurrence of two major epidemics in the Caribbean region within the past decade has raised important questions concerning the geographic origin of the epidemic strains. DEN-2 was recovered by this laboratory associated with outbreaks in Jamaica and Puerto Rico during 1969. DEN-3 strains were first isolated in Puerto Rico in 1963 and were associated with the small outbreak in Jamaica in 1968. These Caribbean type 3 strains possessed markedly lower mouse virulence than the Southeast Asian type 3 strains. It was therefore of considerable interest when in 1964 epidemic dengue occurred in the South Pacific (Tahiti) and was found by Dr. Leon Rosen to be caused by a dengue virus with very low mouse virulence. Subsequent identification of the Tahiti agent as a DEN-3 strain raised the question of its antigenic relationship to the Caribbean DEN-3 strains and to contemporary Southeast Asian strains. In the hope that subtypes of epidemiologic significance could be distinguished, we compared several dengue-2 and dengue-3 strains from the Caribbean with prototype viruses and with strains from Southeast Asia, Africa and Tahiti.

METHODS

Viruses. The strains included in this study are described in Table 1.

Antisera. Hyperimmune mouse ascitic fluids were prepared against the mouse-adapted strains by a modification of the method of Brandt. Ascites was induced in the immunized mice by the use of sarcoma 180 cells. Human convalescent sera were obtained in the course of epidemiologic studies in Puerto Rico and Tahiti. For these studies sera were selected from patients who were thought to have had a primary type antibody response to the dengue infection. Selection was made on the basis of absence of detectable HI antibody in the acute phase specimen and a rise to low or moderate levels (1:80-1:320) in the convalescent specimen.

Table 1. Virus Strains Tested

Designation	Original Isolation Year	Original Isolation Location	Passage Level*	Serotype
New Guinea C	1944	New Guinea	sm-27	DEN-2
TR-1751	1954	Trinidad	sm-55	DEN-2
PR-109	1969	Puerto Rico	sm-5	DEN-2
H-11234	1966	Nigeria	sm-27	DEN-2
H-87	1956	Philippines	sm-26	DEN-3
21153	1965	Thailand	sm-10	DEN-3
Tahiti-4	1964	Tahiti	tc-5	DEN-3
PR-38	1963	Puerto Rico	tc-6	DEN-3
PR-6	1963	Puerto Rico	sm-14	DEN-3
J-1007	1968	Jamaica	tc-6	DEN-3

* sm - suckling mouse passage
  tc - tissue culture passage

Neutralization tests. Plaque reduction neutralization tests were carried out in LLCMK-2 cell cultures. Fifty per cent plaque reduction endpoints were estimated by the probit method.

RESULTS

DEN-2 strains. The results of cross neutralization tests with the dengue-2 strains are shown in Table 2. The neutralization titers in each case failed to show any marked difference between the Caribbean and prototype strains. The slightly lower titers of the TR-1751 ascitic fluid and Puerto Rican human serum against the African H-11234 strain are within the variation expected between tests.

DEN-3 strains. The neutralizing antibody titers of the hyperimmune ascitic fluids shown in Table 3 indicate that the PR-6 ascitic fluid, a 1963 Caribbean strain, neutralized the Southeast Asian strains very poorly in comparison to homologous titers and to the titers against other Caribbean and the Tahitian strains. In contrast, the H-87 and 21153 ascitic fluids do not clearly differentiate between the Asian and the Caribbean and Tahitian strains although the H-87 ascitic fluid has somewhat lower titers against the Caribbean strains.

Results with human convalescent sera as shown in Table 4 confirm the results obtained with the mouse ascitic fluids. A significant difference is apparent; the Puerto Rican sera neutralize the Southeast Asian strains to a much lower titer than the titers against Caribbean and Tahitian strains. The Tahitian human convalescent sera, while neutralizing homologous strains and Caribbean strains, fails to neutralize Southeast Asian strains.

DISCUSSION

The above neutralization tests indicate that the DEN-2 strains from Southeast Asia, Africa, and the Caribbean form a relatively homogenous antigenic group. It, therefore, appears impossible to determine by the virologic methods presently available whether the 1969 epidemic of DEN-2 in the Caribbean was caused by an endemic or by an introduced strain of DEN-2.

The observations on the DEN-3 strains are quite different from those seen with DEN-2 and clearly indicate that the Caribbean and Tahitian strains of DEN-3 form a distinct antigenic subtype. However, continued classification of these strains as dengue-3 appears justified since the neutralization of Caribbean and Tahitian strains by antisera to the Southeast Asian strains is so close to homologous titers.

Table 2. Neutralizing Antibody Titers of Hyperimmune Mouse Ascitic Fluids and Human Convalescent Serum . Against Dengue-2 Strains

	Mouse Ascitic Fluids			Human Convalescent
Virus	NG C	TR-1751	PR-109	Puerto Rico 1969
NG C	3000*	1700	2000	300
TR-1751	1300	1300	1200	200
PR-109	2800	1600	1400	400
H-11234	1900	600	1200	110

* Reciprocal of 50% plaque reduction titer.

Table 3. Neutralizing Antibody Titers of Hyperimmune Mouse Ascitic Fluids Against Dengue-3 Strains.

	Ascitic Fluids		
Virus	H-87	21153	PR-6
H-87	180*	240	70
21153	200	420	130
PR-6	50	250	>640
PR-38	80	280	>640
J-1007	90	230	1100
Tahiti-4	100	250	>640

* Reciprocal of 50% plaque reduction titer.

Table 4. Neutralizing Antibody of Human Convalescent Sera Against Several Strains of Dengue-3.

	Tahitian Serum - 1964			Puerto Rican Serum - 1963	
Virus	4364	4365	4385	YB-6	YB-4
H-87	<20*	<20	<20	30	<20
21153	<20	<20	20	40	25
PR-6	60	60	60	190	50
PR-38	50	50	30	220	115
J-1007	95	140	70	180	75
Tahiti-4	70	150	50	200	100

* Reciprocal of 50% plaque reduction titer.

Of considerable interest is the fact that differentiation of this subtype by neutralization tests appears to correlate with the biologic marker of low virulence for suckling mice.

The recovery of the Caribbean subtype in Jamaica in 1968 suggests that this subtype has remained endemic in the Caribbean region since at least 1963.

Persistence of serum neutralizing antibody to WEE virus in naturally infected quail

Sentinel quail have been used since 1968 to monitor the seasonal transmission of EEE and WEE viruses in a swamp habitat on the eastern shore of Maryland. Each year since 1968 both viruses have been recovered from mosquitoes and birds. However, mosquito infection rates, antibody prevalence in wild birds and seroconversion of sentinel quail show that the virus (EEE or WEE) predominating one year would be supplanted the following year by the other virus. Birds returning to the swamp each year have shown higher antibody prevalence rates to the virus which predominated the previous summer. Presumably, infections acquired during the previous summer resulted in a level of herd immunity to the homologous virus sufficient to retard transmission the next year. An important factor in this situation is the persistence of neutralizing antibody in birds following natural infection. To examine this aspect a number of uninfected, juvenile quail were exposed from 1 to 14 July 1969 in the swamp, removed to a mosquito-proof environment, and bled at selected intervals over a period of one year and the serum tested for N antibody.

Serum neutralizing antibody was assayed by a plaque reduction test in primary chick embryo cells. Plaque dose was approximately 100 pfu of the 46th mouse brain passage of MacMillan strain of WEE virus. Sera were heated at 56°C/30', and assayed at 1:20, 1:40, 1:160, 1:640, and 1:1280 dilutions. Fifty percent endpoints were estimated by plotting percent reductions on probit paper.

Antibody was not detected in pre-exposure sera. From 2 to 53 weeks post-exposure, all sera had neutralizing antibody (Table 5). Highest titers were observed in 7 of 9 quail at 2 to 3 weeks after exposure. At one year after exposure titers varied from 1:20 to 1:200.

TABLE 5

PERSISTENCE OF SERUM NEUTRALIZING ANTIBODY TO WEE VIRUS IN NATURALLY INFECTED QUAIL

		Reciprocal of Serum Antibody[1]						
Weeks Post-Exposure		0[2]	2	3	12	27	39	53
Quail Number	1	< 20	20[3]	190	300	400	135	200
	2	< 20	520	N T	170	88	45	80
	3	< 20	76	20	20	20	20	20
	4	< 20	560	120	180	98	54	140
	5	< 20	500	280	160	200	78	110
	6	< 20	500	N T	430	340	135	220
	7	< 20	720	640	220	200	150	215
	8	N T	68	120	420	N T	90	88
	9	< 20	380	120	75	70	70	N T

[1]Measured in plaque reduction neutralization test using approximately 100 plaque forming units/0.2 ml of MacMillan strain of WEE virus.

[2]Serum obtained before exposure in nature to arboviruses.

[3]Titer estimated by probit analysis plotting percent reduction of plaque numbers at serum dilutions 1:40, 1:160, and 1:640.

[4]N T = Not Tested.

These data confirm the persistence of appreciable levels of WEE virus neutralizing antibody throughout a one-year period. Presumably these quail would remain refractory to mosquito challenge with WEE virus at one year or more post-infection. They remain at risk to EEE infection as the following observations indicate. During 1969, one sentinel quail was naturally infected in the swamp with WEE virus early in the summer, as shown by serological conversion. This quail was re-exposed as a sentinel and was infected during August by EEE virus, demonstrated by virus isolation and serological conversion.

(P.K. Russell)

REPORT FROM THE U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES, FREDERICK, MARYLAND

Persistence of neutralizing antibody in personnel receiving Venezuelan equine encephalitis vaccine

An attenuated Venezuelan equine encephalitis (VEE) vaccine has been employed for 10 years both for research purposes and for immunization of at-risk personnel. (Berge, T.O., I.S. Banks, and W.D. Tigertt. 1961. Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea-pig heart cells. Amer. J. Hyg. 73:209-218; Alevizatos, A.C., R.W. McKinney, and R.D. Feigin. 1967. Live, attenuated Venezuelan equine encephalomyelitis virus vaccine. I. Clinical effects in man. Amer. J. Trop. Med. 16:762-768). A 5 to 6 year follow-up serological evaluation of 30 individuals receiving this vaccine has recently been completed. All subjects were virgin to VEE at the time of immunization in 1963. Sera were obtained in 1968 and 1969 for mouse neutralization tests. Each serum paired with the individual preimmunization serum was used to determine the $\log_{10}$ serum neutralization index (LNI). These tests were performed by the constant serum-varying virus method. One- to 3-day old mice were inoculated intracerebrally with equal amounts of pre- or postvaccination ($\leq$ 5 years) serum mixed with $\log_{10}$ dilutions of Trinidad strain VEE virus. Fifty per cent endpoints were determined 5 days postinoculation using the method of Reed and Muench. As shown in Table 1 none of the subjects had

TABLE 1.

SERUM NEUTRALIZATION INDICES OF INDIVIDUALS 5 TO 6 YEARS POSTVACCINATION WITH ATTENUATED VEE VACCINE

	Responses:			
	Prevaccination		5-6 Years Postvaccination	
LNI	No./Total	%	No./Total	%
3.0 - ≥ 4.0			8/30	27
2.0 - 2.9			16/30	53
1.7 - 1.9			1/30	3
1.0 - 1.6			5/30	17
0	30/30	100		

demonstrable neutralizing antibody at the time of immunization. Five to 6 years postvaccination 83% (25/30) exhibited significant levels of antibody (i.e. LNI $\geq$ 1.7); none of the remaining individuals had LNI of $<$ 1.0.

Cross-protection in hamsters immunized with Group A arboviruses

Cross-protection between Venezuelan, Eastern, and Western equine encephalomyelitis (VEE, EEE, WEE) viruses was studied in the hamster using challenge responses as indexes of protection. Formalin-inactivated vaccines induced only homologous protection regardless of the sequence of vaccination or the combination of vaccines employed. (Tables 2 and 3). Use of attenuated VEE vaccine alone produced virtually absolute homologous protection as well as 37% and 59% protection against WEE and EEE challenges, respectively (Table 2, line 13). No deleterious or enhancing interaction occurred when attenuated VEE and inactivated WEE and EEE vaccines were employed in various sequences of immunization (Table 3, lines 5-10) and all possible combinations (Table 2, lines 4, 11, 12). Studies with attenuated strains of VEE, EEE, and WEE viruses showed that all elicit excellent homologous protection when administered singly (Table 4, lines 11-13). However use of these live strains in many combinations and sequences resulted in a significant ($p<0.05$) decrease in the protective efficacy of the WEE or EEE strains (Table 4, lines 2, 7-10).

(Francis E. Cole, Jr.)

TABLE 2.

RESPONSE TO IP CHALLENGE IN HAMSTERS VACCINATED WITH COMBINED ATTENUATED (ATTEN) VEE AND/OR INACTIVATED WEE, EEE AND VEE VACCINES[a]

Line	Vaccination[b] schedule	No. of doses	% Surviving challenge with 10^3 LD_{50} of		
			WEE	EEE	VEE
1	WEE	1	78	11	0
2	EEE	1	15	72	0
3	WEE-EEE	1	85	88	0
4	VEE (atten)-WEE-EEE	1	84	84	100
5	WEE	2	100	5	0
6	EEE	2	20	95	0
7	WEE-EEE	2	100	96	0
8	WEE	3	87	4	c
9	EEE	3	4	83	
10	WEE-EEE	3	93	85	
11	VEE (atten)-WEE	1	76	46	100
12		1	40	86	100
13	VEE (atten)	1	37	59	98
14	VEE	1	3	4	83
15	VEE (5 X doses)	2	1	2	100

a Data compiled from results of several experiments.
b Unless otherwise noted, vaccines were formalin inactivated.
c Blank denotes not tested.

TABLE 3.

SEQUENTIAL IMMUNIZATION OF HAMSTERS WITH ATTENUATED VEE AND INACTIVATED WEE AND EEE VACCINES

Line	Vaccination schedule[b]	% Surviving ip challenge with[a]		
		WEE	EEE	VEE
1	VEE	37	59	98
2	WEE(2)[c]	100	5	0
3	EEE(2)	20	95	0
4	WEE-EEE(2)	100	96	0
5	VEE,WEE(2)	98	54	99
6	WEE(2),VEE	100	60	100
7	VEE,EEE(2)	31	100	100
8	EEE(2),VEE	39	97	100
9	VEE,WEE-EEE(2)	100	100	98
10	WEE-EEE(2),VEE	98	100	100

a Data compiled from results of several experiments.
b Comma indicates sequential administration of vaccines; hyphen indicates combined administration.
c Number of doses given in parentheses.

TABLE 4.

RESPONSE TO CHALLENGE IN HAMSTERS VACCINATED WITH ATTENUATED VEE, EEE, AND WEE VIRUSES

Line	Vaccination schedule[b]	% Survivors (No./Tot.) after ip challenge with 10^3 LD_{50}[a]		
		WEE	EEE	VEE
1	WEE, VEE	100 (20/20)	82 (45/55)	100 (10/10)
2	VEE, WEE	84 (42/50)	82 (41/50)	100 (13/13)
3	EEE, VEE	32 (16/50)	100 (25/25)	100 (10/10)
4	VEE, EEE	28 (14/50)	94 (47/50)	100 (12/12)
5	WEE, EEE	100 (45/45)	85 (69/81)	23 (63/275)
6	EEE, WEE	96 (24/25)	100 (25/25)	68 (123/180)
7	VEE-WEE-EEE	40 (32/80)	84 (67/80)	98 (59/60)
8	VEE-WEE	70 (21/30)	40 (12/30)	100 (30/30)
9	VEE-EEE	10 (3/30)	73 (23/30)	97 (29/30)
10	WEE-EEE	80 (28/35)	89 (31/35)	11 (11/100)
11	EEE	6 (8/128)	90 (75/83)	7 (5/70)
12	WEE	95 (133/140)	35 (45/130)	1 (1/94)
13	VEE	37 (97/260)	59 (158/268)	98 (190/194)

a Data are a compilation of results of several experiments.
b Comma indicates sequential administration of "vaccines"; hyphen indicates combined administration.

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

Studies of Venezuelan encephalitis (VE) virus virulence were begun by determining the growth and histopathology of virulent and attenuated strains of the virus in hamsters. Virulent virus began to grow sooner than attenuated virus inoculated subcutaneously and reached higher titers in blood; however, in brain, bone marrow, lymph nodes and spleen, maximal titers of both viruses were similar although peaks of growth curves occurred later with attenuated than with virulent virus. Both viruses grew equally fast in brain after intracranial inoculation and in spleen and bone marrow after intracardiac inoculation. Yet attenuated virus was not pathogenic by any route and produced no distinctive histopathology whereas virulent virus uniformly killed hamsters within 3 to 5 days and produced severe necrosis of hematopoietic tissues. Differences in growth and adsorption rates of virulent and attenuated viruses were found in cultured hamster (primary embryonic and BHK21) cells and in chicken (primary embryonic) cells, but they did not correlate with pathogenicity or with events in the host animal. Pixuna and Ft. Detrick attenuated VE virus vaccine (strain TC-83) inapparently infected hamsters and protected them against disease and death from subcutaneous challenge by virulent VE or Mucambo viruses. Six strains of VE virus did not change in virulence for hamsters, mice and cotton rats with 1 oral or 4 to 6 intrathoracic passages in Aedes aegypti. Plaque sizes in primary chicken embryonic cell cultures increased slightly, but significantly, after 1 passage of a strain from Venezuela and decreased after 5 passages of TC-83 strain. Plaque sizes of the other 4 strains did not change after 1 to 6 passages in A. aegypti. Attenuated VE virus (TC-83 strain) grew in hamster testes after direct intratesticular inoculation but not after subcutaneous injection nor in the uninoculated testis after unilateral testicular injection. Viral growth continued for 2-3 weeks after intratesticular inoculation although viremia disappeared within 6 days and viral neutralizing antibodies appeared in serum. Cytonecrosis, inflammation and depression of germinal cell numbers, atrophy and eventual sterility occurred with or following viral growth in testes. Virus antigen was found in diploid but not haploid testicular cells, and virus did not adsorb to or grow in sperm maintained in vitro. Virus apparently did not grow in uteri and produced no histopathology. However infection of females by intrauterine or subcutaneous inoculation sometimes prevented pregnancy. Virus could be occasionally transferred to the uterus-vagina of females who copulated with males with infected

testes but only rarely did females become infected during such copulation. Usually pregnancy developed and progressed normally and no convincing transfer of virus occurred to the progeny. Initial attempts to infect guinea pigs with VE virus given transintestinally by careful catherization of the duodenum and jejunum were successful since 5 of 9 died at appropriate intervals after intraintestinal inoculation of virulent VE virus.

During 1968 in Guatemala, 11 strains of VE virus, 5 of group C, 4 of group Patois and 4 of eastern encephalitis virus were recovered from sentinel hamsters. The Mexican prototype VE, strain 63U2, possessed common CF antigens with the prototype EE, strain Riche, isolated in Louisiana in 1950. Yet the viruses were distinct by neutralization tests.

During June-November 1969 the first recorded major epidemic-epizootic of Venezuelan encephalitis occurred in Central America, beginning on the Pacific coast of Guatemala near the El Salvador border. Jointly with Dr. J.V. Ordonez of the University of San Carlos Medical School in Guatemala, specimens were collected in Guatemala for isolation of virus and for antibody studies from man, equines, cattle, dogs, domestic poultry, wild mammals and birds. By use of sentinel hamsters a strain of VE virus was recovered from the swamp-marsh area at Avellana, Santa Rosa on the Pacific coast, a habitat which yielded "endemic" strains of VE virus during 1968 and is located less than 50 miles from the initial focus of the 1969 epidemic-epizootic.

Also during 1969, arboviruses were investigated along the Gulf coast of Mexico from Veracruz to the Mexico-USA border in collaboration with Drs. J. de Mucha-Macias and M.L. Zarate and associates of the National Virus Institute of Mexico and with the generous cooperation of public health personnel in the Tampico and Matamoros regions of Mexico. Sera from pigs and cows collected during an exploratory investigation in April 1969 failed to yield convincing evidence of VE virus antibody in the Tamaulipas and northern Veracruz regions. During July-September 1969, sera were collected for antibody tests from man, horses, pigs, cattle, goats, dogs and wild birds; 63 equine sera from Matamoros have been tested and were negative by VE virus neutralization test. Sentinel hamsters were placed at selected habitats from the Mexico-USA border to the endemic areas of VE virus in southern Veracruz. VE virus was recovered as in the past from habitats at Minatitlan and Sontecomapan, Veracruz. For the first time, VE virus was recovered at Tlacotalpan, Veracruz, an area where VE virus antibody had been found in the past, but no virus was isolated from mosquitoes or by use of sentinel hamsters. VE virus was also recovered in a swamp-marsh habitat in northern Veracruz, north of the city of Veracruz, at Chachalacas near Cardel.

Sentinel hamsters yielded no VE virus north of this location although a strain of group C, Nepuyo virus was recovered from a hamster exposed at Tampico; this virus may be a mixture because it reacted by CF test at low antigen titers with VE virus antiserum.

A virus isolated from blood of an American egret nestling obtained in 1965 at Minatitlan, Veracruz, Mexico was identified during this year as St. Louis encephalitis virus by CF and neutralization tests in chicken embryonic cell cultures.

Coxsackie A5 and A7, Rous sarcoma, herpes simplex and vaccinia viruses did not multiply in A. aegypti, following intrathoracic inoculation. In contrast, VE virus grew consistently to high titers in A. aegypti and A. triseriatus although only irregularly in C. tarsalis and rarely in C. quinquefasciatus following intrathoracic inoculation. A candidate arbovirus, Tsuruse, isolated in mice from Blue Magpie blood in Japan, grew regularly in A. aegypti after intrathoracic inoculation but poorly when virus was inoculated orally.

(W.F. Scherer)

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

Arbovirus survey specimens consisting of 44 pools of mosquitoes (318), 8 Peromyscus leucopus, 1 pine vole, 1 shrew, 2 toads, 1 leopard frog, 19 blue jays, 4 doves, 3 flickers, 2 cat birds, 1 shrike and 1 cow bird were collected August 17-20 and August 26, 1970 around the swamp at Corum Airport, Heckscher Park and Connetiquot River, Oakdale in Suffolk County.

A strain of Flanders virus was isolated from a single pool of 10 Culiseta morsitans: no agents were recovered from the remaining pools and 82 blood clots and/or tissues of liver, heart and brain of the wildlife.

Table 1 HI Reactions with Wildlife Sera Collected from Corum Area of Suffolk County

No. Tested	Species	No HI Reactivity	HI Titers					
			EE	WE	POW	SLE	CV	CE
4	Quail	4						
3	Shrike	2		20				
1	Flicker	1						
1	Warbler	1						
1	Thursh	1						
2	Robin	1						
6	Catbird	5	40					
1	Cowbird		10			20		
5	Dove	4	10		20			
1	Grackle	1						
1	Black and White Warbler	1						
24	Blue Jays	17				20		
						20		
						20		
			20					
						10		
						10		
8	Peromyscus leucopus	7				20		
1	Pine Vole	1						
2	Toad	2						
1	Leopard Frog	1						
18	Horses	6					10	
	South Haven Club			20				
				40		20		
				40				
				40				
				40			20	10
			40	20				
							20	
				20				
			10	160				
			20					
				160				
67	Mosquito collectors	61				80	10	
						10		
						10		
						10		
						10		

Table 2 - HI Reactions with Sera from Birds, Wild Mammals, Frogs and Horses

No. Tested	Species	No. Negative HI	HI Titers					
			EE	WE	POW	SLE	CV	CE
13	Yellow shafted flickers	8	2560					
			640					
			640					
			1280					
			1280					
5	Robin	2	2560					
			640					
			2560					
1	Cat Bird		320					
1	Black Bird		20					
1	Heron		40					
1	Bull Frog	1						
1	Rabbit	1						
1	Peromyscus leucopus	1						
1	Mus musculus	1						
1	Pine vole	1						
	Horse #1 sick		1280	160				
	" #2 "		2560	80				
	" #3 "		320	20			10	
37	Horses	14	20	20		20		10
				40				
			20	80				
							40	20
			80	320				
			80	160				
			80	40				
			10	40		10		
			20	40		20		
							10	
			40	160		20		
				20				
				40				
				20		10		
			320	640				
			20	80				
				40				
			320	40				
			10	40				
			40	80				
			20	20		20		
				40				
			20	10				
5	Pheasants	No sera						

Hemagglutination-inhibition (HI) tests with virus antigens of eastern and western encephalomyelitis (EE, WE), Powassan (POW), St. Louis encephalitis (SLE), Cache Valley (CV) and California encephalitis (CE) were performed on sera from 50 birds, 9 wild mammals, 2 toads, 1 frog, 18 horses and 67 mosquito collectors from the same area of Suffolk County. These results are shown in Table 1.

In late September arbovirus specimens from a ranch in the Montauk area on the south shore of Suffolk County were collected. Bloods from three sick horses which became ill September 23, 1970 and brain obtained at autopsy of one horse were received September 24. Further samples were collected September 26-28. One hundred and fourteen blood clots and tissues from the species listed on Table 2 were examined. Six strains of EE virus were isolated from the blood clots and also from liver, heart and brain suspensions of 3 yellow shafted flickers.

The results of the HI tests are given on Table 2.

(Elinor Whitney)

REPORT FROM THE VIRUS LABORATORY, INSTITUTE OF LABORATORIES, COMMONWEALTH OF MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH BOSTON, MASSACHUSETTS

In the summer of 1970 Massachusetts had an epizootic of eastern equine encephalitis. On the basis of a typical eastern syndrome fifty horses were reported as having the disease. The first afflicted horse died on July 16, and the last succumbed on October 13. Of the horse brains received in this laboratory, fourteen yielded virus in chick embryo cultures. Identification of eastern virus was made by plaque reduction with known antisera. At the time of isolation of virus from the brain, the sera of all horses tested were positive for eastern antibody. We were not able to isolate virus from five horse brains. Antibody studies in these animals showed a plaque reduction titer for western encephalitis, but not for eastern. Whether this antibody was due to vaccination or to actual infection with western virus is not known.

Mosquito pools were collected from the horse sites as well as from the regular collection areas. Sixteen pools were positive for eastern virus. One was positive for western virus. The western positive pool consisted of Culiseta melanura collected throughout the summer in the Hockomock swamp.

Those pools which yielded eastern virus were caught at the sites of the horse cases. Ten of these were Culiseta melanura. Two were Coquilletidia perturbans. Two were Culex pipiens. Two were Culiseta morsitans. The three last named species have not been found previously to carry encephalitis viruses in this area.

Seven wild birds captured in the swamp during August were in viremia. One yellow throat and three catbirds were infected with western virus. One robin and two catbirds had eastern virus in the blood stream. The brain of a house sparrow which was found ill in the town of Middleboro was positive for eastern virus. Six pheasant brains from domestic flocks in East Bridgewater and South Easton were also positive for eastern virus.

Of the wild birds tested for antibody 23% showed eastern antibody and 3% were positive by plaque reduction for western antibody.

The only human case was a five-month-old boy who became ill on August 26 in the town of Pembroke. Three weeks after onset his serum showed a four-fold rise in eastern encephalitis plaque-reducing titer.

Annual rainfall has been above normal since 1967. In addition there has been less killing of mosquitoes since the ban on DDT. These two factors together probably have accounted for an increase in the total number of mosquitoes.

A survey for the presence of eastern and western antibody in the sera of humans residing in the epizootic area is in progress.

(Barbara J. Rosenau)

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

Flanders virus

Eight viral agents isolated from Wisconsin Diptera in 1964, 1966, 1967, and 1968 have been neutralized by anti-Flanders mouse ascitic fluid. The arthropod pools from which these isolates were made were: Culex sp., 1; Culex restuans, 1; Culex pipiens, 1; Aedes trivittatus, 3; Aedes cinereus, 1; Hybomitra hinei-hinei, 1.

Tabanid isolates

Eight arboviruses have been recovered from biting flies in Wisconsin. Jamestown Canyon virus was isolated from Chrysops cleris and Hybomitra lasiophthalma in 1965 and from H. nuda in 1968. Three isolates of LaCrosse virus were made from H. lasiophthalma in 1967. Flanders virus and a flanders-like virus were both recovered from H. hinei-hinei in 1967.

(R. Anslow)

Cell line susceptibility

The susceptibility of 4 mammalian cell lines to infection with 61 South American arboviruses was determined using both CPE and plaque titers as criteria. All 61 viruses grew in at least two of the cell lines. Highest CPE titers were obtained in BHK_{21} cells for 23 of the viruses, in Vero cells for 19 viruses, in PS cells for 8 viruses and LLC-MK cells for 5 viruses. Highest plaque titers were obtained in Vero cells for 26 of the viruses, in BHK_{21} for 17, in LLC-MK for 10 and in PS for 8.

(T. Yuill)

Caribou isolate

In the late summer and early fall of 1969, 7 of 14 woodland caribou on a commercial game reserve in northern Wisconsin died of a central nervous system disease. Examination of a three-year-old female caribou which died on August 23, 1969, revealed the presence of the meningeal worm, Pneumostrongylus tenuis, in the medulla of the brain and in the spinal cord. Virological studies yielded the isolation of a Bunyamwera group virus from both the brain and the blood of this caribou (J. Wildl. Dis., 6:483-487, 1970). Subsequent cross-neutralization tests in suckling mice conducted by Dr. C.H. Calisher, CDC, demonstrated that the virus was more closely related to Cache Valley than to Tensaw, Lokern, or Main Drain. Neutralizing antibodies to the virus were found in the sera of deer and elk on the Wisconsin game reserve and in caribou in Alaska.

(G.L. Hoff)

Aedes triseriatus and La Crosse virus

A. triseriatus is highly efficient in transmitting LaCrosse virus to suckling mice after ingesting virus from viremic golden hamsters in 1.0 to 3.8 ($\log_{10}$) $TCID_{50}$ per 0.1 ml. This strengthens epidemiologic evidence that this mosquito is the major vector of this virus in the upper Mississippi Valley.

(D. Watts)

Biotron facilities

The University of Wisconsin has grant funds which may be used for Biotron charges in order to introduce investigators from outside the University to the capabilities of this unique programmable environmental facility for research in biology. The Biotron has a wide and flexible range of selective control for the various physical parameters of the environment. Significance of temperature, atmosphere, relative humidity, light may be evaluated individually or in combination within the spectrum of the earth's climatic range and with a choice of biota scaled from microorganisms to man. Work may be conducted with both healthy

and diseased organisms. Studies on vector transmission by arthropods have already been conducted. Further information may be obtained by writing or calling:

Dr. H.A. Senn, Director
The Biotron
University of Wisconsin
Madison, Wisconsin 53706
Telephone (608) 262-4900

(H.A. Senn)

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

During 1970 serologic studies of arbovirus infections in Wisconsin residents have been pursued with a newly adapted micro-metabolic inhibition neutralization test in BHK_{21} cells employing antigens of four California group viruses; LAC, TVT, JC and SSH, and a Bunyamwera group virus isolate from Wisconsin mosquitoes.

Convalescent serums from 34 cases of California encephalitis hospitalized in Wisconsin during 1970 each had highest titers to LAC virus. The distribution was similar to that of previous years, all in children 1 to 16 years of age, two-thirds boys, with exposures to insects in deciduous forests of southwestern Wisconsin and adjoining states. Representative serologically confirmed cases which occurred in Wisconsin in 1970 are shown in the accompanying table.

Antibodies neutralizing JC virus were found in 12.4% of 289 high school age youths arriving at several state forestry camps in northern Wisconsin during the summer of 1970, 20% of those from the northern third of the state and 10% from the southern portion. Seven youths acquired these antibodies to JC virus while attending the early summer six-week session in these camps, and none during the later session starting in mid-July.

Representative Serologically Confirmed

Cases of California Encephalitis of Children

in Wisconsin in 1970

Serum[1]			date	Neutralization Titer Serotypes LaC	SSH	TVT	JTC
TN	10	M	7-3	40	10	<10	40
			7-24	120	30	10	80
CN	10	F	7-9	<10	<10	<10	<10
			7-17	160	80	<10	40
AL	3	F	7-8	40	10	<10	10
			8-5	320	40	<10	20
RW	7	F	7-21	<10	<10	<10	<10
			8-6	320	80	<10	10
JW	1½	F	7-25	<10	<10	<10	<10
			8-19	640	80	10	10
MM	5	M	7-29	80	20	<10	<10
			8-29	320	40	<10	<10
TR	6	M	8-5	<10	<10	<10	<10
			8-24	160	20	<10	<10
SH	9	M	8-13	10	<10	<10	<10
			8-28	160	40	10	40
KS	5	M	8-11	40	10	<10	<10
			9-3	320	80	<10	20
LM	10	F	8-11	<10	<10	<10	<10
			8-23	320	40	10	80
RL	6	M	8-17	<10	<10	<10	<10
			8-28	160	40	40	80

[1] Serum: Initial of patient followed by age and sex.

Previous antibodies to TVT virus were found in 12 (5.8%) of those from the southern part of the state and in only 1 from the northern portion. Antibodies to Bunyamwera group virus were found in 3% of those from the north and 1% from the southern portion. One youth (from southern Wisconsin) had antibody to LAC virus.

One-third of 20 sentinel rabbits at sites in the city of Madison during 1968 and 1969 acquired antibodies to TVT virus, with first activity noted during July and lasting through September. An isolate of TVT virus was obtained from sentinel rabbit blood. During the unusually dry season of 1970 TVT virus activity was detected in only 3/20 sites, and only during July. Although LAC virus activity was not detected in Madison, similar sentinel rabbits placed in hardwood deciduous forest areas in rural and suburban southwestern Wisconsin commonly acquire antibodies to LAC and/or TVT virus.

An isolate closely related to Cache Valley virus has been obtained from the blood of a horse which died with febrile disease in September, 1969, on a farm at the south edge of Madison, and sera from other horses in the area frequently neutralize this virus. Two cases of western viral encephalitis in horses were serologically confirmed.

Viremia and antibody patterns have been established in chipmunks, squirrels, cottontails and deer; transmission of LAC virus has been accomplished with Aedes triseriatus to white mice from chipmunks and squirrels.

(Wayne Thompson)

REPORT FROM THE BACTERIOLOGY DEPARTMENT,
COLLEGE OF AGRICULTURE AND BIOLOGICAL SCIENCES,
SOUTH DAKOTA STATE UNIVERSITY,
BROOKINGS, SOUTH DAKOTA

Arbovirus surveillance in South Dakota

Since the isolation of EEE (from a pheasant outbreak in 1967), WEE and California group of viruses (from mosquitoes) in eastern South Dakota, an Arbovirus Surveillance Committee was formed and long term research program initiated. The first series of research goals are to determine which arboviruses are present in various South Dakota species of mosquitoes, and the infection rates. Also to be investigated is the seroconversion period, i.e. the high virus activity period under South Dakota's short summer condition; sentinel flocks will be used and collections made of human sera for serological testing against various important encephalitis agents (WEE, EEE and St. Louis) from residents in different geographic and climatological rural areas of South Dakota.

1. Mosquito trapping - During the summer of 1970 utilizing CDC light traps supplemented with CO_2, 40,000 mosquitoes were trapped during 35 trap nights from June 11, 1970 to August 29, 1970. These mosquitoes were trapped on 4 ecologically different rural areas with different types of farming, livestock and potholes or wet land surroundings. Eighty percent of the mosquitoes have been identified and attempts at virus isolation are being made at present.

2. Sentinel flocks - Sets of 25 chickens or pheasants as sentinel flocks were located in four different counties in South Dakota where in the past encephalitis cases had occurred in man, horse or pheasants. These sentinel birds were caged in CDC type sentinel cages from June 15, 1970 to the end of September 1970. These birds showed seroconversion when tested with WEE virus antigen and microtiter HAI tests. Thus, Brookings, Redfield, Madison and Canton areas of South Dakota has WEE-virus activity in mosquito vectors. Detailed results are indicated in Table 1.

Table 1

Conversion of Sentinel Birds to WEE Virus in Eastern South Dakota

Bird number	Site	Sentinel (No)	Serum titer
401	Canton	Pheasant (25)	1:2560
402	"		1:5120
403	"		1:5120
406	"		1:2560
407	"		1:5120
408	"		1:2560
411	"		1:5120
413	"		1:5120
416	"		1:320
425	"		1:1280
426	"		1:2560
427	"		1:2560
428	"		1:640
Lost number	"		1:1280
0140	Redfield	Chicken (25)	1:1280
6720	"		1:1280
9119	"		1:80
5001	Madison	Chicken (25)	1:320
9124	"		1:320
9126	"		1:320
4986	Brookings	Chicken (25)	1:10240
4991	"		1:10240

The pheasant sentinel flock at Canton showed a conversion rate greater to WEE of 54% (14/26) than chicken sentinels. Only eight of the 100 chickens tested (8%) showed this conversion. Some chickens converted on each site except at one site in the Brookings area where WEE virus had been isolated from several pools of Culex tarsalis during the previous summer. The fact that these sentinel flocks converted indicated activity of the WEE virus in that area of South Dakota. From preliminary studies pheasants appeared to be the better sentinel host, although increased activity of arthropod vectors in the Canton area may have been present. Further work is under investigation with pheasant and chicken sentinels on the same site in order to determine which host is better for detection of arbovirus activity. Since over 5 million wild pheasants are present in South Dakota, it would be very exciting to know what role this resident species plays in arbovirus ecology within South Dakota.

3. South Dakota human sera bank for arbovirus surveillance - Over 12,000 human sera (one sample per person) have been obtained from four of South Dakota's largest hospitals and the State Public Health Laboratory. Samples were collected from June 15, 1970 to December 11, 1970. South Dakota's population is mainly rural and has 680,514 people spread over 77,047 square miles. Out of this we have 3,000 samples from 33,000 Indian population and 9,000 samples from 650,000 white population. For each serum sample we have data as to age, sex, race (white, Indian or Negro), county, hometown and date sample collected. All of this information has been put on computer cards. For each sample a separate card has been punched. Computer programs have been made in COBOL language for IBM 360 computers. Computer print-out has been obtained for master inventory. Also, print-out has been accomplished to arrange and to sort out easily for laboratory testing of sera samples. The print-out has been on the basis of county, age group (0-5, 6-14, 15-29, 30-50 and 51 and over) race and laboratory sample number for easy retrieval of sample for laboratory testing. All sera samples (12,000 human and 4,000 wild birds and chickens) have been stored at -20°C. A program has been initiated to prompt rural physicians to obtain paired sera from CNS cases. Of these sera, the first 1000 samples will be first tested for antibody to WEE, St. Louis and California encephalitis via HAI and neutralization tests. One of the first goals to exploit this sera bank would be to determine arbovirus activity, endemicity and point to possible geographical areas where in the future detailed study could be made to understand mechanisms of outbreak in man and perpetuation of arbovirus under the North Central regional environmental condition.

4. EHD deer hemorrhagic fever virus study - In order to classify EHD and determine the difference between various strains of EHD virus, tissue culture and electron microscopic studies were conducted. South Dakota, New Jersey, Alberta, Michigan and North Dakota strains of EHD virus were grown in BHK-21, Vero, Aedes albopictus, deer kidney and duck embryo cell lines. These agents produced CPE in these cell lines and were plaqued (4 to 7 days) on Vero and or duck embryo cell lines under 45% CO_2 and 1.45% Noble agar with MEM medium (without serum) overlay. Plaque size difference was noticeable between strains, Table 2. Intracytoplasmic basophilic inclusion bodies were observed in infected cells. When examined electron microscopicallv the infected cells showed various sized inclusion bodies, mitochrondrial degeneration and virus particles 32-35 nm in size. Virus particles have electron dense center and single membrane envelope. CPE was noticed between 4 to 14 days depending on the number of viral passages. EHD virus strains showed 0.5 log to 1.0 log decrease in titre when treated with ether and chloroform. Since EHD agent grew in albopictus cell line it is possible to classify EHD as an arbovirus and it can be grouped with limited lipid solvent sensitive group of arboviral agents. The unusual characteristic of South Dakota EHD agent observed was deer kidney cell chromosomal breakage (61+ fragments to 80+ fragments). Normal white tailed deer cells were found to have a diploid chromosomal number of 70.

5. Procedure of genetron extraction of WEE virus and successful utilizing of the extract as an antigen source for HAI tests in sero epidemiology studies - The Genetron-extracted antigen yield a titer of 1:320 in the HA test. A 1:40 dilution was prepared to obtain a working antigen with four HA units per 0.025 ml of antigen. The antigen was titrated in duplicate sets of eight tubes. Two ml of the antigen would be used for this titration. Seven dilutions were used for each serum sample in microtiter "U" plates. For each dilution, 0.025 ml of antigen was dispensed yielding a total of 0.175 ml of antigen per serum sample. The antigen prepared could thus be enough to test approximately 8000 different serum samples. The antigen has been tested in human and bird sera that showed antibody to this antigen preparation. It was compared and found positive when tested with sucrose acetone extracted WEE antigen. Procedure for preparation is shown in Table 3.

(G.C. Parikh)

Table 2 Plaque Characteristics of EHD Strains in Vero Cell Lines

	Alberta	Michigan 55
Plaque Size: (averaged 20-25)		
4 days	0.45 mm	0.29 mm
7 days	1.12 mm	1.02 mm
Plaque Titer:		
4 days	$1 \cdot 10^4$/ml	$3 \cdot 10^4$/ml
7 days	$6 \cdot 10^3$/ml	$3 \cdot 10^4$/ml
Remarks:		
plaques -	- rough edges - size increase, from 4-7 days	- rough edges - size increase, from 4-7 days
range -		
4 days	0.41 - 0.50 mm	0.28 - 0.32 mm
7 days	1.08 - 1.15 mm	0.99 - 1.03 mm
plaque comparison -		
4 days	1.53 X > than M55	0.65 X size of Alb.
7 days	1.09 X > than M55	0.91 X size of Alb.

Table 3. Extraction of WEE with Genetron 113

Time required and quantities obtained from one hundred, 3-day old WEE virus infected mice.

	Procedure	Time Involved	Yield
1.	Inject 0.02 ml of 10% infected SMB intercerebrally into 3-day old mice	30 min.	
2.	Harvest brains from convulsing or dead mice after a 3-day holding period	60 min.	10 ml of infected SMB
3.	Prepare a 20% suspension of infectious SMB	15 min	50 ml
4.	Mix 1 part Genetron* in M-199 medium at pH 7.6 with five parts of 20% SMB suspension	15 min	60 ml
5.	Shake vigorously	2 min	
6.	Centrifuge at 1000 x g	15 min	
7.	Remove supernatant which contains Antigen	10 min	40 ml
8.	Centrifuge Ag at 25,000 x g	60 min	
9.	Remove supernatant containing Antigen	10 min	38 ml
10.	Store at -70°C		

Total time for antigen preparation 3 hr & 37 minutes

* Genetron - influorotrichloroethane ($C.Cl_2$ F-C Cl F_2) purple label 113 from General Chemical Division of Allied Chemical, Morristown, N.J.

REPORT FROM THE ARBOVIRAL DISEASE SECTION, ECOLOGICAL INVESTIGATIONS PROGRAM, CDC, USPHS, FORT COLLINS, COLORADO

Colorado

Colorado sentinel chickens in 1970 reflected a low year for WE and SLE virus activity. There were only 4 WE conversions and no SLE conversions among 63 sentinel chickens in two flocks in Ft. Collins. A single flock of 32 chickens at a farmsite in Larimer County also had 4 WE conversions, and 3 SLE conversions. Two sentinel flocks were placed out in Monte Vista, Colorado at an elevation of 8000 feet. There were two conversions among the 61 chickens at this higher elevation. One of the conversions occurred between bleedings on 7-29 and 8-26, and the second between bleedings on 9-23 and 10-20.

Virus isolation studies in Colorado mosquitoes substantiated the low level of WE virus activity as reflected in low conversion rates in sentinel chickens. Single isolates of WE virus were made from 8300 C. tarsalis and 2625 C. pipiens tested. Two Turlock virus isolations were also made from the C. tarsalis, and 2 unidentified isolates with plaquing characteristics similar to the Bluetongue-like isolates previously recovered in Oregon, Colorado, and Texas from C. tarsalis.

West Texas

No California encephalitis virus was recovered in Hale County, Texas from a limited number of Lepus californicus (jack rabbits) tested in 1970 or from mosquitoes. In the course of these studies, 2 Lokern-like and 2 Main Drain virus isolations were made from 36 L. californicus blood samples collected during the last half of June. Two Main Drain isolates were also made from Aedes nigromaculis, and a Lokern-like virus was recovered from a pool of Psorophora signipennis.

WE virus activity in C. tarsalis continued at a high rate as evidenced from virus isolations presented in Table 1. Virus isolations from nestling house sparrows bled in the area of the majority of the mosquito collections continued to decline. Virus isolations from the nestling house sparrows have been at a low level since the last epidemic years of 1965 and 1966 in Hale County. This despite the continuing, relatively high isolation rate of WE virus in C. tarsalis.

TABLE 1

Seasonal pattern of development of HI antibodies
in sentinel chickens & virus isolations from Culex tarsalis
& nestling house sparrows (Passer domesticus), Hale County, Texas-1970

BLEEDING DATES		RURAL FARMSITE No.	No. (%) Pos.			RURAL COMMUNITY No.	No. (%) Pos.			Virus Isolations From C. tarsalis No. Mosq.	MIR/1000[a]			Virus Isolations P. domesticus No.		
		No. Test	WE	SLE	Tur	No. Test	WE	SLE	Tur	Tested	WE	SLE	Tur	Tested	WE	Turlock
JUNE	6-2	34	0	0	0	67	0	0	0	31				20		
	6-18	34	0	0	0	66	0	0	0	123				20		
JULY	7-2	34	0	0	0	65	0	0	0	468	6.4			20		
	7-16	34	0	0	0	64	0	0	0	1129	8.9	.9	1.8	20	1	
	7-30	34	1(3)	1(3)	0	65	4(6)	0	0	778	12.8			20	1	1
AUG	8-13	34	2(6)	1(3)	0	65	12(18)	0	0	1686	6.5	.6		20		
	8-27	34	2(6)	1(3)	0	64	21(32)	1(2)	0	2235	13.0		.4	20	2	
SEPT	9-10	34	3(9)	1(3)	0	63	42(65)	5(8)	2(3)	1874	13.3	1.1				
	9-24	34	4(12)	2(6)	0	64	44(68)	13(20)	2(3)	738	2.7	1.4	1.4			
OCT	10-15	34	6(18)	2(6)	0	64	44(68)	16(25)	4(6)	207	4.8					
	10-29	34	6(18)	2(6)	0	64	44(68)	15(23)	4(6)							

a - MIR = minimum infection rate obtained by assuming only 1 infected mosquito in each positive pool of 25.

One hundred and two sentinel chickens were placed at seven sites in and around Lubbock, Texas by the Lubbock City-County Health Department. Chickens were bled on October 12, and the sera tested for HI antibodies to WE, SLE, and Turlock viruses. Thirty percent had WE antibody, 15% SLE antibody, and 2% Turlock antibody.

Burrowing owls from Hale County have consistently been negative for WE antibody, although the prevalence of SLE antibodies has been high. Since C. tarsalis is believed to be the principal vector of both WE and SLE viruses in this area, the lack of WE antibody with a high prevalence of SLE antibody did not seem consistent with the hypothesis that burrowing owls were being infected with SLE virus by C. tarsalis bite. Possible explanations for these observations were that burrowing owls were becoming infected with SLE virus by another means, or that burrowing owls were refractory to WE virus infection. In considering the latter explanation, a series of burrowing owls were trapped and inoculated with WE virus. Eight owls serologically negative to WE virus were inoculated with WE virus; four received 250 pfu each and the other four received 2550 pfu each. None of the eight owls had viremia at 24-hr. bleeding intervals for nine days following inoculation, and none developed HI or neutralizing antibody.

Two of the above owls and 13 additional seronegative owls were then inoculated with $10^{6.1}$ pfu of WE virus. Table 2 summarizes results of tests following this large inoculum of virus. All owls remained healthy for at least two months following inoculation, suggesting that the absence of antibody was not due to high mortality among naturally infected owls.

It is concluded that the absence of WE seropositive burrowing owls in the field is probably due to their unusually high resistance to infection with WE virus.

Montana

In response to a request for assistance in investigating the possibility of an arboviral encephalitis epidemic in the vicinity of Glasgow, Montana, mosquitoes were collected and processed for virus isolation. Culex tarsalis and Aedes vexans were the principal mosquitoes collected and single isolates of WE and Turlock viruses were recovered from 2920 C. tarsalis tested in 65 pools. No virus was recovered from 2848 A. vexans tested. The other mosquitoes collected and tested in lesser numbers included A. nigromaculis, A. dorsalis, A. increpitus, Anopheles earlei, and Culiseta inornata.

TABLE 2

Summary Table of Results Obtained Following Inoculation of $10^{6.1}$ DECC PFU of WEE Virus into Burrowing Owls

Leg Band	Previous Inoculation WEE Virus (2/26/70)	WEE Viremia (pfu/0.2 ml whole blood) 24-hr.	48-hr.	WEE-HI Serum Pre-inoc 12/8/70	Serum Post-in 1/14/7
61-62	2550 pfu	Neg	Neg	<10	±20
69-70	None	50	Neg	<10	80
71-72	None	Neg	Neg	<10	±80
73-74	None	220	25	<10	40
75-76	None	15	15	<10	80
79-80	None	5	10	<10	20
81-82	None	115	Neg	<10	±40
83-84	None	Neg	Neg	<10	±40
85-86	None	Neg	Neg	<10	20
87-88	None	40	20	<10	20
89-90	None	35	5	<10	20
91-92	None	5	5	<10	±160
93-94	None	5	Neg	<10	20
95-96	None	5	Neg	<10	20
63218	253 pfu	5	Neg	<10	40
53-54[a]	253 pfu	Neg	Neg	<10	<10
65	2550 pfu	Neg	Neg	<10	<10

a - 53—54 and 65 did not receive the challenge dose of $10^{6.1}$ pfu.

South Dakota

In cooperation with Dr. G.C. Parikh and Mr. D.R. Larson of the South Dakota State University at Brookings, an arbovirus HI antibody survey was made of 20 commercially reared and 96 wild pheasants trapped in eastern South Dakota in late September 1970. The major purpose of the survey was to determine if EEE virus activity was still evident in that area three years after the reported outbreak of 1967 (G.C. Parikh, Z.D. Colburn, and D.R. Larson, 1967, Eastern encephalitis outbreak on a South Dakota pheasant farm, Bacteriological Proceedings - 1969, V64). As shown in the following table, HI antibodies were found against WEE and Turlock viruses but not against EEE and SLE viruses:

Habitat/Age	Ratio HI-Positive			
Commercial	WEE	Turlock	SLE	EEE
Immature	1/20 (5%)	5/20 (25%)	0/20	0/20
Wild				
Immature	7/70 (10%)	9/67 (13%)	0/70	0/70
Mature	5/26 (19%)	6/25 (24%)	0/26	0/26
Total	13/116 (11%)	20/112 (18%)	0/116	0/116

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY,
WESTERN COLLEGE OF VETERINARY MEDICINE,
UNIVERSITY OF SASKATCHEWAN AND THE CANADA DEPARTMENT OF AGRICULTURE,
RESEARCH STATION,
SASKATOON, SASKATCHEWAN, CANADA

Study of collection data and examination for arboviruses of the specimens collected in 1970 has continued. Suckling mice are the primary hosts used for all attempted virus isolations.

Mosquito light trap catches indicated that Culex tarsalis populations in Saskatchewan in 1970 were well above the average levels observed from 1963 to 1968 but were below those of the epidemic years of 1963 and 1965. A number of virus isolations were made from mosquitoes, all from C. tarsalis taken at Outlook and Weyburn. These isolations included 7 of WE virus, 9 of HP-FLA and 3 strains as yet unidentified, but not WE or HP-FLA. The pools positive for WE virus were collected between August 11 and August 30 and the pools positive for HP-FLA virus between July 30 and September 1. The isolations of WE virus are the first that have been made from Saskatchewan mosquitoes since 1966.

In addition to the routine mosquito light traps operated at Saskatoon, Outlook and Weyburn, sentinel chicken flocks were also operated in each of those districts. The chickens were housed in the sheds recommended by Rainey et al. (Mosquito News 22:337. 1962). At Outlook the light trap and chicken shed were about 1 mile apart, at Saskatoon 2 1/2 miles apart and at Weyburn about 4 miles apart. The mosquito traps in the chicken sheds were reversed to trap mosquitoes attempting to leave the sheds. This procedure probably did not trap all the mosquitoes that had entered the sheds and not all of the C. tarsalis taken in the shed traps were blood-engorged. The numbers of C. tarsalis taken in each trap for the season are given in Table 1. Only at Saskatoon was the number taken in the shed trap greater than the number taken in the light trap. No virus isolations were made from any of the C. tarsalis taken in the Saskatoon traps. HP-FLA virus was isolated from C. tarsalis taken in both traps at Outlook and Weyburn. At Outlook, WE virus was isolated only from C. tarsalis taken in the light trap (2 isolations) and at Weyburn only from C. tarsalis taken in the shed trap (5 isolations). The only explanation that can be offered at present for the differences in incidence of C. tarsalis infected with WE virus in the catches of the two traps at Outlook and Weyburn is that WE infections in mosquitoes must be a very localized phenomenon under non-epidemic conditions.

Table 1. Female Culex tarsalis Taken in Light Traps and Shed Traps at Three Locations in Saskatchewan, 1970

Location	Number of Specimens	
	Light Trap	Shed Trap
Saskatoon	115	167
Outlook	852	635
Weyburn	1470	1373

The light traps at Saskatoon, Outlook and Weyburn were operated nightly from June 2 to September 28. The shed traps, in which the chickens were the bait, were emptied daily from June 2 to September 28 at Saskatoon, from June 2 to September 11 at Outlook and from June 2 to September 12 at Weyburn. During the last week of light trap operation (week ending September 28) C. tarsalis and Culiseta inornata were still flying at Outlook and Weyburn. At Saskatoon, in the last week, only C. inornata was still flying, the last specimen of the season of C. tarsalis having been taken on September 19. The dates of latest occurrence of C. tarsalis and C. inornata in the light traps and shed traps at each of the three locations in 1970 are given in Table 2. At Weyburn, one specimen of C. tarsalis was found in the shed trap on the last day that the trap was emptied but during the preceding 5 days only five specimens of that species had been found in the trap, hence its occurrence in the shed trap was virtually at an end. Although C. tarsalis and C. inornata were still flying during the last week of September (as indicated by the light traps), blood-seeking activity (as indicated by the shed trap catches) and incidentally virus transmission had ceased between August 24 (Outlook) and September 12 or 13 (Weyburn). This suggests also that the latest individuals of those two species to emerge and which go into hibernation do not take a blood meal before going into hibernation.

In addition to the WE conversions in our sentinel flocks and the isolation of WE virus from the brain of a ground squirrel mentioned in our previous report, six virus isolates have been obtained to date from wild nestling birds collected from July 29 to August 5, 1970. Brains, blood and livers of the birds were examined for virus. The blood and liver of a house sparrow yielded WE virus; the other four isolates have not yet been identified but they are not WE.

The accumulated evidence indicates that WE virus activity in Saskatchewan increased in 1970 over that of the preceding 3 years.

(A.N. Burton and J. McLintock)

Table 2. Dates of Latest Seasonal Occurrence of Culex tarsalis and Culiseta inornata in Traps at Three Locations in Saskatchewan, 1970

	Light Trap	Shed Trap
	Saskatoon	
C. tarsalis	Sept. 19	Sept. 5
C. inornata	Sept. 28	Sept. 7
	Outlook	
C. tarsalis	Sept. 28	Aug. 24
C. inornata	Sept. 28	Sept. 5
	Weyburn	
C. tarsalis	Sept. 23	Sept. 12
C. inornata	Sept. 24	Sept. 5

*Light traps operated nightly from June 2 - September 28. Shed traps operated from June 2 - September 28 at Saskatoon; from June 2 - September 11 at Outlook; from June 2 - September 12 at Weyburn.

☆ U.S. GOVERNMENT PRINTING OFFICE, 1972—745-353/1585.

EDITORIAL NOTE

Contributions for Issue Number 23 of the Arthropod-borne Virus Information Exchange will be due September 1, 1972. They should be addressed to:

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