Number 24 April 1973

Table of Contents

Page

Report from Chairman of the Subcommittee for Arthropod-borne Virus Information Exchange 1

Listing of Available Arbovirus Reference Reagents, Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 2

Reports From:

Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan 5

Department of Microbiology, Kobe University School of Medicine, Kobe, Japan 650 8

Virology Department, Calcutta School of Tropical Medicine, Calcutta, India 11

Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg, South Africa 13

Arbovirus Laboratory, Institut Pasteur and Orstom, Dakar, Senegal 15

Medical Institute, Tbilisi, USSR 18

Arbovirus Department, D.I. Ivanovsky Institute of Virology, USSR Academy of Medical Sciences, Moscow, USSR 25

Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia 33

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

Reports from (continued): Page

Arbovirus Laboratory, Institute of Hygiene and Tropical Medicine, Lisbon, Portugal 39
Trinidad Regional Virus Laboratory, Port-of-Spain, Trinidad 41
San Juan Tropical Disease Laboratories, Ecological Investigations Program, Center for Disease Control, San Juan, Puerto Rico 43
Arbovirology Section, Virology Branch, Center for Disease Control, Atlanta, Georgia 51
Department of Microbiology, Cornell University Medical College, New York, New York 67
Arboviral Disease Section, Ecological Investigations Program, CDC, USPHS, Fort Collins, Colorado 69
Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley, California 78
Animal Pathology Division, Health of Animals Branch, Agriculture Canada, Sackville, New Brunswick, Canada 81
Division of Medical Microbiology, University of British Columbia, Vancouver, Canada 82
Comment from the Editor 84

REQUEST FROM CHAIRMAN OF THE SUBCOMMITTEE FOR ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

It is strongly urged that as much pertinent supportive data as possible be included when registering new arboviruses in the Catalogue. The Subcommittee on Evaluation of Arthropod-borne Status (SEAS) have noted that some recent registrations have contained little information beyond the barest circumstances concerning the isolation of the virus from an arthropod or from a vertebrate animal. Such sparse information does not provide the Subcommittee on Evaluation of Arthropod-borne Status (SEAS) with sufficient basis for reasonable judgement as to whether or not the virus in question should be considered an arbovirus. Please refer to pages 4-7, Arthropod-Borne Virus Information Exchange No. 23, for the criteria being used by SEAS in evaluating the arthropod-borne status of the various registered viruses. Most of the viruses which must now be considered only "possible arboviruses" could be elevated to the category of "proven arboviruses" merely by experimentally demonstrating infection of the arthropod species by ingestion and transmission by bite.

(T.O. Berge)

LISTING OF AVAILABLE ARBOVIRUS REFERENCE REAGENTS
RESEARCH RESOURCES BRANCH
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
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 January 3, 1973. 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-30, Bethesda, Maryland, 20014.

1. Arbovirus Seed Virus

Anopheles A
Anopheles B
Bebaru
Bimiti
Bluetongue
Bussuquara
Buttonwillow
Bwamba
California Encephalitis
Candiru
Caraparu
Catu
Chagres
Changuinola
Chikungunya
Cocal
Colorado Tick Fever
EEE
EHD
Getah
Guama
Guaroa
Hart Park
Hughes
Highlands J
Ilheus
Junin
Kern Canyon
Lone Star
Manzanilla
Mayaro
Microtus
MML
Modoc
Naples SFF
Nepuyo
Oriboca
Oropouche
Patois
Powassan
Punta Toro
Rio Bravo
Ross River
Sawgrass
Semliki Forest
Sicilian SFF
Silverwater
Sindbis
SLE
Tacaribe
Tembusu
Tensaw

Trivitattus
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
Candiru
Catu
Chagres
Chikungunya
Colorado Tick Fever
EEE
Guama
Guaroa
Hart Park
Hughes
Ilheus
Kern Canyon
Manzanilla
Mayaro
Melao
MML
Modoc
Naples SFF
Oropouche
Patois
Punta Toro
Rio Bravo
Ross River
Sicilian SFF
Silverwater
Turlock
VSV (Indiana)
VSV (New Jersey)
West Nile
Wyeomyia
Yellow Fever
Zegla

3. Arbovirus Grouping Fluids

Group A
Group B
Group C
Group Bunyamwera
Group California
Group Capim
Group Guama
Group Kemerova
Group Phlebotomus
Group Quaranfil
Group Simbu
Group Tacaribe
Group VSV
Polyvalent Anopheles A
Polyvalent Bwamba
Polyvalent Congo
Polyvalent Patois
Polyvalent Polyam
Polyvalent 1
Polyvalent 2
Polyvalent 3
Polyvalent 4
Polyvalent 5
Polyvalent 6
Polyvalent 7
Polyvalent 8
Polyvalent 9
Polyvalent 10
Polyvalent 12

4. <u>BPL Inactivated CF Antigens</u>

Lanjan
Semliki Forest
Capim
Mermet
Caraparu
Tacaribe
Yellow Fever
Tensaw
Bimiti
Cocal

(E.C. Chamberlayne)

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE,
RESEARCH INSTITUTE FOR MICROBIAL DISEASES,
OSAKA UNIVERSITY,
OSAKA, JAPAN

1. Cross-protection of mice immunized with Chikungunya virus against challenge with Semliki Forest virus

Cross-protection tests were performed by intracerebral challenge with Semliki Forest virus (SFV) of mice immunized with live Chikungunya (Chik) virus or with Formalin-killed Chik virus vaccine.

An inbred strain of C3H mice and plaque-purified Chik virus were used. The virus was grown in BHK21 cells and purified to have a titer of 10^8 PFU/ml before formalin-inactivation. Immunization of mice was performed by 2 intraperitoneal inoculations of 10^7 PFU Chik virus 4 weeks apart or by 4 intraperitoneal inoculations of 0.5 ml of killed vaccine at 2 week intervals. Four weeks after the last immunization, mice were bled and challenged with serial dilutions of SFV.

Before challenge, no significant HI or N antibodies against SFV, nor significant interferon titers, were observed in mice sera, although antibody titers against Chik virus were appreciably high (Table 1). The LD_{50} titers in Table 2 show that mice immunized with live Chik virus were protected to some degree; however, less protection was observed in mice immunized with formalin-killed Chik vaccine. Three weeks after challenge, slight serum N titer against SFV was observed in mice preimmunized with live Chik virus, without any detectable N titer against SFV in surviving mice preimmunized with killed Chik vaccine (Table 2).

The results suggest that N antibody formation plays an important role in this cross-protection system.

Table 1. Serum antibody and interferon titers (log) in mice before challenge with SFV.

Immunization	Serum antibody against				Interferon
	Chik virus		SFV		
	HI	N	HI	N	
Live Chik Virus	2.66	4.28	$<$1.3	$<$0.70	0.77
Killed Chik Virus Vaccine	2.28	3.54	$<$1.3	$<$0.50	0.74

Serum N antibody titers were assayed by 50 % plaque reduction method.

Table 2. Serum antibody titers (log) after challenge with SFV and LD_{50} titer (log) of challenge virus calculated in immunized mice.

Immunization	Chik virus		SFV		LD_{50} of
	HI	N	HI	N	SFV
Live Chik virus	2.40	4.31	1.3	1.62	4.58
Killed Chik virus vaccine	2.08	3.14	1.3	0.60	5.71
Control	NT	NT	NT	NT	6.10

NT: not tested.

2. Intracellular components associated with Chikungunya virus-specific RNA's in infected BHK21 cells

Cytoplasmic extracts of BHK21 cells infected with Chikungunya virus were analyzed by sucrose gradient sedimentation. Besides 140 S nucleocapsid associated with 45 S virion RNA, 65 S component associated with 26 S single-stranded RNA was labeled with ^{3}H-uridine in Actinomycin-treated infected cells. This component accumulated with decreased labeling of 140 S nucleocapsid, when labeling was performed after inhibition of protein synthesis. The 65 S component was remarkable when cytoplasmic extract was prepared with solutions containing EDTA. After glutaraldehyde fixation, this component had a density of 1.48 - 1.50 g/cc in CsCl. Pulse-chase experiments did not show any precursor-product relationship between the 65 S component and the 140 S nucleocapsid. Rather the experimental results were more smoothly explained if we consider that 65 S component containing 26 S RNA and 140 S nucleocapsid associated with 45 S RNA were synthesized on separate pathways, and that inhibition of protein synthesis directly blocks the breakdown of the 65 S component and indirectly inhibits the formation of 140 S nucleocapsid through depletion of virus-specific proteins.

(Dr. A. Igarashi, Dr. Sasao and Dr. K. Fukai)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY,
KOBE UNIVERSITY SCHOOL OF MEDICINE,
KOBE, JAPAN 650

Metabolic changes of mammalian cells infected with arboviruses

The rate of ^{3}H-uridine incorporation into RNA in African green monkey kidney (AGMK) cells infected with WEE virus reached a maximum at about 5 hrs after infection. In contrast, the RNA synthesis induced by JE or DEN-1 virus did not increase appreciably during the first 15 hrs, and a maximum was reached at 24-30 hrs. after infection. Cytoplasmic large- and small-particle fractions from the WEE or JE-infected cells catalyzed the incorporation of 4 nucleoside triphosphates into acid-soluble products. In the WEE-infected cells, the enzyme activity was associated almost solely with the small-particle fraction, whereas the nuclear and large-particle fractions of JE-infected cells still contained 15-30% of the total enzyme activity 24 hrs after infection. Rapid inhibition of cellular DNA synthesis was observed 4 hrs after infection, with each of the three kinds of arboviruses used. Heat-inactivated WEE virus was unable to suppress the host cell DNA synthesis, whereas the infection by UV-irradiated virus did result in a clear inhibition of DNA synthesis which, however, was apparently less pronounced than that by the active virus.

Protein synthesis in BHK-21 cells infected with CHIK virus was markedly inhibited. Strongest inhibition was observed in the nuclear and microsomal fractions. The same effect, however, was not noted in the cells infected with DEN-1 virus. DNA-dependent RNA polymerase activity in BHK-21 cells was inhibited by the infection of both CHIK and DEN-1 viruses, although the effect by the latter was apparently less profound than that by the former. The inhibitory factor was found mostly in the microsomal fraction of infected cells.

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Takehara, M.:
Comparative studies on nucleic acid synthesis and virus-induced RNA polymerase activity in mammalian cells infected with certain arboviruses. Arch. f. ges. Virusforsch., 34, 266-277, 1971.

Takehara, M.:
Inhibition of nuclear protein synthesis in BHK-21 cells infected with arboviruses. Arch. f. ges. Virusforsch., 39, 163-171, 1972.

Japanese encephalitis virus-specific RNA synthesized in BHK-21 cells

Under the conditions in which 1 ug/ml actinomycin S_3 selectively inhibited the synthesis of host cell RNA, the synthesis of JE-specific RNA began around 12 hrs, reaching a maximum at 15-17 hrs, after infection. The sucrose sedimentation analysis of viral RNA from infected cells labeled with ^{3}H-uridine during the maximum incorporation period showed that the RNA was composed of 42S, 20S, and 10-15S forms. The 42S form was infective and absolutely sensitive to RNase; its S value was the same as that from purified virions. By sepharose 2B chromatography and benzoylated diethylaminoethyl cellulose chromatography, the 20S RNA was shown to contain RNase-resistant and RNase-sensitive components.

The 26S RNA form, as usually found in the group A CHIK virus-infected BHK cells (refer to our previous report, INFORMATION EXCHANGE No. 23, October 1972, p. 105), was not obviously detected in the present JE-BHK system. In the presence of actinomycin, the JE-infected cells synthesized RNA of low molecular weight (10-15S), whose characteristics remain to be clarified.

REFERENCE

Fukui, K.:
Characteristics of Japanese encephalitis virus-specific RNA synthesized in BHK-21 cells. Kobe J. Med. Sci., Vol. 19, 1973, in press.

Ionic effects on the multiplication of arboviruses

Low ionic strength of culture medium resulted in decrease of extracellular yields of arboviruses from infected cells. This effect was due to the ionic strength rather than osmorality of culture media. No substances other than the salt, such as amino acids or culture medium constituents, were regarded to be related. The effect, which was reversed rapidly by placing the cells from "low" to "normal" medium, was not affected by metabolic inhibitors such as cycloheximide, KCN, NaN_3, NaF, iodoacetic acid, etc. Incorporation of ^{3}H-uridine and ^{14}C-amino acids into infected cells in the presence of Actinomycin D was not affected significantly by changing the media from "low" to "normal" or by maintaining the medium in "low." Electron microscopic observations of these cells showed that the low ionic strength appeared to involve the inhibition of the terminal stage of virus maturation (such as budding process of virus precursors acquiring the cell membrane) rather than the earlier stages.

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Matsumura, T., Shiraki, K., Hotta, S. and Sashikata, T.:
Release of arboviruses from cells cultivated with low ionic strength media. Proc. Soc. Exp. Biol. & Med., 141, 599-605, 1972.

Matsumura, T., Shiraki, K. and Hotta, S.:
Effect of metabolic inhibitors on the release of arboviruses from cells. In preparation.

(S. Hotta)

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

Dengue haemorrhagic fever as sporadic cases

Epidemics of Dengue Haemorrhagic Fever (DHF) had been reported from Philippines, Bangkok, Calcutta and a few other places. Classical dengue is characterized by fever, body-ache, prostration, rash and occasional petichial haemorrhage. Severe haemorrhage with or without shock is not a feature of classical dengue. Some time ago, two epidemics of dengue in two neighboring towns, about one hundred miles from Calcutta, were investigated and during the investigation a few cases with haemorrhage and/or shock were encountered and in these cases dengue virus appeared to be the aetiologic agent. The epidemic in one of the towns has already been described in Info-Exchange No. 22.

Out of many cases of classical dengue there were eight cases with haemorrhage and/or shock. Dengue virus could not be isolated from the acute blood samples of any of these cases. Unfortunately, convalescent blood could be procured from only 2 out of the 8 cases. The results of serological examination and other details about the 8 cases are shown in the table to follow.

The results in the Table suggest that the illness in the 8 patients was due to dengue virus infection. It is important for the clinicians to remember the possibility of sporadic cases of DHF.

(J.K. Sarkar, S.K. Chakravarty and R.K. Sarkar)

Table - I.

Showing presenting features and serological response of eight cases.

Case No.	Age in years and sex	Day of illness	H.I. titre against				CF titre against				Presenting features, in addition to fever.
			Chik	Dengue Type 2	JE	WN	Chik	Dengue Type 2	JE	WN	
1.	7; Male	8	<20*	>2560	640	640	<4*	>512	256	256	Haematemesis 4-5th days; Platelet count 7th day - 225000/cmm.
2.	25; Male	2	< 20	640	320	160	< 4	8	<4	<4	Shock. Platelet count 85,000/cmm 2nd day.
3.	9; Female	2	20	5120	5120	5120	< 4	>512	128	64	Repeated bleeding per vagina 2nd-3rd day; platelet count on 2nd day 100,000/cmm.
		36	<20	2560	1280	2560	< 4	256	128	64	
4.	30; Male	4	< 20	1280	320	320	< 4	> 128	4	4	Haematemesis 3rd day. Platelet count 200,000/cmm on 4th day.
5.	35; Male	5	< 20	320	320	320	< 4	32	4	4	Haematemesis and malaena 4-6th day; shock. Platelet count 240,000/cmm on 4th day.
6.	14; Male	5	<20	640	640	640	< 4	>128	<4	<4	Repeated bleeding from gum 3-6th day. Platelet count on 5th day - 70,000/cmm
7.	45; Male	2	< 20	160	20	20	< 4	128	8	4	Haematemesis 2-3rd day. Platelet count 3rd day - 250,000/cmm.
8.	28; Male	6	< 20	160	20	20	< 4	128	8	8	Haemoptysis 4-6th day. Shock Platelet count on 5th day 110,000/cmm.
		30	< 20	640	40	40	< 4	512	32	8	

* Highest dilution of serum inhibiting haemaglutination or fixing complement.

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

CULEX (NEOCULEX) RUBINOTUS Theobald

Within the last year further information on the viral associations of *rubinotus* has been obtained. The following extract from our Annual Report for 1972 deals with our recent findings:

"Although the 9,266 mosquitoes and 506 *Culicoides* collected at Port Shepstone yielded neither Rift Valley fever nor Wesselsbron viruses, the collections there revealed a most welcome and much sought-after source of *Culex rubinotus* from which multiple isolations of virus were obtained, including strains of Germiston, Witwatersrand, Banzi and Arumowot viruses. These isolations, viewed together with the earlier isolations from *rubinotus* mentioned last year, now make it reasonably sure that *rubinotus* is an important vector of viruses, probably rodent-associated, and suggest that an intensive study of this mosquito is required.

Observations suggest that *rubinotus* has a limited flight range, tending to remain completely within, or at the most, in the immediate periphery of its specialized and inaccessible habitat which consists of dense stands of tall reeds or rushes growing in moist situations, or as emergent vegetation in water. Successful collection of this species apparently requires the siting of traps actually within this habitat and omission to do so previously probably explains the rarity of this species among mosquito collections during earlier years. Recent collections indicate that *rubinotus* is widespread in Southern Africa, being found both on the inland plateau in South Africa and Rhodesia as well as along the eastern coastal region of Natal and Mocambique. It seems that this species tends to occur in small numbers, even under favourable circumstances, since continual trapping in the same area soon reduces numbers.

The belief that *rubinotus* feeds significantly on rodents, which are the probable source of the viruses isolated, is suggested by the identification of 9 (53%) natural blood-meals as being of rodent origin as well as the fact that 36 sentinel hamsters exposed over a nine-day period in a locality at Port Shepstone where infected *rubinotus* was shown to be present yielded 10 strains of Germiston virus and 8 of Witwatersrand virus. Furthermore, bird-baited traps in the same locality failed to collect *rubinotus*.

From collections of rubinotus made at Port Shepstone during February and April, 1,352 females were tested for virus in 215 lots. This resulted in the isolation of 23 strains of Germiston virus, 18 each of Witwatersrand and Banzi viruses and 2 of Arumowot virus, a uniquely high infection rate for South African mosquitoes."

(B.M. McIntosh)

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

Investigations on yellow fever and other arboviruses have been the principal field activities during 1972, especially in the new station of Kedougou. Following serological evidence of yellow fever activity in this area in 1965, 947 human sera were collected in 1970 and 1971 mainly from children under 6 years of age. This survey showed that children under 2 years of age had been infected with yellow fever virus. A surveillance network for yellow fever and other arboviruses has been established in this area in cooperation with the ORSTOM entomological team.

However studies initiated in Bandia field station have been continued but it appears that the lack of rainfall in this northern part of Senegal, which has precluded large mosquito hatches, has had its effect on arbovirus build up.

1. VIROLOGICAL STUDIES

1.1. Human blood samples

405 human blood specimens were collected from febrile patients (308 in Bandia, 56 in Dakar, 42 in Kedougou).

A strain of Zika virus was isolated from a febrile mosquito catcher bled in Kedougou in November 1972.

1.2. Wild vertebrate samples

260 specimens were processed for virus isolation. One strain of Chikungunya virus was isolated from the blood of *Cercopithecus aethiops* shot in Kedougou in September 1972.

One strain of Congo virus was isolated from a sentinel goat bled in Bandia in December 1972.

1.3. Arthropods

1.3.1. Mosquitoes

69,212 mosquitoes were processed in 583 pools. A strain related to West Nile virus was isolated from a pool of *Aedes minutus* collected in June 1972 in Kedougou.

A strain of Chikungunya virus was isolated from a pool of Aedes luteocephalus captured in Kedougou in September 1972.

A strain of Zika virus was isolated from Aedes vittatus captured on human bait in Kedougou in September 1972. The mosquito catcher became ill four days later and his serum yielded a strain of Zika virus.

A strain of a virus related to Uganda S was isolated from a pool of Aedes furcifer-taylori captured in the same area in December 1972 (see 1.1. Human blood samples).

1.3.2. Ticks

22,348 ticks were collected chiefly at the Dakar abattoirs and processed in 1420 pools. They yielded three strains of Jos virus from Amblyomma variegatum in January 1972. Another strain of Jos virus was isolated in July 1972 from Amblyomma variegatum also.

2. SEROLOGICAL STUDIES

2.1. Human sera Bandia

269 sera have been collected from febrile children attending the dispensary of Bandia. From June to August, 16 paired sera were collected at the same dispensary. These sera were tested for HI antibodies to the following viruses: CHIK, ONN, YF, UGS, DB, WN, ZIKA, BUN. They were tested for CF antibodies to Ilesha, Tataguine and Bandia. The last three viruses without HA antigens have been formerly isolated in Bandia, the first two from febrile children and the third from wild rodents.

Serological evidence that a virus related to Zika has been circulating in this area was obtained.

A 13-year-old boy had a positive CF test with Ilesha in January 1972.

2.2. Wild vertebrate sera

310 sera taken from wild vertebrates were tested for HI antibodies. 202 of these sera were collected from monkeys, 116 of which had been shot in Upper Volta in a survey on the yellow fever enzootic area in West Africa. HI studies showed a high percentage of group B polyinfection. 86 were trapped, bled, marked by tattooing and released in Kedougou.

Serological studies point out an incidence of yellow fever recent infection in this area.

Equine encephalitis; Serological data from horse sera

Equine encephalomyelitis syndrome appeared in June 1971 among horses in the ranch of Dara (Senegal). Blood samples were taken only in September 1971 by Dr. P. Bourdin. Preliminary serological studies on 7 samples showed specific serological responses (HI, CF and NT) for Semliki Forest virus antigen. This surprising specificity was confirmed at YARU (Dr. J. Casals).

Following these results, blood samples were collected from 1265 horses in different areas of Senegal. The sera were tested by HI, CF and NT with Semliki Forest virus. The survey has shown evidence that Semliki Forest virus or a very closely related virus is active in the equine population in Senegal.

These results are surprising because SFV has never been reported as causing disease in human beings or animals and has never been yet isolated in Senegal.

(G. Le Gonidec and Y. Robin, Institut Pasteur, Kakar; R. Taufflieb and M. Cornet, ORSTOM, Dakar)

REPORT FROM THE MEDICAL INSTITUTE,
TBILISI, USSR

(Translated from Russian by Mr. Warren L. Armstrong, CDC Library)

Serological investigation of arboviruses in the Georgian Republic

Results of both serological and virological surveys in recent years have revealed a fairly wide distribution of arbovirus infections in the southern republics of the USSR (Gaidamovich et al., 1968).

In the territory of Georgia there exist diseases of obscure etiology with neurological syndromes which are compatible with arbovirus infections, and there are many natural conditions which can foster the existence and circulation of arboviruses: hot climate, diversified terrain, an abundance of bloodsucking arthropods and vast reservoirs of birds on a major migratory route. Despite this, practical work on arboviruses in this region had not been conducted.

In view of the absence of information concerning the distribution of arboviruses in the territory of Georgia, it was decided before beginning the work on isolation of viruses to conduct preliminary investigations of antibodies among the local human population, farm animals and birds.

Abkhaziya, Adzhariya and other regions of Georgia, representing different terrains and geographical zones, were selected for collection of materials. In all, 4955 blood sera were collected (3376 from people, 1312 from farm animals and 267 from domestic and wild birds). The sera were examined by the hemagglutination-inhibition (HI) reaction with antigens of group A arboviruses -- western and eastern equine encephalomyelitis (WEE, EEE), Sindbis, Chikungunya and Semliki Forest; with antigens of group B arboviruses -- tick-borne encephalitis (TBE), Japanese encephalitis (JE), and West Nile encephalitis (WNE), St. Louis encephalitis (SLE), Omsk hemorrhagic fever (OHF) and Ntaya; and the group Bunyamwera-Bunyamwera. The specificity of the isolated anti-hemagglutinins was confirmed by the complement fixation test (CFT) and by biological neutralization in tissue culture (NT).

Analysis of the composite results (Table 1) showed presence of antihemagglutinins in blood sera of a considerable number of people, characterized by antigenic proximity to viruses of group A (WEE, EEE and Semliki Forest, respectively 5.2, 6.3 and 6.0%), and also to some viruses of group B (West Nile, SLE and Ntaya, respectively 6.9, 5.1 and 15.8%). The distribution of antibodies against viruses of different groups in the separate regions proved to be different. Antibodies against viruses antigenically related to WEE were more often observed (and at higher average geometrical titers) in South Osetii,

in the regions of Tbilisi, Kutaisi and Zugdidi, (respectively 5.0, 5.4, 8.1 and 9.6%). Against viruses antigenically close to EEE, antihemagglutinins were often shown in populations of Abkhazii and Adzharii, and also of the Telavsk region, in the regions of Zugdidi and Gori (respectively 9.4, 12.2, 14.5, 5.5 and 5.1%). Antihemagglutinins against viruses antigenically related to Semliki Forest were found relatively often in sera of populations of Adzharii, and also of Tbilisi and Telavsk regions (respectively 6.1, 8.7 and 11.9%). Antibodies against the Chikungunya and Sindbis viruses in all regions were low titered and in an insignificant number of sera, probably reflecting only intra-group cross reactions. Noteworthy from group B were the sera positive to West Nile and Ntaya viruses; a large number were found in all regions with high average geometrical titers. However, in Abkhazii, Gori region and South Osetii the West Nile antibody rate was not high, nor in the Telavsk region, against the Ntaya virus. Relatively high immune strata against St. Louis virus appears in Abkhazii, and in the regions of Tbilisi and Gori.

Analysis of the composite results obtained upon examination of blood sera of farm animals of 6 regions of the republic shows frequent presence of antibodies of group A against virus antigenically close to WEE (9.1%) and EEE (8.2%). Of the viruses of group B, antihemagglutinins were found against West Nile, Ntaya and St. Louis (respectively 4.5, 4.1 and 3.8%). A small number of sera showed antihemagglutinins against other viruses of this group. The distribution of antibodies against viruses of different groups, and also their titers in animals and in people, were different in the separate regions (Table 2). In 2.8 to 17.2% of the sera of different species of birds antibodies were found against WEE, EEE, West Nile and Ntaya (Table 3).

To confirm the specificity of the HI results of positive human blood sera, 230 were also tested by the CFT and 149 by the NT. They were thus examined to evaluate specificity of infection with certain arboviruses (WEE, EEE, Semliki, West Nile and St. Louis) and also to evaluate the possibility of heterologousness of TBE and Ntaya antibodies.

In the NT tests 78.2% of sera protected against West Nile virus and 66.6% against WEE virus, indicative of high specificity of the detected antihemagglutinins antigenically close to these viruses (Table 4). Circulation of viruses antigenically related to EEE and SLE can also be assumed from the NT results (48.4 and 40% of the sera were positive, respectively, against these viruses).

The presence of CF antibodies in 13.5 to 20.8% of sera of examined people is indicative of infection or even reinfection with viruses antigenically similar to EEE, WEE, Semliki Forest, West Nile and St. Louis.

The hypothesis expressed by us concerning the heterologousness of anti-hemagglutinins to viruses of TBE and Ntaya is confirmed by the results of the NT and CFT.

Thus, in the territory of Georgia we succeeded in detecting antihemagglutinins and virus-neutralizing antibodies against a series of arboviruses of groups A and B in a significant number of sera. These findings indicate the existence in the republic of natural foci of arboviruses of these groups or of viruses antigenically related to them. Positive results of the CFT, usually indicative of recent infection, confirm what has been said.

(D.G. Merabishvili and V.I. Bakutashvili)

Table 1. Results of HI tests of sera from human populations against arboviruses of group A, B and Bunyamwera

Таблица I. Результаты РПГА с сыворотками населения с арбовирусами группы А,В и Буниамвера

Место сбора материала Locality	Показатель Index *	Результат реакции с антигенами (Results of tests with antigens): группы А (Of group A) ВЭЛ WEE	ВЭЛ EEE	Семлики Semliki	Чикунгунья Chik	Синдбис Sindbis	группы В (Of group B) КЭ Tick Enc.	ОГЛ Omsk hem. fev.	ЯЭ Jap. Enc.	ЗН West Nile	СЛ St. Louis Enc.	Нтайя Ntaya	Буниамвера (Bunyamwera)
(Abkhaziya) Абхазия	I	352	352	352	352	352	352	352	352	352	352	352	-
	II	3,I	9,4	2,0	2,0	2,0	5,I	I,4	I,7	2,5	9,I	II,3	-
	III	5,0	5,7	5,0	4,8	4,4	4,9	4,7	4,7	4,7	5,6	5,2	-
(Adzhariya) Аджария	I	590	590	590	590	590	590			590			
	II	3,7	I2,2	6,I	2,0	I,2	2,9	435	499	3,9	499	499	
	III	4,9	5,7	5,2	4,6	4,7	4,7	2,7	0,8	5,8	3,0	25,3	
(South Osetiya) Южная Осетия	I	I00	I00	I00	I00	I00	I00	I00	I00	I00	I00	I00	
	II	5,0	I,0	2,0	5,0	0	6,0	I,0	I,0	0	0	I6,0	
	III	5,5	4,3	4,3	4,3		4,6	4,3	4,3			6,3	-
(Tbilisi region) Тбилисский район	I	I304	I304	I304	I304	952	720	720	720	I30	952	I062	400
	II	5,4	3,4	8,7	I,6	2,8	3,5	I,3	3,2	9,5	8,2	I0,4	0
	III	5,7	5,4	5,3	4,7	4,9	4,8	4,6	4,8	6,I	5,4	5,3	
(Kutaisskii region) Кутаисский район	I	343	343	343	343		343	343	343	343	343	343	
	II	8,I	6,I	3,4	4,0		3,4	0,5	0	I0,4	3,2	27,9	
	III	6,I	4,7	5,I	4,6	–	4,7	4,8		6,0	5,2	5,2	
(Zugdidi region) Зугдидский район	I	236	236	236	236		236	236	236	236	236	236	
	II	9,6	5,5	2,5	I,2		0,8	0,4	0	5,0	I,2	I9,4	
	III	5,6	5,9	4,9	4,6		4,8	4,3		5,7	4,9	5,8	
(Gori region) Горийский район	I	275	275	275	275	275	275	275	275	275	275	275	
	II	4,3	5,I	3,6	5,4	I,I	2,8	I,4	I,4	I,6	3,6	I6,0	-
	III	5,5	5,9	4,8	4,7	4,6	4,5	4,5	4,5	4,7	6,0	6,0	-
(Telavsk region) Телавский район	I	II7	II7	II7	II7	II7	II7	II7	II7	II7	II7	II7	II7
	II	3,4	I4,5	II,9	3,4	2,5	I,7	0	0	6,8	5,I	0	0,8
	III	4,8	5,9	5,2	4,8	4,6	4,3			5,7	4,6		6,3
(Poti region) Потийский район	I	59	59	59	59	59	59	59	59	59	59	59	
	II	3,4	0	I,7	0	0	0	0	0	25,4	0	6,8	-
	III	4,8	-	4,3						6,5		5,3	
(Total) ВСЕГО:	I	3376	3376	3376	3376	2445	2792	2637	270I	2202	3024	3043	5I7
	II	5,2	6,3	6,0	2,4	I,5	3,2	I,2	I,4	6,9	5,I	I5,8	0,2

* See footnote, Table 2

Table 2. Results of HI tests of animal sera against A and B group arboviruses

Таблица 2. Результаты РПГА с сыворотками животных с арбовирусами групп А и В

Место сбора материала (Locality)	Показатель (Index)	Результаты реакции с антигенами (Results of tests with antigens)									
		группы А (Of group A)					группы В (Of group B)				
		ЗЭЛ WEE	ВЭЛ EEE	Семлики Semliki	Чикунгунья Chik	Синдбис Sindbis	КЭ Tick	ЯЭ Jap.Enc.	ЗН West Nile	СЛ SLE	Нтайя Ntaya
Абхазия (Abkhaziya)	I	I69	I69	I69	I69	I69	I69	I69	I69	I69	97
	П	7,I	I0,6	3,0	3,0	I5,4	3,0	I,7	5,3	3,0	5,I
	Ш	5,3	5,6	4,7	4,3	4,8	4,7	4,3	4,8	5,7	5,5
Аджария (Adzhariya)	I	279	279	279	279	279	279	279	279	208	279
	П	5,4	4,3	I0,3	2,9	0,7	0	0	I,8	3,8	3,2
	Ш	5,0	5,3	5,0	4,5	4,8			5,5	4,8	5,4
Южная Осетия (South Osetiya)	I	II5	II5	II5	II5	II5	II5	II5	II5	II5	II5
	П	3,5	7,0	0	I,7	0	2,6	0	0	0	7,8
	Ш	6,0	5,0		4,3		4,6				5,3
Тбилисский район (Tbilisi region)	I	262	262	262	262	262	262	262	262	262	262
	П	20,2	8,4	2,2	8,0	I0,3	I,I	0	7,6	3,0	7,2
	Ш	5,5	5,3	4,6	4,5	5,0	4,6		5,7	4,5	5,0
Горийский район (Gori region)	I	223	223	223	223	223	223	223	223	223	223
	П	9,4	8,0	0	3,6	3,6	I,3	I,8	3,6	6,3	2,7
	Ш	5,I	5,3		4,7	4,4	4,6	4,3	4,7	5,4	5,4
Телавский район (Telavsk region)	I	264	264	264	264	264	264	264	264	264	264
	П	5,7	I0,9	6,0	I,I	3,0	I,9	0	6,4	77	0
	Ш	4,9	5,4	4,9	4,3	4,7	4,5		5,6	4,8	
ВСЕГО: (Total)	I	I3I2	I3I2	I3I2	I3I2	I3I2	I3I2	I24I	I3I2	I024	I049
	П	9,I	8,2	4,2	3,5	5,4	I,4	0,5	4,5	3,8	4,I

Обозначения: те же, что и в табл.I. I-число исследованных сывороток; П- число положительных результатов (в %); Ш - среднегеометрические титры антител.

Symbols: the same that are in Table 1. I. Number of sera examined II. Number of positive results (in percent-%)

III. Average geometrical titer of antibody

Table 3. Results of HI tests of bird sera against arbovirus groups A and B

Таблица 3. Результаты РПГА с сыворотками птиц с арбовирусами групп А и В

Results of tests with antigens

Виды птиц / Species of bird	Число особей / Number of specimens	Результат реакции с антигенами x) группы А: ЗЭЛ WEE	ВЭЛ EEE	Семлики Semliki	Чикунгунья Chik	Синдбис Sindbis	группы В: КЭ Tick	ЯЭ Jap.	ЗН WN	СЛ SLE	Нтайя Ntaya
(Rook) Грачи	23	0	0	0	0	0	0	I	0	I	0
(Pigeon) Голуби	20	0	2	0	0	0	0	0	I	0	0
(Field sparrow) Полевые воробьи	48	0	0	3	0	0	0	I	3	0	I
(Crow) Вороны	29	4	I	0	0	0	0	0	5	0	2
(Domestic bird) Домашние птицы	I04	3	0	I	0	0	0	I	4	0	3

Здесь и в табл.4: числа - количество положительных результатов; 0 - отрицательный результат.

Here and in Table 4: figures are the number of positive results. 0 = negative results

Table 4. Results of CFT and NT

Таблица 4. Результаты РСК и РН

	Вирус Virus	Р С К (CFT)		Р Н (NT)	
		всего сыворок All sera	из них положительных No. positive	всего сыворок All sera	из них положительных No. positive
WEE -	З Э Л	37	7	24	I6
EEE -	В Э Л	37	7	33	I6
Semliki -	Семлики	37	5	23	7
Tick -	К Э	I6	0	I2	0
West Nile -	З Н	48	I0	23	I8
St. Louis -	С Л	32	6	20	8
Ntaya -	Нтайя	22	0	I4	0

REPORT FROM THE ARBOVIRUS DEPARTMENT,
D.I. IVANOVSKY INSTITUTE OF VIROLOGY,
USSR ACADEMY OF MEDICAL SCIENCES,
MOSCOW, USSR

Application of ultrasound for increasing activity of arbovirus antigens in serologic reactions in vitro

In 1969 Ardoin, Clarke and Hannoun (1) reported the application of ultrasound for preparation of hemagglutinating antigens of arboviruses. Ultrasonic treatment made it possible for the authors not only to increase the hemagglutination titers but to establish hemagglutinating properties in arboviruses which previously had been considered to be nonhemagglutinating. The task of our investigation was to study the action of ultrasound on the activity of antigens of arboviruses belonging to different groups not only in the hemagglutination (HA) test but also in the complement fixation (CF) and agar diffusion precipitation (ADP) tests.

Twenty-one arboviruses were used in the present study: Sindbis, Western equine encephalomyelitis, Aura, Chikungunya of group A; West Nile (strains IG 2266 and Eg 101), yellow fever (Dakar), Dengue II, Tuleniy, Japanese encephalitis of group B; Tribeč, Kharagysh, Kemerovo of the Kemerovo group; Uukuniemi, Sumakh, Potepli of the Uukuniemi group; Bujaru, Chagres, Sicilian and Naples phlebotomus fever of the phlebotomus fever group; Čalovo of the Bunyamwera group; Tahyna of the California group.

Antigens were prepared from suckling mouse brain by sucrose-acetone and Freon extraction; besides, Aura and Sindbis viruses were represented by tissue culture antigens prepared from the infected chick fibroblast culture medium (medium 199 without serum) without any pretreatment. The mouse brain material was homogenized in the Lourdes homogenizer (USA) at 2,000-3,000 rev/min for 2 minutes. In the process of sucrose-acetone antigen preparation an 8.5 per cent solution of sucrose was added to the mouse brain material and the mixture was homogenized. The homogenate was treated with acetone three times accordingto the method of Clarke and Casals. Chemically pure acetone obtained after additional distillation at 56°C was used. For Freon antigen preparation, the brain material was homogenized in a borate buffered saline, added to make up 10 per cent brain suspension, and an equal volume of Freon 113 was simultaneously introduced into the homogenizer. Then Freon was removed by centrifugation at 3,000 rev/min for 30 minutes.

Ultrasonic treatment was carried out by means of the MSE 150 watt ultrasonic disintegrator, using the 34041 titanium exponential micro probe. Antigens prepared by the above methods were subjected to ultrasonic treatment. In some experiments sucrose-acetone antigen was subjected to ultrasonic treatment at the preparatory stage preceding final centrifugation.

The process of ultrasonic treatment consisted in the following:

1. A glass tube was added with 3-5 ml of antigen, sealed with a rubber cap with the hole in the middle and placed into a beaker containing ice.

2. The probe was inserted through the sealing cap so that its end was immersed not more than 3-4 mm in the suspension.

3. Ultrasonic treatment was carried out at a frequency of 20,000-25,000 hertzs for 30-40 seconds, the procedure was repeated 3 times at 3-minute intervals.

The HA and HI tests were done by a micromethod in the Takatsy microtitrater at pH optimal for each virus. The CF test was also carried out by a micromethod in a modification of Gaidamovich and Kirjushchenko (2); in accordance with this modification all the reaction ingredients were prepared in 0.01M Tris buffer solution (0.01 M Tris (hydroxymethylaminomethan) HCl) at pH 7.3. The ADP test was carried out in 1% Difco agar in borate buffered saline, pH 9.0. Mouse immune ascitic fluid prepared as described previously was used in all tests.

Efficiency of ultrasonic treatment was evaluated by the method of antigen titration in one experiment before and after ultrasonic treatment in the HA, CF and ADP tests. Specificity of hemagglutination antigen was also tested in the HI test. In experiments with group A arboviruses (Table 1) a 4-8 fold increase in the hemagglutination titer of sonicated antigens was regularly observed irrespective of the material and pretreatment. Antigen titers in the CF test correspondingly increased 4 to 8 times. In the ADP test both sonicated and nonsonicated antigens reacted similarly. Analogous data were obtained with antigens of group B arboviruses. Of all antigens tested only one series of Japanese encephalitis antigen was found to be refractory to ultrasonic treatment (Table 2). Ultrasonic treatment of antigens of the Kemerovo group viruses resulted in a 2-4 fold increase in the antigen titer in the CF test. Viruses of this group are not known to possess hemagglutinating properties. Nor did the application of ultrasound produce positive results (Table 3).

Ultrasonic treatment of antigens of phlebotomus fever virus group was found to be effective (Table 4). Hemagglutinins of this group are known to be very inhibitor-sensitive and unstable. Under the specific conditions of these experiments Freon antigens of series 2 and 3 of Naples phlebotomus fever virus were inactive in the HA test and ultrasonic treatment produced no positive results, nor was the antigen titer of this virus, series 13, increased. Other cases showed a 2-8 fold increase in the titer in the HA test, whereas sucrose-acetone antigen of Bujaru virus acquired hemagglutinating properties only after ultrasonic treatment. High titers of unsonicated antigens in the CF test were

similar to those of the sonicated, and a 2-4 fold increase in the titer of less active antigens was observed. In single cases the antigen titer increased in the ADP test.

Antigens of the Uukuniemi group viruses are attributed to the hemagglutinating antigens that are relatively difficult to reproduce. They are usually prepared by the treatment of sucrose-acetone and Freon antigens with Tween-80 and ether. In our experiments ultrasonic treatment permitted the hemagglutinin titers to increase slightly but a clear-cut effect of ultrasound on an increase in antigen titers was manifested in the CF and ADP tests (Table 5).

Thus, our experiments with 21 arboviruses have corroborated that ultrasound stimulates the activity of antigens in the CF test. At the same time, the data obtained indicates that the antigen titer in the CF test can increase more regularly than that in the ADP test. A possible explanation is that under the influence of ultrasound there occurs dispersion of antigens and the resulting release of more active groups interacting with antigens in the CF and ADP tests. In addition, the ability of antigens to diffuse in the ADP test seems to increase. This has been manifested by the formation of precipitin lines several hours earlier with sonicated antigens than with nonsonicated ones. It was often that the absolute antigen titers after ultrasonic treatment did not exceed those obtained by other methods; however, each specific case showed an increase in initial titers of antigens. Therefore, ultrasound can be applied not only for preparation of high-titered antigens but can be also used as an additional method for increasing the titer of antigens with low activity, which point is of primary importance in mass antigen production.

References

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2. Gaidamovich S., Kirijushchenko T., Voprosy Virusologi, 1972, No. 3, p. 358.

(S.Ya. Gaidamovich)

Table I

EFFECT OF ULTRASOUND ON ACTIVITY OF ANTIGENS OF GROUP A ARBOVIRUSES

Virus and strain	Series of antigen	Method of preparation	Titer in HT before	HT after	CFT before	CFT after	ADPT before	ADPT after
Sindbis	1	B, sucrose-acetone	128	1024	320	1280	undiluted	
	2	B, Freon	8	64	80	320	0	0
	3	C, nontreated	0	16	undiluted	1:4	0	0
Western American Equine Encephalomyelitis	1	B, sucrose-acetone	1024	2048	640	2560	2	2
		B, Freon	1280	5120	160	2560	undiluted	
	2	C, nontreated	320	1280	16	64	undiluted	
Chikungunya	1	B, sucrose-acetone	1280	10240	320	1280	not tested	
	2	B, sucrose-acetone	2560	10240	320	1280	not tested	
Aura	1	B, sucrose-acetone	1280	10240	160	320	2	2
	2	B, Freon	160	160	320	320	2	2
	3	C, nontreated	40	160	2	16	0	0

Designations for Tables 1-5:

B - antigen from the suckling mouse brain

C - antigen from chick fibroblast culture

O - negative results

Table 2

EFFECT OF ULTRASOUND ON ACTIVITY OF ANTIGENS OF GROUP B ARBOVIRUSES

Virus and strain	Series of Antigen	Method of preparation	Titer in HT		Titer in CFT		Titer in ADPT	
			before	after	before	after	before	after
West-Nile IG 2266	I	B, sucrose-acetone	320	1600	320	640	4	4
West Nile Eg 101	2	B, sucrose-acetone	1280	6400	320	2560	4	4
Japanese encephalitis	I	B, sucrose-acetone	640	640	80	80	0	0
Japanese encephalitis	2	B, sucrose-acetone	10240	20480	160	320	undiluted	
Yellow Fever (Dakar)	3	B, Freon	160	640	40	40	0	0
Tuleniy	I	B, sucrose-acetone	1280	5120	160	320	2	2
Denge II	I	B, sucrose-acetone	160	640	40	80	not tested	

Table 3

Effect of Ultrasound on Activity of Antigens of Arboviruses belonging to Kemerovo, Čalovo and Tahyna groups

Virus	Series	Method of preparation	Titer in CFT		Titer in ADPT	
			before	after	before	after
Kemerovo	I	B, sucrose-acetone	80	160	undiluted	
	2	B, sucrose-acetone	160	640	undiluted	
Tribeč	I	B, Freon	40	80	undiluted	
Kharagush	I	B, sucrose-acetone	40	80	0	0
Čalovo		B, Freon	160	640	undiluted	
Tahyna	I	B, sucrose-acetone	64	256	not tested	
	2	B, sucrose-acetone	64	256	not tested	
	3	B, sucrose-acetone	64	256	not tested	

Table 4

EFFECT OF ULTRASOUND ON ACTIVITY OF ANTIGENS OF ARBOVIRUSES OF PHLEBOTOMUS FEVER GROUP

Virus	Series	Method of preparation	Titer in HT		Titer in CFP		Titer in ADPT	
			before	after	before	after	before	after
Naples	1	B, Freon	64	1024	not tested		not tested	
	2	B, Freon	0	0	80	320	0	0
	3	B, Freon	0	0	1280	1280	undiluted	1:4
	8	B, sucrose-acetone	8	64	320	640	undiluted	2
	13	B, sucrose-acetone	32	32	80	160	0	0
Sicilian	9	B, sucrose-acetone	4	8	1280	1280	undiluted	4
	10	B, Freon	2	4	1280	1280	4	4
	12	B, sucrose-acetone	8	32	5120	5120	2	4
Chagres	7	B, sucrose-acetone	128	512	1280	1280	2	2
	11	B, sucrose-acetone	256	512	160	160	0	0
	14	B, sucrose-acetone	128	512	1280	1280	4	8
Bujaru	15	B, sucrose-acetone	0	64	not tested			

Table 5

EFFECT OF ULTRASOUND ON ACTIVITY OF ANTIGEN OF ARBOVIRUSES OF THE UUKUNIEMI GROUP

Virus	Series	Method of preparation	Titer in HT before	HT after	CFP before	CFP after	ADPT before	ADPT after
Sumakh	1	B, sucrose-acetone	2	16	64	256	0	0
	2	B, sucrose-acetone	4	32	256	1024	2	4
	3	B, Freon	0	2	512	1024	0	2
Uukuniemi	1	B, Freon	0	0	256	1024	0	4
	2	B, sucrose-acetone	0	0	256	512	0	2
Potelpi	1	B, Freon	0	2	256	1024	0	2
	2	B, sucrose-acetone	0	2	256	512	undiluted	2
Manawa	1	B, sucrose-acetone	16	128	256	256	undiluted	

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

1. Localization of tick-borne encephalitis (TBE) virus in alveolar cells of salivary gland of DERMACENTOR MARGINATUS and HAEMAPHYSALIS INERMIS ticks

The salivary glands of ticks constitute the major route through which pathoergonts are transmitted to susceptible hosts and they are also a source of toxine. The aim of our study was to elucidate the virus development in the tick and to demonstrate the presence of virus in the cells of the salivary gland. The new approach to this problem is electron microscopy.

The TBE virus was observed in alveolar cells of salivary gland on the 5th day after feeding of *Dermacentor marginatus* and *Haemaphysalis inermis* females on viraemic white mice. The presence of virus in granule-secreting alveoli on the 5th day after beginning of feeding was confirmed by isolation of virus from glandular tissue, and by electron micrographs. The first presence of virus was observed on the second day in a *Haemaphysalis inermis* female by immunofluorescence. The maturation of TBE virus was observed on the smooth intracytoplasmic vacuolar membranes of "e" cells of granular alveoli III infected ticks. The mature virus was accumulated in lumen of intracytoplasmic vacuoli.

It seems that arboviruses of groups A and B mature by budding from intracytoplasmic membranes, or the nucleocapsids are assembled in the cytoplasmic matrix of infected cells, migrate to the cell surface, and are wrapped tightly in envelop derived from the cell surface membrane as they are extruded from the cell.

(J. Nosek, F. Čiampor, O. Kožuch, J. Rajčáni)

2. Haemagglutination-inhibiting antibodies against arboviruses in animal sera, collected in some regions in Austria

In 1968 a close cooperation between WHO Regional Reference Centre for arboviruses in Bratislava and from the Institute of Hygiene in Graz, Austria, was initiated. Some results of common work carried out by W. Sixl, M. Batikova, D. Stunzner, M. Sekeyova, B. Sixl-Voigt and M. Gresikova are presented. Blood samples of 378 randomly chosen domestic animal and 83 wild animal sera collected in Karnten, Steiermark and Burgeland were examined for the presence of HI antibodies to some arboviruses.

Antibodies against Sindbis virus were found in the sera of dogs (16.6%), of swine (8.6%), in cattle (5.4%). A high percentage of positive reactions with TBE virus antigen was found in dogs (22.2%). On the other hand, only one out of 244 swine sera reacted with TBE virus. Antibodies to TBE virus were also found in cattle (10.7%). All the sera of rabbits tested reacted with West Nile antigen. A high percentage of positive reactions with West Nile antigen was found in dogs (33.3%) and cattle (26.7%). The sera of swine reacted with West Nile antigen in 6.9%. The titres of antibodies are summarized in Table 1. No haemagglutination-inhibiting antibodies to Uukuniemi virus were found in domestic animal sera. From wild animals only a limited number of sera were tested. HI antibodies to Sindbis virus were found in the sera of *Erinaceus europeus* (1 positive of 29 tested). Antibodies to TBE virus were found in rabbits (2 from 4 tested), in *Anguis fragilis* (1 from 3 tested), in *Erinaceus europeus* (2 of 29 tested), in *Apodemus flavicollis* (1 of 2 tested) and in *Citellus citellus* (1 of 10 tested). Antibodies to West Nile antigen were found in *Natrix natrix* (5 positive of 10 tested), in *Myotis myotis* (3 positive of 15 tested), in *Erinaceus europeus* (9 positive of 29 tested), in *Citellus citellus* (3 of 10 tested), in *Vulpes vulpes* (2 of 4 tested). The titres of the antibodies are summarized in Table 2.

The cross reactions with B group arboviruses (TBE virus and West Nile virus) are presented in Table 3.

Sera of 2 dogs reacted specifically with TBE antigen in a titre of 1:20 and 1:640, respectively. Sera of 5 dogs reacted specifically with WN antigen in titres of 1:10 - 1:20 and 1:640, respectively. No cross reactions in swine sera with group B antigens were observed. On the other hand cross reactions with group B arboviruses were observed in cattle sera.

In wild animal sera cross reactions with TBE and WN antigens were observed in two instances in the sera of Erinaceus europeus; the titre of antibodies against TBE virus was higher (1:80 and 1:320). The cross reactions in sera of rabbits was observed in two instances; the titre of antibodies against West Nile virus was the highest; one serum reacted with West Nile antigen only in the titre 1:320. In other wild animal sera no cross reactions with group B arboviruses were observed.

The presence of haemagglutination-inhibiting antibodies against West Nile virus is of interest. High antibody level to West Nile virus (1:320 and or 1:640), in rabbits sera and low titer (1:10) to TBE virus may lead to the conclusion that very likely another virus of B group is circulating in the studied area. Antibodies to West Nile virus have been found also in the sera of dogs, swine, cattle. In wild animals antibodies to this virus were detected in the sera of Cunicullus cunicullus, Natrix natrix, Myotis myotis, Erinaceus europeus, Citellus citellus and Vulpes vulpes.

(W. Sixl, M. Batíkova, D. Stünzner, M. Sekeyová, B. Sixl-Voigt and M. Grešíková)

Table 1

HI antibodies against some arboviruses in domestic animal sera collected from Kärnten, Steiermark, Burgeland.

Species	No.of sera examined	HI antibody titre against arbovirus antigen			
		Sindbis	TBE	WN	Uukuniemi
Canis familiaris	36	2 x 1:20 2 x 1:80 1 x 1:160	1 x 1:10 4 x 1:20 2 x 1:80 1 x 1:160	2 x 1:10 5 x 1:20 2 x 1:40 1 x 1:80 1 x 1:320 1 x 1:640	-
Sus	244	4 x 1:20 5 x 1:40 6 x 1:80 6 x 1:160	1 x 1:40	1 x 1:10 11 x 1:20 3 x 1:40 2 x 1:160	0
Bos	94	1 x 1:10 2 x 1:20 1 x 1:40 1 x 1:80	6 x 1:20 3 x 1:40 1 x 1:80	6 x 1:20 7 x 1:40 10 x 1:80 2 x 1:160	0

TBE = Tick - borne encephalitis
WN = West Nile

0 = Negative results
- = Not tested

Table 2

HI antibodies to some arboviruses in wild animal sera collected from some regions in Austria.

Species	No of examined sera	HI antibody titre with antigens		
		Sindbis	TBE	WN
Cuniculus cuniculus	4	0	2 x 1:10	2 x 1:320 1 x 1:640
Anguis fragilis	3	0	1 x 1:20	0
Natrix natrix	10	0	0	1 x 1:10 3 x 1:20 1 x 1:80
Lacerta agilis	1	0	0	0
Myotis myotis	15	0	0	2 x 1:10 1 x 1:40
Erinaceus	29	1 x 1:20	1 x 1:80 1 x 1:320	5 x 1:10 3 x 1:20 1 x 1:40
Cricetus cricetus	5	0	0	0
Clethrionomys glareolus	6	0	1 x 1:10 1 x 1:20 1 x 1:80	0
Apodemus flavicollis	2	0	1 x 1:640	0
Citellus citellus	10	0	1 x 1:20	1 x 1:10 2 x 1:20
Vulpes vulpes	4	0	0	1 x 1:40 1 x 1:80

TBE = Tick - borne encephalitis
WN = West Nile

0 = Negative results

Table 3

Cross - reactions in HI tests with animal sera tested for the presence of TBE and West Nile antibodies.

Species	Serum	HI titre with TBE antigen	HI titre with WN antigen
Cuniculus cuniculus	2	1 : 10	1 : 320
	4	1 : 10	1 : 640
Canis familiaris	13	1 : 10	1 : 20
	16	1 : 20	1 : 40
	25	1 : 20	1 : 20
	59	1 : 80	1 : 320
	60	1 : 80	1 : 80
Bos	39	1 : 20	1 : 40
	40	1 : 40	1 : 80
	57	1 : 40	1 : 80
	58	1 : 20	1 : 40
	59	1 : 20	1 : 40
	6	1 : 40	1 : 80
	8	1 : 80	1 : 80
	10	1 : 20	1 : 160
Erinaceus eusopeus	2	1 : 320	1 : 40
	29	1 : 80	1 : 20

TBE = Tick - borne encephalitis
WN = West Nile

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

1. STUDIES DONE IN PORTUGAL

1.1. Antibodies to arboviruses in the human population of Portugal

After the serological surveys done in order to study the epidemiology of the arboviruses in Portugal and the subsequent isolation of West Nile virus in south Portugal (Acta Virol. 16, 361, 1972) it was decided to do other surveys in order to correlate the presence and activity of these viruses and the immunological responses of the human population.

About 1649 human sera have been studied by the haemagglutination-inhibition test using 14 antigens extracted from viruses of group A, B, Sicily, Tahyna and Calovo. The HI test showed that 3.3% of the studied sera had antibodies against arboviruses from group B. Several of the positive sera by the HI test were also studied by the mouse neutralization test and they protected the inoculated animals when they were challenged with West Nile virus.

The results obtained show that probably in certain areas of the country some febrile diseases of unknown origin observed in the summer may be caused by arboviruses.

1.2. A presumptive epizootic by West Nile virus

In 1970 we had the information that some years ago several cases of equine encephalomyelitis occurred on a farm of south Portugal. In the same year we bled 24 horses that survived from the disease. All the sera were examined by the neutralization test against West Nile. The results of the test have shown that 29.1% of the studied animals had neutralizing antibodies against this virus. Serological surveys done in the same general area some years before this outbreak had revealed antibodies against West Nile virus in several domestic animals living in places not very far from the infected farm. Also, in 1969, this virus was isolated from a pool of mosquitoes captured near a water dam, situated to the north of the farm where the illness appeared.

2. STUDIES DONE IN ANGOLA

2.1. Serological survey with human sera taken before the Yellow Fever and Chikungunya epidemic of Luanda, 1971

In 1972 we had the opportunity to study a group of 408 sera from women who had lived in Luanda, Angola, for more than 1 year; the sera were collected between April and June 1970. These sera had been collected for other immunological studies, but fortunately they could also be studied to obtain information concerning the activity of arboviruses in Luanda almost 1 year before the start of the Yellow Fever and Chikungunya epidemic which occurred in 1971. The sera were examined first by HI test using antigens from the following viruses: Sindbis, Chikungunya, Semliki, West Nile, Ntaya, Zika, Yellow Fever, Wesselsbron, TBE, Dengue 1, Dengue 2, Banzi, Oriboca, Marituba, Sicilia, Tahyna, Bunyamwera and Rift Valley Fever. The sera with HI antibodies against Yellow Fever and Chikungunya were also studied by the CF test.

The results obtained with this serological survey showed that 5.6% of the examined sera had HI antibodies against Yellow Fever while in the following year a group of sera very similar showed that 22.2% of the studied sera were positive against the same virus. The results obtained with the Chikungunya antigen were 5.3% positive in 1970 and 13.7% positive in 1971, during the "Katolu-Tolu" (Chikungunya) epidemic. The sera obtained in 1970 did not show the high-titered antibodies to group B virus characteristic of secondary infections which were found during the Yellow Fever epidemic. It seems that the antibodies found in this survey correspond to old infections. The CF test confirmed that most of the positive cases revealed by the HI test were in reality old infections.

2.2. Serological survey for arboviruses in Angola

Since the serological survey done by Kokernot et al (Trans. Roy. Soc. Trop. Med. Hyg. 59, 563, 1965) in 1960, no other general survey has been done in Angola.

In collaboration with the Instituto Provincial de Saúde Pública de Angola and the Instituto de Investigacao Cientifica de Angola, our laboratory started in 1972 a general survey with 5500 human sera collected in 17 selected areas of Angola. The first part of this study was done with the HI test using 19 antigens. It is not possible yet to mention the final results, but at this moment we can inform you that this survey has disclosed several areas where the Chikungunya virus is particularly active, as are other group B viruses. Details of this survey will be published as soon as possible.

(A.R. Filipe)

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

Endemic EEE at Aripo-Waller Field

The field programme which was designed to study the natural history of EEE at Aripo-Waller Field in 1971 was stopped in January 1972 but was restored in November on a limited basis.

Three small "forest islands" in one of the savannahs were chosen for exploratory work. It was thought that if EEE was found on the "islands", it would be more informative to study the natural history of the virus in a small area with definite boundaries than over larger and less defined forests.

The largest of the three "islands" is about 3.8 acres in which five stations were sited. Two stations were also selected in each of the two other islands. Sentinel mice under hoods were exposed twice per week at each of the stations, while on alternate nights the sentinels were exposed in No. 17 traps to catch blood-sucking arthropods which may be transmitting the virus to the sentinels.

Three stations A2, A10 and A10A which were in operation in 1971, and where EEE was known to be active, were also operated in a similar manner to the "island" stations for comparative purposes.

Two strains of EEE virus were isolated but only from sentinel mice. Both strains came from station A2, within the main forest, on November 29 and December 14. Although the strain (TRVL 122949) of December 14 was from sentinels exposed in No. 17 traps, it was not possible to isolate the virus from mosquitoes which were collected from the same trap.

During the two-month period we collected some 3800 mosquitoes. Sixty-one percent of these were Culex portesi, but C. taeniopus the next most abundant mosquito accounted for only 9 percent of the total catch. Most of the mosquitoes collected were inoculated in mice in 58 pools, but EEE virus was not isolated.

Although we failed to detect EEE activity in the "forest islands", we cannot yet conclude that it does not exist there. Investigation during the next rainy season may assist in giving the answer.

Other arboviruses

In association with these EEE studies, 36 strains of viruses were isolated from sentinel mice and two from mosquitoes. The Table summarizes the isolates from sentinel mice by stations. While viruses were isolated from all stations, A2 appears to be the best in terms of total number of sentinel mouse groups infected.

Although VEE, Group C and Guama group viruses are known to be rodent-associated, we were only able to trap one mammal in the A15-A19 3.8 acre area in 640 trap nights. We wonder at this stage if our mammal trapping techniques are adequate, that the water logged conditions had an adverse effect, or indeed that some other group vertebrates may also be associated with these viruses.

(E.S. Tikasingh, C.O.R. Everard, J.B. Davies and M.C. Williams)

Arboviruses isolated from sentinel mice in Trinidad,
Nov.-Dec. 1972

STATIONS	Mouse groups Exposed	EEE	VEE	Group C	Guama Gp.	Total
A2	14	2	2	3	2	9
A 10	15		2	1		3
A 10A	13		6			6
A15-19	74		3	4	5	12
A20-21	28		1	3		4
A22-23	28		2	2		4
TOTALS	172	2	16	13	7	38

REPORT FROM THE SAN JUAN TROPICAL DISEASE LABORATORIES, ECOLOGICAL INVESTIGATIONS PROGRAM, CENTER FOR DISEASE CONTROL, SAN JUAN, PUERTO RICO

Dengue surveillance

During 1963-64 and 1968-69, Puerto Rico experienced explosive epidemic activity of dengue-3 and dengue-2 virus, respectively. During the inter-epidemic period, endemic dengue was not observed. However, since the 1969 epidemic, a surveillance system focusing on the western and southwest region of the Island has revealed the persistence of dengue-2 transmission through February 1973 (Figure 1). The surveillance system depends primarily upon the collection of acute and convalescent sera from suspected cases seen by local physicians and public health nurses in outlying health centers. In addition, a "sentinel population" of hospital and health center employees from selected communities has cooperated by providing serum specimens at intervals of 3-6 months for "passive" serological surveillance.

Investigation of a dengue outbreak

In July 1972, the surveillance mechanism indicated an increase in the incidence of febrile illness with rash in the Guánica-Ensenada area of southwestern Puerto Rico.

On August 22-24, September 12-13, October 16-17, and November 21-22, house-to-house surveys were conducted in Guánica-Ensenada to determine the incidence of all febrile illnesses since June 1, 1972, and to establish Aedes aegypti population indices. Six geographic subdivisions were defined and mapped, and every fifth occupied house was visited. The same houses were visited on each survey, during which an average of 263 families were interviewed. Data was obtained on a mean of 1,130 persons each survey, representing approximately 13% of the population. From data obtained during the four surveys, a composite epidemic curve was drawn (Figure 2). The overall febrile illness attack rate for the 5-1/2-month period was 331/1,000 population. There was no striking predilection for a specific age group, and no significant differences in sex-specific attack rates were noted. No hemorrhagic manifestations were reported.

Blood specimens were obtained from persons who had experienced fever within 10 days of the interview. Dengue-2 infection was confirmed serogically in 35 (56%) of 62 febrile patients in the survey population from whom paired sera were obtained. Positive serology was no more frequent in patients with fever and rash or with fever, rash, and body pain than in patients with fever alone.

Premise indices (percent of premises positive for A. aegypti larvae) were determined for the six geographical subdivisions on each survey (Table 1). The types of containers positive for A. aegypti larvae were also determined (Table 2). Numbers of adult A. aegypti collected per man-hour during the 1st, 2nd, 3rd, and 4th surveys were 1.3, 2.7, 4.0, and 0.7, respectively.

Larval and adult A. aegypti control efforts began 1 week before the 3rd survey. Two groups of premises were examined in the 2nd and 3rd surveys to evaluate the efficacy of treatment of water containers with an organophosphate larvicide. Larvicide was applied to containers in a group of 96 premises between the 2nd and 3rd surveys, while another group of 134 premises received no treatment. Notable success was achieved in eliminating larvae from the 50-gallon barrels (Table 3) in the treated group. Part of the increase in positive containers in the untreated group (Table 4) is attributable to unusually heavy rains which fell during the 3 weeks prior to the 3rd survey.

The acute serum of a resident of Guánica yielded dengue-2 virus isolated and identified by Dr. Sonja Buckley and Jordi Casals of the Yale Arbovirus Research Unit.

Small foci of dengue-2 activity have now been identified in Sabana Grande and Juana Díaz, west and east of Guánica. An additional focus has been identified in Villalba, in a mountainous region to the north of Juana Diaz. A. aegypti control measures are in progress in these towns.

Dengue in a traveler from Puerto Rico to Colombia

On June 19, 1972, a 27-year-old woman living in San Juan, Puerto Rico, developed general malaise progressing to chills, fever (temperature 102.4°F), severe generalized muscular and bone pain, and headache by the following day. Subsequent examination revealed a white blood cell count of 4,200 with an unremarkable differential count. Thick smears were negative for malaria parasites.

On June 23, a fine red macular rash appeared on the patient's face and trunk. By the next day, the rash became coarse, slightly elevated, and more confluent, and spread to most of the body. No hemorrhagic manifestations were noted, and the patient had a complete recovery by June 28.

Two serum specimens were collected from the patient on June 20 and yielded dengue type 2. A diagnostic rise in the titer of HI and CF antibody was observed in paired acute and convalescent sera.

Epidemiologic investigation revealed that the patient had traveled to Colombia on June 9 to join her husband, an epidemiologist temporarily in Colombia to observe the 1971-72 dengue outbreak. On June 10, for approximately 6 hours, the patient visited the small community of Buena Vista, Cordoba, which was experiencing an outbreak of suspected dengue. The remainder of her stay in Colombia was spent in areas known to be free of *Aedes aegypti*.

Note: Although sporadic endemic dengue cases have been recognized in Puerto Rico since the 1969 epidemic, it is almost certain that this traveler was infected while in Colombia. Endemic activity in Puerto Rico during the past year appears to be limited to the southwest part of the Island, and the patient had not traveled to this region for several months.

Acknowledgments

The above studies have been carried out in collaboration with the Puerto Rico Health Department; the Arbovirology Section, Virology Branch, Laboratory Division, Center for Disease Control; and Epidemic Intelligence Service Officer; and the University of Puerto Rico (Mayaguez Campus) Entomological Pioneering Research Laboratory.

(B.L. Cline)

Table I

AEDES AEGYPTI LARVAL SURVEY IN GUANICA-ENSENADA, PUERTO RICO (AUG-NOV. 1972)

SUBDIVISION	NO. HOUSES EXAMINED				PERCENT HOUSES POSITIVE FOR LARVAE			
	1ST. SURVEY	2ND SURVEY	3RD. SURVEY	4TH. SURVEY	1ST. SURVEY	2ND. SURVEY	3RD. SURVEY	4TH. SURVEY
GUANICA (1)	70	81	83	78	12.8	9.8	28.9	12.8
ENSENADA (2)	67	50	47	43	23.8	28.0	40.4	13.9
GUAYPAO (3)	26	25	25	25	19.2	24.0	52.0	4.0
MONTALBA (4)	38	38	38	35	23.6	21.0	28.9	11.4
COLINAS (5)	26	26	28	27	30.7	11.5	39.2	7.4
LA JOYA (6)	29	29	28	27	20.6	17.2	17.9	3.7
	256	249	249	235	20.7	17.8	33.3	10.2

Table 2

SUMMARY OF CONTAINERS WITH *AEDES AEGYPTI* LARVAE IN GUANICA-ENSENADA, PUERTO RICO. (AUG-NOV. 1972)

TYPE OF CONTAINER	1ST. SURVEY		2ND. SURVEY		3RD. SURVEY		4TH. SURVEY	
	NO. POS.	% OF TOTAL	NO. POS.	% OF TOTAL	NO. POS.	% OF TOTAL	NO. POS.	% OF TOTAL
50 GALLON BARRELS	22	35.4	29	35.9	18	14.2	8	21.0
1-5 GALLON CANS	12	19.4	12	14.8	20	15.7	7	18.4
ANIMAL WATERING PANS	10	16.2	4	4.9	18	14.1	4	10.5
OLD TIRES	6	9.6	16	19.8	27	21.2	7	18.4
BUCKETS	4	6.4	8	9.8	14	11.2	2	5.2
MISCELLANEOUS	8	13.0	12	14.8	30	23.6	10	26.5
	62		81		127		38	

TABLE 3. SUMMARY OF CONTAINERS WITH AEDES AEGYPTI LARVAE FROM 96 MATCHED PREMISES BEFORE AND AFTER TREATMENT (2ND AND 3RD SURVEYS).
GUANICA-ENSENADA, PUERTO RICO
SEPT.-OCT. 1972

TYPE OF CONTAINER	NUMBER OF POSITIVE CONTAINERS	
	2ND SURVEY	3RD SURVEY
50 GAL. BARRELS	16	0
1-5 GAL. CANS	7	5
ANIMAL WATERING PANS	1	6
OLD TIRES	9	6
BUCKETS	4	4
MISC.	1	6
	38	27

TABLE 4. SUMMARY OF CONTAINERS WITH AEDES AEGYPTI LARVAE FROM 134 MATCHED, UNTREATED PREMISES OF THE 2ND AND 3RD SURVEYS.
GUANICA-ENSENADA, PUERTO RICO
SEPT.-OCT. 1972.

TYPE OF CONTAINER	NUMBER OF POSITIVE CONTAINERS	
	2ND. SURVEY	3RD SURVEY
50 GAL. BARRELS	13	16
1-5 GAL. CANS	9	15
ANIMAL WATERING PANS	3	12
OLD TIRES	7	18
BUCKETS	1	9
MISC.	3	12
	36	82

FIG. 1

FOCI OF CONFIRMED DENGUE-2 IN

PUERTO RICO DURING 1970 THRU FEBRUARY 1973

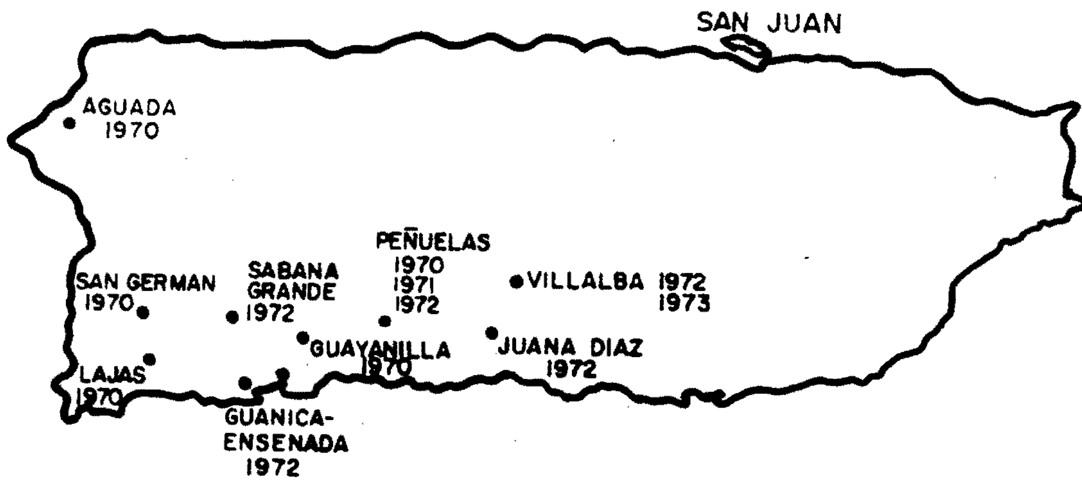


FIG. 2

FEBRILE ILLNESS ATTACK RATES, BY DATE OF ONSET

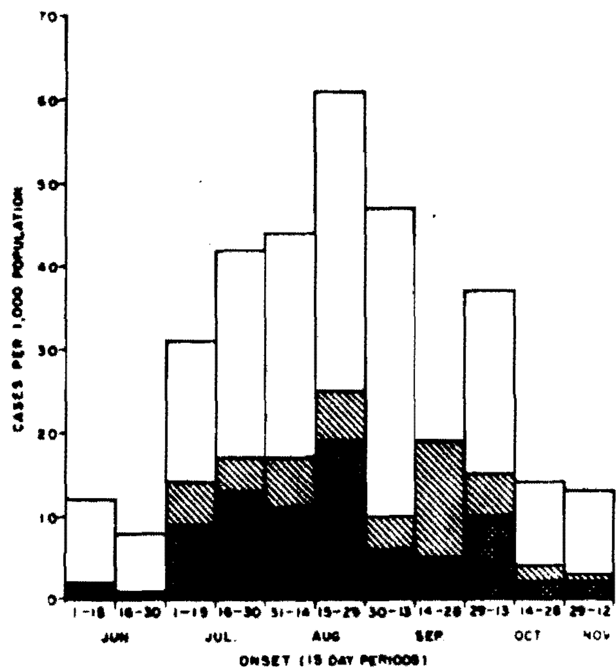
GUANICA-ENSENADA, PUERTO RICO

JUNE 1 - NOV. 12, 1972

All Febrile Illness ☐

With Rash ▨

With Rash and Body Pain ■



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

Arbovirus vector ecology studies in Mexico: 1972-1973

VE occurred in epizoodemic form in the State of Durango in 1971. In June and July 1972 virus vector studies were conducted in the State of Durango, Chihuahua and Tamaulipas, Mexico. The summary of mosquitoes collected in June in the State of Durango is presented in Table 1, those collected in Chihuahua, in Table 2, and the mosquitoes collected in Tamaulipas in July are presented in Table 3. Approximately 50,000 mosquitoes were tested for arboviruses. Viruses isolated included VEE, WEE, SLE, Turlock, Flanders, Trivittatus and a member of the Bunyamwera Group. These are the first reports of SLE virus from mosquitoes in Mexico, and of the isolation of a California Group virus (Trivittatus), and of WEE, Turlock and Flanders viruses in Mexico.

Apparently only a low level of VE virus transmission to equines occurred in Durango, probably due to the scarcity of mosquitoes in the affected area. The causative agent was confirmed by the isolation of the epidemic variant of VE virus from two pools of Anopheles p. pseudopunctipennis (Theo.), and from one symptomatic equine, a horse with high fever. The low mosquito population and the low incidence of equine cases observed, together with the absence of reports of human disease due to VE from the outbreak area, suggested an enzootic situation. It is postulated that VE virus persistence from one epidemic period to another may occur in a low-level mosquito-equine transmission cycle in a similar manner.

Mosquito collections made in 1972 in Durango, Chihuahua and Tamaulipas (areas which had been affected by the epidemic of VE in 1971) failed to reveal evidence of VE virus persistence.

A cooperative Mexico-USA arbovirus field study was started in January, 1973 to investigate the ecology of VE between epidemics in Mexico.

Mosquitoes were collected at several potential study sites. VE virus was isolated from Culiseta inornata collected in a wooded area near Xochomilco, Mexico on January 18, 1973. The strain isolated was pathogenic for guinea pigs; definitive subtyping has not yet been done. Equine cases were reported in the same general vicinity in late November 1972. This isolation of VE virus during an inter-epidemic period is of considerable interest since C. inornata is not generally present until cool weather sets in. Collection of domestic and small wild mammal specimens and further mosquito captures are presently underway in the Xochomilco area.

(W.D. Sudia, V.F. Newhouse, CDC; L. Fernandez, R. Sainz, Mexico)

Arbovirus studies, Evans County (Claxton), Georgia

Previous virus-vector studies in south Georgia (Waycross) conducted by the Arbovirology Section indicated the presence of three California group viruses (LaCrosse (LAC), Trivittatus (TVT), and Keystone (KEY). KEY was most often isolated from mosquitoes.

In 1972 studies were initiated in Evans County, approximately 50 miles west of Savannah, with the ultimate goal to define the role, if any, of KEY virus in human febrile illness. Preliminary results indicated a high prevalence of California infection; of 102 sera collected during the period 1962-71 by Dr. Curtis Hames, 68 (67%) contained N antibodies to one or more California viruses tested. 14 of the 102 sera were positive for KEY antibodies but not for TVT or LAC (Table 5).

In August, 1972, mosquitoes and wild mammals were collected in Evans County. The mosquito species captured are shown in Table 6. From 4687 mosquitoes, 23 virus isolations were made, all KEY virus. 21 isolations were from *Aedes atlanticus*-*tormentor*, and 1 each from *A. infirmatus* and *A. canadensis*. No virus isolations were made from 50 small wild mammals and 2 reptiles.

A prospective study of humans with febrile "viral" illness was conducted between August-November, 1972. Specimens for virus isolation were received from 49 patients; no isolations were made by ic inoculation of SM. Serologic studies are incomplete.

(Dr. C. Hames, Claxton, Ga. and K. Murphy, S. Bowen, W.D. Sudia, V.F. Newhouse, C. Calisher and T. Monath, CDC)

Infection and transmission of VEE-IB virus by various mosquito species

A great many different species of mosquitoes have been found infected in the field, but field infection does not necessarily indicate their true vector potential. This is best determined by laboratory transmission studies. Unfortunately, most of the species incriminated in the field have not yet been colonized and are only seasonally available for experimental use. The current studies were conducted with colonized species presently at hand.

Mosquitoes were infected by feeding them on 3-4 day old suckling mice inoculated with approximately 1000-3000 $SMicLD_{50}$ of VEE virus (subtype IB, strain GJ9-1BJ). The time of mosquito feeding upon the inoculated mice was judged by a pre-determined viremia curve so that expected peak viremia (approximately 10^9 $SMicLD_{50}$/0.02 ml blood) would be reached just prior to feeding.

The "infected" mosquitoes were incubated at 80°C and transmissions to normal suckling mice attempted at 7, 14, 21 and 28 days incubation. Individual mosquitoes were fed upon individual suckling mice secured to a tongue blade in a gauze wrap. Mosquitoes which were observed to feed were immediately killed by freezing and tested for virus to confirm that they actually were infected. Mice upon which they fed were code-marked, returned to their mothers and checked 3 times daily for the following 2 days and once a day thereafter for a two-week period. Brains of mice which died were passed to confirm the specificity of death.

Results of studies completed to date using available colonized mosquitoes are presented in Table 7. Of interest is the high rate of infection of An. quadrimaculatus but its poor vector efficiency as measured by the transmission experiments. For C. tarsalis (a mosquito common in areas of the United States potentially susceptible to incursions of VE), the infection rate declined with time, although the percentage of surviving mosquitoes able to transmit virus increased.

(V.F. Newhouse, D.L. Miller)

Experimental infection of Rhesus monkeys with VEE Type I, subtypes A-E

Five subtypes of VEE Type I virus are currently recognized, although the antigenic distinctions as determined by short-incubation HI test now appear to be less clear than originally described by Young & Johnson, 1969. Subtypes IA, B, and C have been associated with epizoodemic disease, are transmitted by various Aedes, Anopheles and Mansonia spp., and are highly virulent for humans and equines. Subtypes ID and E, in contrast, are enzoodemic strains, are transmitted in nature by Culex (Melanoconion) mosquitoes, and are generally non-pathogenic for equines. Human CNS disease caused by an endemic tropical strain (ID, 3880) has been described and is analagous to the sporadic clinical infections with the Everglades strain (VEE Type II), endemic in Florida and also generally non-pathogenic.

Experimental infection of rhesus monkeys was undertaken in order to compare the clinical, virological and serological responses to epizoodemic and enzoodemic VEE subtypes. Two young adult Macaca mulatta monkeys with no preexisting group A antibodies were inoculated with each of the following viruses:

Type	Subtype	Strain	Passage	Inoculum ($SMicLD_{50}$)
I	A	Trinidad	GP_1 $Vero_2$	16,000
I	B	PTF-39	SM_1 $Vero_2$	20,000
I	C	P676	SM_1 $Vero_2$	160,000
I	D	3880	SM_3 $Vero_3$	1,600
I	E	Mena II	SM_3 $Vero_2$	20,000

Monkeys were bled daily for 10 days and at intervals thereafter. Pharyngeal swabs were obtained and expressed into 1 ml 0.75% bovalbumin in PBS containing antibiotics. Rectal temperatures were recorded, and clinical examinations were performed by a veterinarian.

Figure 1 shows the relationship of viremia and pharyngeal virus excretion to febrile response and total white cell count in animals infected with each subtype. All animals developed high viremias (approx. 9.0 dex/ml), beginning on the first post-inoculation (PI) day and ending on day 4-6.

Febrile responses were uniformly recorded in monkeys infected with the epizoodemic subtypes but not with VEE ID or IE. The incubation period varied from 2 to 6 days, and was shorter for IA and IB than for IC infected monkeys (despite a ten-fold higher inoculum in the latter). Febrile responses generally appeared as the viremia declined or shortly after viremia disappeared, suggesting that pyrogenic substances different from the virus itself was responsible for fever. In this regard it may be significant that leukopenia also occurred at the time of disappearance of virus and appearance of fever. Leukopenia was, however, demonstrated in some VEE ID and IE infected animals despite absence of fever.

Clinical manifestations in IA, IB, and IC infected monkeys coincided with fever, and included anorexia, irritability, lethargy, and loose stools. Results of biochemical studies (SGOT, SGPT, and amylase) are pending.

Sera collected at varying intervals were tested for N antibodies. N antibodies were first detectable 13 days PI at low titer.

In order to investigate the ability of rhesus antisera to differentiate VEE Type I subtypes, serum dilution plaque reduction N tests and short incubation HI tests were performed. The HI tests were also done using rhesus antisera fractionated by sucrose gradient centrifugation into IgM and IgG components. The results of N tests are shown in Table 8. By this method, a close relationship between the three epizootic subtypes is demonstrated; they are clearly differentiated from VEE ID and IE. Subtype IE antiserum reacts specifically with the homologous virus, and thus appears to be antigenically distinct.

Short incubation HI tests (Tables 9, 10) provided similar information. However, by this test, subtype IC could be distinguished from the IA-IB complex.

Monkeys were challenged with 5000 LD_{50} VEE IB on day 112 PI. No viremia or fevers were observed.

(T. Monath, C.H. Calisher, M. Davis and G. Sather)

Table 1
Summary of Mosquitoes collected in the
State of Durango, Mexico, June, 1972, tested for arboviruses

Mosquito Species	Barranca	Cienega	Charcon	Leona Vicario	Gomez Palacio	Delila	Parras de la Fuenta	Santa Rosa	TOTAL
Aedes									
angustivittatus	80(80)*	76(76)	420(420)	95(95)		20,841(227)	1210(13)	4267(48)	26,989(959)
dorsalis									
nigromaculis	1(1)		1(1)			654(32)	1224(50)	24(4)	1,904(88)
sollicitans					1(1)				1(1)
vexans	15(15)	2(2)	8(8)	5(5)	737(17)	12,185(501)	137(6)	1145(50)	14,234(604)
sp.									
Anopheles									
franciscanus						1(1)			1(1)
pseudopunctipennis	21(21)	33(33)	72(72)	20(20)		1(1)	2(1)		149(148)
punctipennis						80(10)		19(4)	99(14)
quadrimaculatus						3(1)			3(1)
Culex									
peus				2(2)	3(1)			2(1)	7(4)
quinquefasciatus					35(3)				35(3)
salinarius				14(14)	1(1)	255(16)	1(1)	10(4)	281(36)
tarsalis		2(2)	10(10)	5(5)	10(2)	967(48)	781(33)	365(18)	2,140(118)
spp.			1(1)	15(15)					16(16)
C. (Mel.) sp.							1(1)		1(1)
Culiseta									
inornata								1(1)	1(1)
Psorophora									
ciliata									
confinnis	1(1)	7(7)	1(1)						9(9)
cyanescens									
discolor									
signipennis	1(1)	9(9)	1(1)	10(10)		18(8)	338(15)		377(44)
Total	119(119)	129(129)	514(514)	166(166)	787(25)	35,005(845)	3694(120)	5833(130)	46,247(2048)
Trap Nights	7	9	18	18	10	6	6	6	80
Ave. Mosq./Trap Night	17	14	29	9	79	5,834	616	972	578

*No. Mosquitoes/No. pools tested.

Table 2

Summary of mosquitoes collected in the State of Chihuahua, Mexico, June, 1972, tested for arboviruses

Species	San Diego	Julimes	Total
Aedes			
angustivittatus	409 (19)*	5 (2)	414 (21)
dorsalis	11 (1)		11 (1)
sollicitans		1 (1)	1 (1)
vexans	1185 (50)	217 (10)	1402 (60)
sp.	3 (1)		3 (1)
Anopheles			
pseudopunctipennis	12 (2)	18 (1)	30 (3)
punctipennis	40 (2)	5 (1)	45 (3)
Culex			
peus	1 (1)		1 (1)
salinarius	27 (3)	1 (1)	28 (4)
tarsalis	195 (10)	90 (5)	285 (15)
spp.	2 (1)		2 (1)
Psorophora			
ciliata	3 (1)		3 (1)
confinnis	88 (4)	1 (1)	89 (5)
cyanescens	3 (2)	1 (1)	4 (3)
discolor	5 (1)		5 (1)
signipennis	159 (8)	8 (1)	167 (9)
Total	2143 (106)	347 (24)	2490 (130)
Trap Nights	16	12	28
Ave. Mosq./Trap Night	134	29	89

*No. Mosquitoes/No. pools tested.

Table 3

Sumary of
Mosquitoes collected at Palo Blanco, Tamaulipas, Mexico, July, 1972, and tested for arboviruses with negative results

Mosquito Species	7/21/72
A. sollicitans	46 (3)*
taeniorhynchus	1 (1)
An. albimanus	6 (1)
crucians	15 (1)
quadrimaculatus	14 (1)
p. pseudopunctipennis	15 (1)
C. coronator	12 (1)
salinarius	1 (1)
tarsalis	1 (1)
thriambus	2 (1)
C. (Mel.) sp.	102 (6)
P. confinnis	749 (32)
discolor	7 (1)
U. lowii	2 (1)
Total	973 (52)
Trap Nights	31
Ave. Mosq./Trap Night	31

*No. mosquitoes/No. pools tested.

Table 4
Summary of arboviruses isolated from mosquitoes collected in the States of Durango and Chihuahua, Mexico, June, 1972.

Mosquito Species	Durango						Chihuahua
	Cienega	Santa Rosa	Parras de la Fuente	Dalila	Gomez Palacio	San Diego	Julimes
Aedes augustivittatus		2 Buny Gp., 1 Calif. Gp.					
Aedes vexans				1 SLE			
Anopheles p. pseudopunctipennis	2 VEE*						
Culex tarsalis		4 SLE	7 SLE	8 SLE, 1 SLE-1 Turlock*	1 Flanders	1 WEE	1 Flanders
Total isolations (30)	2	7	7	11	1	1	1

*Mixed infection.
*VEE = Venezuelan equine encephalitis virus, Buny Gp. = Bunyamera Group virus, Calif. Gp. = California Group virus, SLE = St. Louis encephalitis virus, Turlock = Turlock virus, Flanders = Flanders virus, and Unid. = virus as yet unidentified.

Table 5

Results of plaque N test in BHK using 1:5 diluted serum and approximately 100 PFU California viruses, adult humans,* Evans County, Georgia

KEY **	TVT	LAC -	LAC +
-	-	34	1
	+		
+	-	14	9
	+	1	43

	No. Tested	No. (%) POSITIVE KEY	LAC	TVT	TOTAL
TOTAL	102	67 (66%)	53 (52%)	44 (43%)	68 (67%)

*Sera collected by Dr. Hames, Claxton, Georgia 1962-71.

** KEY= Keystone virus, TVT= Trivittatus, LAC= LaCrosse

Table 6

Mosquitoes collected in Claxton, Georgia during August 8-11, 1972

Species	Total (Pools)
Aedes aegypti	6 (2)
atl-tor.	2531 (63)
canadensis	336 (11)
dupreei	33 (5)
fulvus pallens	238 (14)
infirmatus	18 (3)
triseriatus	40 (10)
vexans	66 (11)
Anopheles crucians	145 (10)
punctipennis	77 (6)
quadrimaculatus	12 (5)
Culex nigripalpus	297 (16)
quinquefasciatus	76 (5)
restuans	2 (1)
salinarius	51 (8)
territans	1 (1)
C. (*Mel.*) sp.	217 (12)
Culiseta melanura	172 (7)
Mansonia perturbans	193 (12)
Orthopodomyia signifera	1 (1)
Psorophora ferox	163 (13)
Uranotaenia sapphirina	12 (6)
Total	4687 (222)
TN	58
Avg/TN	81

*No. mosquitoes tested/No. Pools tested.

Table 7
Infection and Transmission of GJ9-1BJ (IB) Virus by Mosquitoes

Species	C. tarsalis		An. quadrimaculatus		A. triseriatus		A. aegypti		An. albimanus	
Days of Incubation	% Inf	% Trans	% Inf	% Trans	% Inf	% Trans	% Inf	% Trans	% Inf	% Trans
7	85%	10%	88%	3%	100%	36%	100%	79%	100%	INC**
14	71%	29%	68%	11%	100%	74%	100%	86%	INC	INC
21	45%	40%	87%	7%	100%	100%	100%	79%	N.S.	N.S.
28	N.S.*	N.S.	93%	8%	100%	100%	100%	85%	N.S.	N.S.

* No surviving mosquitoes

** Not yet completed

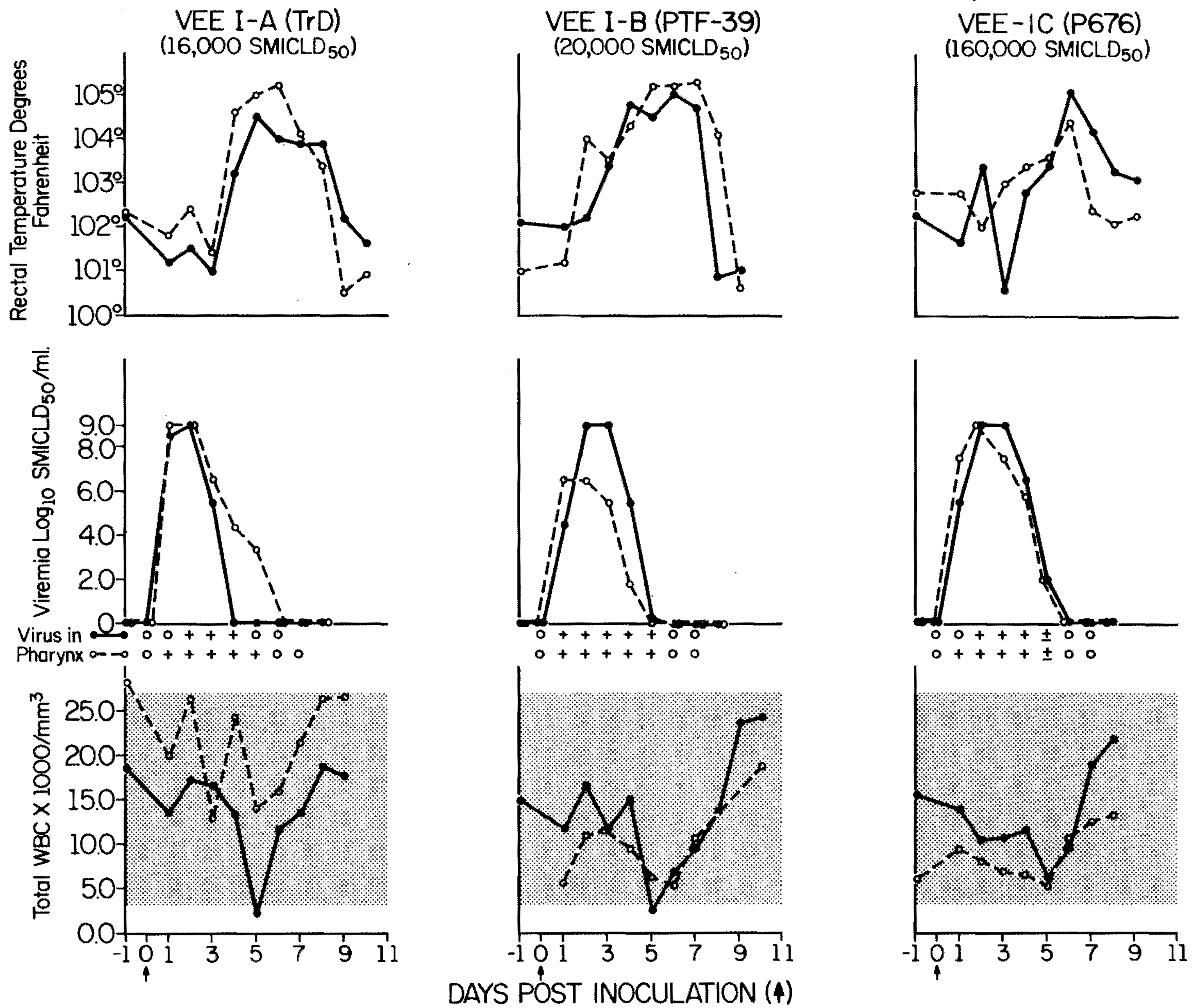


Figure 1

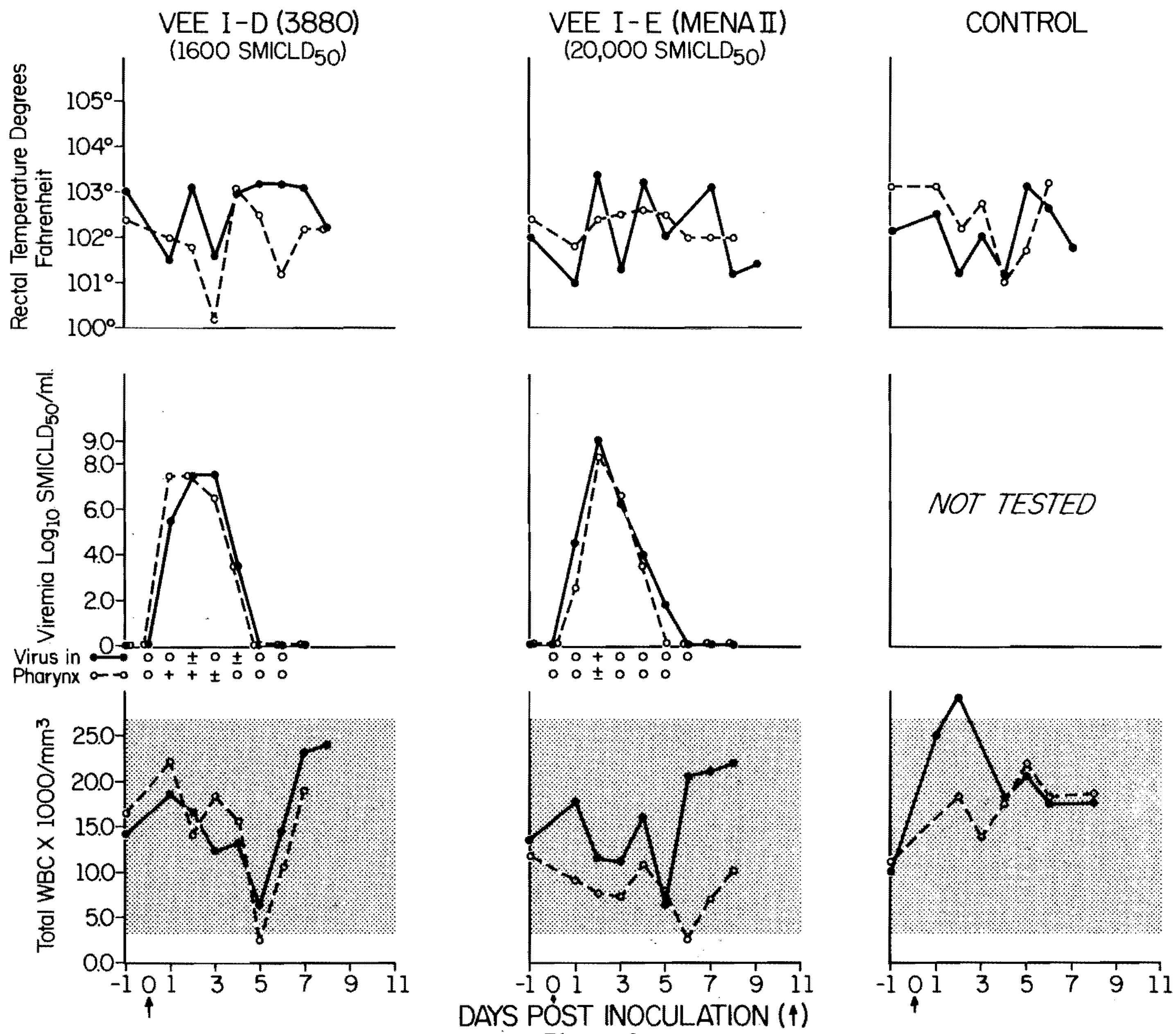


Figure 2

Table 8
Comparison of VEE Subtypes by Serum Dilution Neutralization Test in DECC Using Rhesus Antisera (Day 45 P.I.)

Antiserum	Virus IA (TrD)	IB (PTF-39)	IC (P676)	ID (3880)	IE (Mena II)
IA (TrD)	64*	64	32	4	4
IB (PTF-39)	128	128	64	8	4
IC (P676)	64	128	256	32	8
ID (3880)	16	4	16	16	16
IE (Mena II)	8	<4	4	<4	32

*Highest dilution of serum inhibiting ≥90% plaques (constant virus dose ≃ 100 PFU).
Tests performed without fresh serum factor.

Table 9
Results of VEE Short Incubation period HI tests using rhesus monkey whole serums.

Antiserum	Dilution	One Hour Reading Units of Indicated VEE Antigen Inhibited							
		IA	IB	IC	ID	IE	II	III	IV
IA	1:640	32	32	4	<4	<4	16	<4	8
IB	1:320	128	128	<4	<4	<4	4	<4	8
IC	1:80	64	128	512	4	4	32	16	4
ID	1:160	16	32	<4	16	4	8	32	8
IE	1:40	8	32	<4	<4	16	16	16	8

Table 10
Results of VEE short incubation period HI tests using rhesus monkey Ig fractions.

Serum		Dilution	One Hour Reading Units of Antigen Inhibited							
			IA	IB	IC	ID	IE	II	III	IV
(IgM)	IA		4	4	<4	<8	<8	<8	<4	<4
	IB		16	8	<4	<8	<8	<8	<4	<4
	IC		8	8	16	<8	<8	<8	<4	<4
	ID		8	8	<4	8	<8	<8	8	<4
	IE		8	8	<4	16	8	<4	16	<4
(IgG)	IA	1:64	64	32	16	<8	<8	8	8	<8
	IB	1:128	32	32	16	<8	<8	<8	<4	<8
	IC	1:64	8	8	256	<8	<8	<8	<4	<8
	ID	1:64	64	64	16	64	<8	16	64	<8
	IE	1:32	<8	<8	<8	<8	16	<8	<4	<8

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

Subtype I, variety B, Venezuelan encephalitis (VE) virus first appeared north of South America in Guatemala during the equine epizootic-epidemic of 1969. To search for persisting virus in Guatemala, 28 strains were recovered from various parts of the country in 1970 by exposure of sentinel hamsters and 20 strains were isolated from mosquitoes on the Pacific coast; in 1971, an additional 28 strains were obtained from sentinel hamsters exposed on the Pacific coast. These strains are currently under analysis to learn whether they are the pre-outbreak, enzootic, subtype I, variety E, or epizootic subtype I, variety B.

Investigations of possible periodic VE virus movement from the endemic Avellana marsh habitat on the Pacific coast of Guatemala were begun in 1971. The basic approach is to attempt to demonstrate seasonal changes in the presence of VE virus at various distances from the marsh which would suggest waves of virus movement. To begin the study, sentinel hamsters and sentinel horses were placed at selected locations between the coastal marsh and the inland foothills. During July and August 1971, VE virus could be demonstrated only two kilometers inland from the marsh by use of sentinel hamsters, but a sentinel horse became infected in August as far as 13 kilometers inland. During the winter of 1971-1972, additional horses developed VE virus antibodies at other locations including one 14 kilometers north and inland from the marsh in February 1972. These first experiments were designed only to give a general idea of geographic and seasonal distribution of VE virus in the region, and obviously more detailed and precise documentation of seasonal fluctuations of virus in appropriate micro-habitats are now needed.

As a baseline for studying the possible role of bats in the ecology of VE virus at Avellana, Guatemala, bats and terrestrial mammals were collected in 1971 for antibody and viremia tests which are under way. These studies included sera from dogs and humans. In addition to group C (Nepuyo) and group Patois (Patois and Zegla) viruses, at least two new, as yet unidentified viruses were isolated by use of sentinel hamsters on the Pacific coast of Guatemala during 1970 and 1971.

Mosquitoes and sentinel hamster specimens collected in the Amazon region of Peru east of the Andes during September 1970-1971 have so far yielded VE virus (nine strains), Eastern encephalitis (two strains), group C arboviruses (nine strains) and viruses not in arbovirus groups A, B or C (five strains). The VE viruses seem to be in subtype I, but their varieties have not yet been determined; they are virulent for adult hamsters and adult mice inoculated peripherally. One strain is peculiar in that it is a VE virus by CF test, but is not neutralized well by subtypes I, II, III or IV VE virus antisera. The group C arboviruses so far identified are Oriboca-Itaqui (one), Caraparu-Ossa (five) and probably Marituba (three). Antibody studies of equine sera in 1970 revealed no evidence of VE virus activity in the southern dry coast of Peru nor did sentinel hamsters yield evidence of current activity in Tumbes on the coast of northern Peru, just south of Ecuador.

Studies of virulence of VE viruses have characterized the homogeneity and stability of four strains which represent the spectrum from fully virulent to avirulent for hamsters. This was done by examining 100 individual clones from each strain. Two strains were homogeneous on this basis, one had 2% of clones with different virulence from the parental virus, and the attenuated TC83 vaccine strain although over 99% homogeneous, was unstable since hamster-virulent virions could easily be selected from parental virus by passage in hamsters. Clearance rates of virulent and benign VE viruses for hamsters revealed that three hamster-lethal strains of subtype I, one of subtype II and two of subtype III were cleared slowly from hamster plasmas during 90 minutes whereas three benign strains (two of subtype IV and the TC83 vaccine strain) were cleared rapidly.

VE viremia occurred in some young indigenous Guatemalan bovines after subcutaneous inoculation of two strains of subtype I, variety B virus, but not after inoculation of one strain of subtype I, variety E virus. Hemagglutination-inhibiting and neutralizing antibodies appeared in serum taken one to three weeks after infection. Bovines remained healthy except for transient fevers in those with viremia or virus in the throat.

A survey for dengue virus neutralizing antibodies in human sera from the Caribbean coast of Mexico, Guatemala, British Honduras and Honduras, revealed that antibodies to type 3 virus were present in the older age groups on the Caribbean coast of these countries and on the Pacific coast of Guatemala. These data suggested that dengue virus, possibly type 3, was active there some years ago, but that there has been probably little, if any, recent activity. Consequently, there are significantly large susceptible human populations there.

(W.F. Scherer)

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

The arbovirus surveillance established in Arizona, Colorado, Louisiana, New Mexico, Oklahoma, and Texas (Arbovirus Information Exchange 23:13, 1972) was continued through November 1972. The surveillance was undertaken because of the widespread concern regarding the potential for renewed and expanded Venezuelan equine encephalitis (VEE) virus activity within the United States. In addition to collections of mosquitoes and other biting flies for virus testing, VEE-susceptible burros were used as "sentinel" animals.

Periodic visits were made to 41 study areas through November 1972. None of 177 blood specimens from the sentinel burros was positive for virus or indicated serologic conversion for VEE antibody; however, three of the sentinel burros (10 percent) had diagnostic rises in titer for WEE antibody that provided evidence of infection with that Group A arbovirus. Viruses were isolated from mosquitoes collected in each of the six states and included western equine encephalitis (WEE), St. Louis, Turlock, Hart Park, California and Bunyamwera Group arboviruses; no VEE virus was isolated (Table 1). Nine virus strains were isolated from one species of biting gnat, Culicoides variipennis, but none was VEE virus; whereas, two strains of VEE virus had been isolated in our laboratory from C. arubae collected in Texas during the 1971 outbreak.

The current surveillance for arboviruses provided timely evidence that VEE virus was not active in these major river areas during the 1972 season, it provided a service to the states that they were unable to undertake, and it provided insight into the widespread potential of future WEE epizootics and epidemics.

The finding of high VEE antibody prevalence among Lepus californicus (Blacktail Jackrabbit) in Texas during 1971 (Arbovirus Information Exchange 23:13, 1972) indicated that they may serve as vertebrate hosts for this virus. Laboratory studies have been completed to determine the levels of viremia and the serologic response of this widely distributed rabbit species. Virus was circulating in the blood by 24 hours after inoculation and was present on days 1 and 2 in all jackrabbits inoculated (Table 2). Blood samples tested concurrently in both suckling mice and duck embryo cell culture demonstrated the marked difference in sensitivity of the two assay systems. The results indicate that L. californicus could serve as infective host for feeding mosquitoes, although the low viremia titers are indicative that it probably would not be an efficient host.

The hemagglutination-inhibition (HI) antibody results summarized in Table 3 show that the jackrabbits had good antibody responses and that the titers persisted for at least 8 weeks after infection.

Studies have been initiated to determine susceptibility levels in common mosquito species of the western U.S. to a Texas epizootic strain of VEE virus. Table 4 summarizes data from Culex tarsalis reared from field collected pupae and fed on pledget suspensions of virus. Mosquitoes were refed on suckling mice after 18 days extrinsic incubation, and although three of the positive mosquitoes ingesting the highest concentration of virus fed on suckling mice, no virus was transmitted. Testing of other mosquito species and studies comparing pledget and viremic animals as a virus source for mosquitoes are currently underway. The results suggest that C. tarsalis are not particularly susceptible to the VEE Texas equine isolate, presumably 1B strain, used in these studies.

Viremia patterns from SLE virus-inoculated Passer domesticus (House Sparrow) nestlings, 1 to 10 days-old, reared from immune and nonimmune parent birds, demonstrated marked difference (Tables 5 and 6). Although viremia was a consistent result of inoculation of nestlings from nonimmune parents, the level of viremia was not great. There was a marked difference in survival through 20 days between nestlings from immune and nonimmune parents. Comparable survival of diluent-inoculated nestlings from nonimmune parents (38%) and virus-inoculated nestlings from immune parents (50%) indicates that the poor survival of virus-inoculated nestlings from nonimmune parents was due at least in part to the virus. Further evidence of this deleterious effect of the virus was obtained by the isolations from nestling sparrow brains. The persistence of virus in the brain for 20 and 24 days, and the high levels of virus present in brain harvests are of considerable interest. The results indicate that although nestling house sparrows have sufficient viremia to infect mosquitoes, they are probably not of major importance as amplifying hosts for SLE virus under most circumstances. This hypothesis is based upon the low levels of viremia detected, the observed poor survival of nestlings from nonimmune parents, and the high levels of virus recovered from the brains of dead nestlings. The high mortality rate among inoculated nestlings also may help explain the relatively low prevalence of SLE antibody usually found among adult house sparrows.

Forty Colorado tick fever (CTF) cases were diagnosed using the fluorescent-antibody (FA) procedure on 65 blood specimens submitted by private physicians and the Colorado Department of Health. Among the diagnosed cases was a single fatality in a 10-year-old female from Colorado Springs. This is only the second recorded death due to CTF virus. The continuing studies of confirmed CTF cases have lead to the realization that the clinical spectrum of this disease may be much broader than generally surmised.

A second year of field trials using seed treated with Ornitrol as a chemosterilant for house sparrows (Passer domesticus) was completed in Hale County, Texas. Comparison of embryonated eggs in nests at the treated and the untreated sites along with other indices revealed no effect of the Ornitrol. In an aviary colony of house sparrows used as a control, the feeding of treated seed inhibited reproduction during the period of the field studies. Inspection of the feed residue composed of seed hulls left in the feeders at the treated sites suggested an explanation for the failure to reduce reproduction in the house sparrows. The hulls from "shelled" seeds caught below the feeder were seen to be covered with crystals of the Ornitrol; thus, the chemosterilant probably was not ingested in sufficient quantities by the birds to be effective. Sparrows in the treated area were observed to regularly use the feeders containing the treated seed and the consumption of seed at the untreated and treated sites was similar. The sparrows in the treated area undoubtedly utilized alternative food sources; whereas, the aviary birds had no other food source. This may explain why the treated seed was effective in suppressing reproduction only in the aviary colony.

(R.O. Hayes and D.B. Francy)

TABLE 1.

Arbovirus isolations from mosquitoes by state from April through November 1972.

State	No. Mosq. Tested	No. Pools Tested	WEE	Turl.	SLE	Bun. Group.	CEV	Other*	Totals
Arizona	37,877	734	20	16	5		1	21	63
Colorado	8,556	413	12	3	1			7	23
Louisiana	2,462	46						2	2
New Mexico	44,442	970	63	12	3	2		37	117
Oklahoma	2,428	120		1				1	2
Texas	66,298	1,675	43	4	1	3	2	29	82
Totals	162,063	3,958	138	36	10	5	3	97	289

* These are not Group A arboviruses; preliminary identification tests indicate that many of these are Bunyamwera, California Group, B Group, and Hart Park arboviruses.

TABLE 2

Viremia in *Lepus californicus* subcutaneously inoculated with VEE virus

Animal Number	Test System	Days Post Inoculation			
		1	2	3	4
LC 954	DECC[a]	1.2[b]	<0.5	<0.5	<0.5
	SM	3.7	1.7	<0.5	<0.5
LC 956	DECC	<0.5	<0.5	<0.5	<0.5
	SM	1.7	3.1	4.7	<0.5
LC 960	DECC	1.3	0.8	<0.5	<0.5
	SM	4.8	NT[c]	1.7	<0.5
LC 964	DECC	2.1	1.5	<0.5	<0.5
	SM	5.0	3.8	<0.5	<0.5
LC 965	DECC	1.1	0.8	0.5	<0.5
	SM	3.7	1.9	2.7	<0.5
LC 969 (Control)	SM	<0.5	<0.5	<0.5	<0.5

a - DECC, primary duck embryo cell culture; SM, suckling mice.

b - Log_{10}PFU or $SMICLD_{50}$/0.1 ml blood.

c - NT, not tested

TABLE 3

Hemagglutination-inhibition antibody response in *Lepus californicus* inoculated subcutaneously with VEE virus.

Animal Number	Days Post Inoculation					
	0	7	14	21	28	56
LC 954	<10	20	160	160	160	80
LC 956	<10	80	160	160	160	160
LC 960	<10	160	320	320	5120	-
LC 965	<10	20	40	160	160	320
LC 969 (Control)	<10	<10	<10	<10	<10	-

TABLE 4

Summary of infection and transmission studies with wild *Culex tarsalis* and VEE virus[a]

Dilution Fed	Amount Virus Ingested/0.02 ml	No. Mosq. Tested	No. Mosq. Positive[b]	No. Transmissions/ No. Positive Mosq. Refeeding
10^{-2}	$10^{7.8}$[c]	22	10(45%)	0/3
10^{-3}	$10^{6.5}$	12	2(17%)	0/1
10^{-4}	$10^{5.5}$	19	2(11%)	0/2
10^{-5}	$10^{4.5}$	14	0(0%)	-

a Mosquitoes fed for approximately 1 hour on cotton pledgets saturated with each virus dilution in defibrinated hamster blood.

b Tested individually in suckling mice after 18 days extrinsic incubation.

c Based on titration in suckling mice of an aliquot of each suspension frozen at time of mosquito feedings.

TABLE 5

Viremia in nestling Passer domesticus reared from SLE immune parents following subcutaneous inoculation with SLE virus.

Day Post Inoculation	Log_{10}PFU/0.1 ml blood		
	<0.7	Trace[a]	0.7 - 1.7
1	8[b]	1	
2	3		2
3	1	1	4
4	6		2
5	4	2	
6	3	2	
7	5	2	
8	2		1
9	4	1	
Totals	36	9	9

a - A single plaque in 1 of 2 bottles inoculated with a 1:5 blood dilution.

b - Total number of blood samples tested from all nestlings bled on each day post-inoculation.

TABLE 6

Viremia in nestling *Passer domesticus* reared from SLE non-immune parents following subcutaneous inoculation with SLE virus.

Day Post Inoculation	Log_{10}PFU/0.1 ml blood						
	<0.7	Trace[a]	0.7-1.7	1.8-2.7	2.8-3.7	3.8-4.7	Ave. Mean Geometric Titer
1	5[b]		5	5			1.1
2	1			5	7	2	2.8
3	1			4	6	2	2.9
4			2	2	5	1	2.7
5	1		2	4	3		2.0
6		1	3	1	1	2	1.9
7	3		2	2			1.0
8	1	1	5				0.9
9	4		1				<0.7
Totals	16	2	20	23	22	7	

a - A single plaque in 1 of 2 bottles inoculated with a 1:5 blood dilution.

b - Total number of blood samples tested from all nestlings bled on each day post-inoculation.

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

Extensive surveillance to detect the possible introduction of Venezuelan equine encephalomyelitis virus into California and to monitor activity of the known endemic arboviruses was carried out during 1972 in collaboration with other units of the Department; the Schools of Public Health of the University of California, Berkeley and Los Angeles; the University of California, Davis; the State Department of Agriculture; the U.S. Department of Agriculture; county health departments; mosquito abatement districts; the Center for Disease Control; and various other agencies.

At least 851 persons in the State suspected of having an arbovirus infection were screened serologically by this laboratory or the four county laboratories which have begun arbovirus testing. There were two human cases of Venezuelan encephalitis infection, a 22-year-old man from Santa Cruz, California, exposed in Nayarit and Jalisco States, Mexico (onset August 9) and an entomologist from U.C.L.A. exposed in Sinaloa State, Mexico (onset August 24). There were five human cases of St. Louis encephalitis: a 25-year-old man from San Diego County (onset July 14) who was exposed either in Yuma, Arizona, Imperial County or eastern San Diego County, or in Peru or Brazil; an 11-year-old boy from Tehama County (onset September 13); a 25-year-old man from Yolo County (onset September 14); a 55-year-old man from Tulare County (onset September 22); and a 17-year-old boy from Kern County (onset October 5). There were three human cases of western encephalitis: a 4-month-old girl from Madera County (onset September 8); a 14-year-old boy from Fresno County (onset September 8); and a 35-year-old man from Fresno County (onset September 10). All cases recovered and no serious sequelae were reported. Cases were confirmed serologically by CF, HAI, neutralization tests and indirect fluorescent antibody tests. The value and specificity of the IFA test and a metabolic inhibition test in cell cultures has been further confirmed in our studies and sometimes can prove the diagnosis where CF or HAI tests fail to show a rising antibody titer.

There were three cases of dengue detected (undoubtedly there were additional unsuspected cases in travelers): a 20-year-old woman from Santa Cruz exposed in Somoa (onset January 13, 1972); a 50-year-old woman from Marin County, exposed in Haiti (onset January 19, 1972); and a 29-year-old woman from Los Angeles, exposed in Southeast Asia (onset July 27). The CDC and Walter Reed laboratories assisted in identifying these cases.

There were 68 clinically suspected cases of encephalitis in equines reported to the Department in 1972, but vaccination or earlier naturally-acquired antibodies prevented a definitive diagnosis in all but one case (Kern County, onset July 24). Tissue samples from 31 equines were tested in suckling mice, with negative results. In addition, tissue samples from 65 bats, 7 cats, 8 skunks, 2 cows, 9 squirrels, 2 racoons, 5 foxes, 1 dog, 1 chipmunk, 2 weasels and 13 sentinel hamsters were tested for arboviruses with negative results.

Serologic sampling by metabolic inhibition test for WEE and VEE antibodies, of 283 rodents of other mammals trapped in San Diego, Los Angeles, Imperial, Riverside, Stanislaus and Colusa counties, revealed only low titers (1:4 to 1:8) of antibody in a few: 2 *Neotoma lepida* from San Diego County, May 11, and June 14, and 10 *Sigmodon hispidus* in Yuma, Arizona or Imperial County, June 14 through August 10, had WEE antibody. Three *Rattus norvegicus* in Colusa County, September 21, had WEE titers and two of these also had 1:4 VEE titers, but the significance of this is uncertain.

A total of 6,336 mosquito pools (over 194,737 individuals) were tested in suckling mice or duck embryo cell cultures, yielding 180 viruses, as shown by county and month in the attached table. Direct fluorescent antibody staining was of great value in rapidly identifying viral isolates.

There were seven cases of Colorado tick fever identified during 1972: a 33-year-old man in Modoc County exposed in Gerlach, Nevada (onset March 18); a 57-year-old man in Napa County, exposed in Nevada (onset May 4); a 34-year-old man in San Francisco, exposed in Idaho (onset May 16); a 56-year-old man in Marin County, exposure unknown (onset May 20); a 72-year-old woman in Sacramento, exposed in Lassen or Modoc counties (onset June 5); a 48-year-old man in Sacramento, exposed in Lassen County (onset July 8); and a 62-year-old woman in Mono County exposed near home (onset August 23). Direct fluorescent antibody staining of blood clots was used to rapidly identify the cases, subsequently confirmed by virus isolation and IFA serologic tests.

There were 116 tick pools tested in suckling mice for arboviruses, including *Ixodes pacificus*, *I. signatus*, *I. uriae*, *Haemaphysalis leporis-palustris*, *Ornithodoros coriaceous*, *Dermacentor occidentalis*, *D. parumapertus*, and *D. variabilis*. The only virus isolates have been three agents suspected to be in serogroup B or the Kemerovo-Chenuda-Mono Lake group, from *Ixodes uriae* ticks collected by Dr. Harald N. Johnson from Flat Iron Rock off the coast of Humboldt County, California, June 22, 1972.

(R.W. Emmons)

Viruses Isolated from Mosquitoes by the Viral and Rickettsial Disease Laboratory,
California State Department of Public Health, by County and Month of Collection
1972

County	June	July	August	September	October*
Colusa		SLE (1) Turlock (1)	SLE (8) Unid (7) Turlock (2)	WEE (1)	
Fresno		Turlock (1)		WEE (3)	
Imperial	SLE (8) WEE (3) Turlock (6)	SLE (10) WEE (8)	SLE (2) WEE (1)	WEE (2) SLE (8) Turlock (2)	SLE (1) Turlock (3)
Kern		Turlock (3)	WEE (2)		
Kings		WEE (1)			
Los Angeles			Turlock (1)		
Madera		WEE (2)	WEE (10) SLE (1) Turlock (3)	WEE (1)	WEE (2) SLE (1)
Merced	Unid (1)	Unid (1)	Turlock (2) Unid (1)		
Modoc	Unid (1)				
Placer			Turlock (1)	Turlock (1) Unid (1)	
Plumas			Turlock (1)		
Riverside	WEE (1)		SLE (1)		
Sacramento		SLE (1)			
San Diego		Unid (2)	Turlock (2) Unid (2)	SLE (1)	
Shasta		SLE (1)	SLE (2) Turlock (1)		
Siskiyou			Turlock (1) Unid (1)		
Stanislaus		Turlock (2) Unid (2)	Turlock (5)		
Sutter		Turlock (1)	Unid (1)	WEE (1)	
Tehama	Turlock (2)	Unid (2)	SLE (6)	SLE (4) Unid (1)	
Tulare			SLE (2) WEE (2) Turlock (2) Unid (1)	WEE (1)	SLE (1)
Yolo	Turlock (1) Unid (2)		SLE (1)	Turlock (1)	SLE (1)
Yuma, Arizona	Turlock (2) WEE (1)		SLE (1)	SLE (2)	
TOTALS	SLE (8) WEE (5) Turlock (11) Unident. (4)	SLE (13) WEE (11) Turlock (8) Unident. (7)	SLE (24) WEE (15) Turlock (21) Unident. (13)	SLE (15) WEE (9) Turlock (4) Unident. (2)	SLE (4) WEE (2) Turlock (3)

* In addition, (1) Turlock in November from Imperial County. Results of additional tests on unidentified viruses pending (WEE, SLE, and Turlock viruses ruled out).

REPORT FROM THE ANIMAL PATHOLOGY DIVISION, HEALTH OF ANIMALS BRANCH, AGRICULTURE CANADA, SACKVILLE, NEW BRUNSWICK, CANADA

Isolation of eastern encephalitis virus in Canada

During September, 1972, several cases of eastern equine encephalomyelitis occurred in Brome and Compton counties in the Eastern Townships region in the Province of Quebec. These laboratory-confirmed cases followed earlier reports of cases suspected by veterinarians on the basis of clinical findings. They constituted the first laboratory-confirmed equine cases diagnosed in Canada in many years. A total of five equine cases were confirmed by isolation of virus from the brain. These five cases were presented between September 19 and 28 and all exhibited encephalitis to varying degrees. One animal was coincidentally positive on agar-gel precipitation test for equine infectious anemia, but all cases reacted negatively on our CF test for EEE.

No conclusion has yet been reached with respect to the source of the infection, although on the basis of epidemiological reports, it is believed to have occurred as an extension from bordering States of the northeastern region of the U.S.A.

Since the diagnosis, attempts are being made to uncover serological evidence in other area horses and it is planned to extend these studies to include HI tests.

Arbovirus surveillance in the Atlantic area

During the summer of 1972, mosquitoes were light-trapped and netted for the purpose of monitoring possible virus activity. The area chosen is on the border between salt marsh and upland topography, and sites representative of each were chosen for collecting.

At the same time, a sentinel chicken flock was maintained on the salt marsh location and serum samples were collected at two-week intervals throughout the season.

Examinations on mosquitoes and serum are presently underway.

(D.P. Gray)

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

The Marsh Lake 23 strain of California encephalitis (CE) virus (Snowshoe Hare subtype) which was isolated from Aedes canadensis mosquitoes collected near Whitehorse, Yukon Territory, Canada (61°N, 135°W) during July 1971 replicated both in salivary glands and thoraces of A. canadensis and Culiseta inornata mosquitoes (collected wild in the Yukon during summer 1972), following intrathoracic inoculation and incubation at temperatures of 80°, 50° and 40°F.

Aedes aegypti mosquitoes, bred in a laboratory at the University campus, transmitted Marsh Lake 23 virus by biting newborn mice after 20 and 24 days of extrinsic incubation at 80°F, following intrathoracic injection of 300 mouse LD_{50} of virus. No transmission was observed between 4 and 18 days after injection, or after 28 and 40 days, although all mosquitoes contained infective virus. At 55°F, both salivary glands and thoraces of all mosquitoes tested between 12 and 34 days after injection yielded virus, but transmission by biting upon mice was observed only after 26 days of extrinsic incubation.

After mosquitoes inbibed 15 mouse LD_{50} of Marsh Lake 23 virus whilst feeding on newborn mice rendered viremic by intracerebral injection immediately beforehand, salivary glands and thoraces of all mosquitoes contained infective virus after 14 to 27 days' extrinsic incubation at 80°F, but salivary glands from only one of 4 mosquitoes contained virus at 7 days. At 27 days, 1 of 7 mosquitoes transmitted virus by biting upon mice. At 55°F, infectivity was first detected in salivary glands at 21 days, but by 28 days 3 of 3 mosquitoes transmitted virus by biting upon mice.

We had already demonstrated that mouse-adapted dengue-1 (Hawaii) and dengue-4 (H 241) viruses were transmitted by biting upon mice two weeks after intrathoracic injection and incubation at 80°F, but that the rate of viral replication was reduced after incubation at 70°F and 55°F. We now report the ability of A. aegypti bred at U.B.C. to transmit zero-passage dengue viruses after different temperatures of incubation. Mosquitoes were injected with 1 mouse LD_{50} of dengue-2 virus present in serum from patients with clinical dengue, and with two strains of dengue-2 virus after one intrathoracic passage in mosquitoes.

At 75°F, all four dengue-2 strains were transmitted to mice 10 to 14 days after mosquitoes were inoculated. Although virus was not detected in salivary glands at 3 and 6 days, by 10 days all mosquitoes contained infectivity both in salivary glands and thoraces.

At 90°F and 95°F, infectivity was demonstrated in salivary glands as early as 3 days after injection.

At 55°F, mosquitoes injected with a Pacific isolate after one mosquito passage transmitted virus by biting mice after 25 days of extrinsic incubation, but infectivity was detected 13 days after injection with a Caribbean isolate after one mosquito passage.

(D.M. McLean)

COMMENT FROM THE EDITOR

Many thanks for your continued cooperation in helping to maintain the high standards of the Info-Exchange. Contributions for the next issue (Number 25) will be due September 1, 1973.

The quality of copy submitted has been exceptionally good. I respectfully request, however, that contributions be in English, as translating service is generally not available to me. Also, tables should be of such dimensions that they can conveniently be reproduced on the size of page used in the Info Exchange. Please bear in mind that tables and graphs are photographically reproduced as submitted and of necessity must be clear copy, black on white, to come through in readable condition.

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