



# ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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*Dengue "myocarditis"  
- Philippines*

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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.

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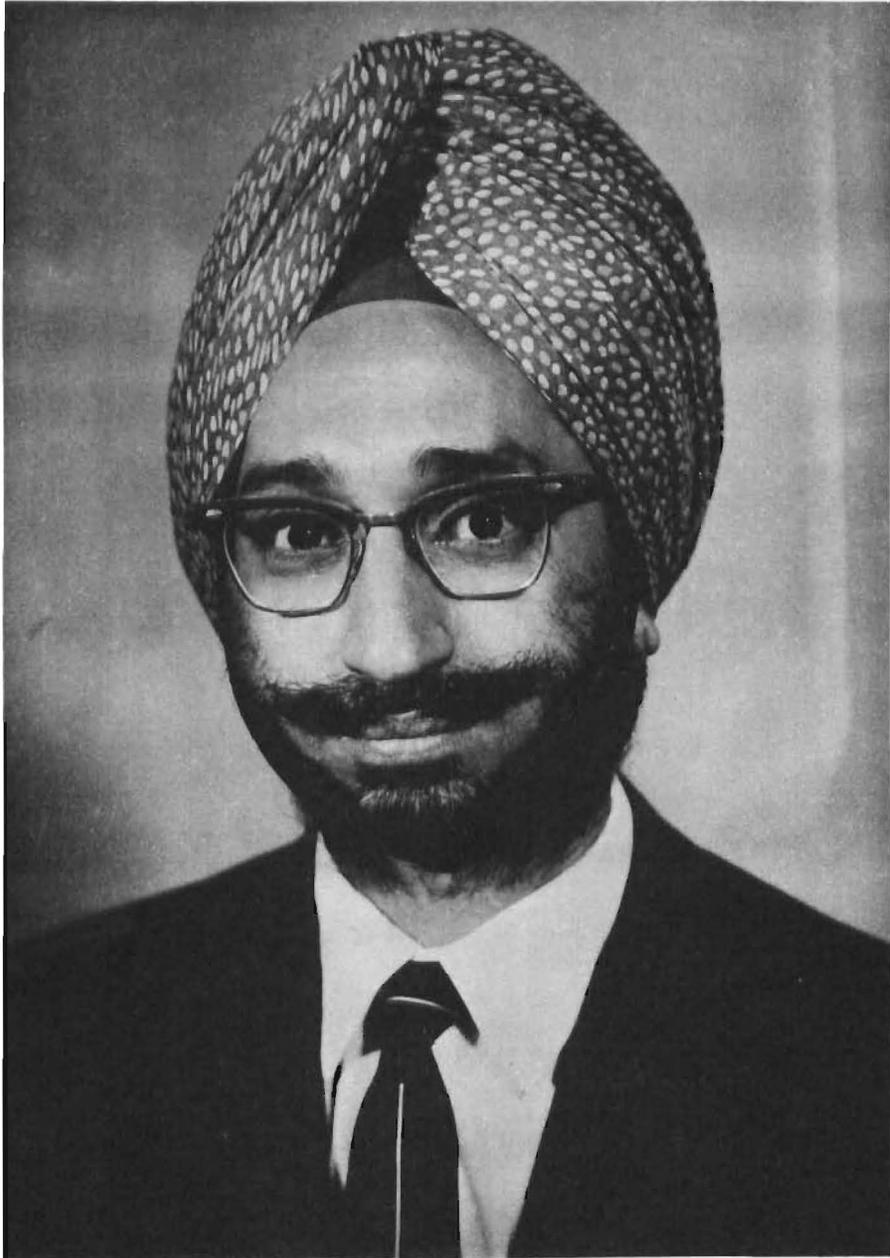
COMMENTS FROM THE EDITOR

Several recipients of the Arthropod-borne Virus Information Exchange have informed me that attempts to contact particular contributors have failed, that their letters were returned because of incomplete or incorrect address. To alleviate this condition in the future a complete mailing address will be included for at least one contributor in each report, either as a complete-address report heading (when brief enough) or as an addendum at the end of the report.

Also, I have had several of my pre-publication reminder letters returned because the individuals to whom they were addressed had apparently moved to some other location. In such cases I have no recourse but to drop them from the mailing list. If you transfer, please inform me promptly and indicate whether or not you will still have need for the Info-Exchange in your new assignment.

Reports for the spring issue (No. 34) will be due March 1, 1978. Please mark your calendars. The address:

Roy W. Chamberlain, Editor  
Arthropod-borne Virus Information Exchange  
Virology Division  
Center for Disease Control, Atlanta, Georgia 30333, U.S.A.



Dr. K. R. P. Singh

April 22, 1930 - May 20, 1977

Dr. K. R. P. Singh, Deputy Director, Vector Control Research Centre, passed away on May 20, 1977, at the Command Hospital, Poona, India. Dr. Singh had a distinguished academic record. He obtained an M. Sc. degree in the first class from the University of Agra, India, in 1952, and a Ph.D. from the University of Western Ontario, Canada, in 1956 under the supervision of Dr. A. W. A. Brown. He worked for some time in the U.S.A.; then he joined the Virus Research Centre, Poona, India, in 1959. There he worked with many problems pertaining to vectors of virus diseases, and distinguished himself as one of India's leading medical entomologists. His investigations on the vectors of dengue, chikungunya and Kyasanur Forest Disease viruses were outstanding.

His greatest contribution to science in general and arbovirology in particular was the establishment of technics for initiation and propagation of cell lines from mosquito tissue using a medium developed by Mitsuhashi and Maramorosch in 1964. A relevant portion from the 1967 annual report of the Virus Research Centre is reproduced here.

"The development of mosquito cell lines has been an ambition of many workers and the success obtained by Grace in Australia in developing such cell lines of Aedes aegypti reported in 1966 had created great interest. However, the technical difficulty in connection with the Grace's cell lines was the need of hemolymph of a moth in the growth and maintenance medium. Attempts made a year ago at the Virus Research Centre to obtain such hemolymph from the Tassar moth (Anthera paphia) has not been very encouraging. Using a medium without an insect hemolymph developed by Japanese workers for the growth of other insect tissues, it has now been possible at this Centre to develop and establish several cell lines from mosquito tissues, both from Aedes albopictus and Aedes aegypti. Not only have these cell lines grown exceedingly well but it has also been possible to grow several arboviruses in them. Preliminary studies have indicated that the mosquito cell lines now developed are quite susceptible to mosquito-borne viruses but not to tick-borne viruses tested so far. The development of these cell lines using a medium which can be easily prepared from commercially available ingredients has opened a new vista for the study of arboviruses and has been acclaimed as a major breakthrough."

Prior to his experiments to establish mosquito cell cultures, Dr. Singh, under a Rockefeller Foundation travel grant, visited various laboratories in different countries where work on insect tissue culture was being done. Although financial support for his work came from the Indian Council of Medical Research and the Rockefeller Foundation, it was Dr. T. Ramachandra Rao and Dr. C. R. Anderson, then Director and Research Director of Virus Research Centre who provided the constant encouragement and support which led Dr. Singh to persist in spite of past history of failures on the part of those who had previously tried to establish insect tissue culture cell lines.

Dr. Singh joined the WHO - ICMR Unit on Genetic Control of Mosquitos (now the Vector Control Research Centre) in 1970, and was engaged in mass production of sterile male mosquitos for mosquito control processes. He was later deputed to Mauritius through the F.A.O. for studies on the genetic control of Stomoxys. On return to India, he was taken ill and his condition deteriorated rapidly. He is survived by his wife, a daughter and a son.

Pravin N. Bhatt  
Sonja M. Buckley

Yale University School of Medicine  
New Haven, Connecticut

REPORT FROM THE ARBOVIRUS RESEARCH UNIT, EGYPTIAN ORGANIZATION FOR BIOLOGICAL AND VACCINE PRODUCTION, AGOUZA, CAIRO, AND MEDICAL ZOOLOGY DEPARTMENT, NAMRU-3, AMERICAN EMBASSY, CAIRO, EGYPT

Serological survey of Egyptian mammal sera for three tickborne viruses of public health importance.

A total of 1,174 sera from humans (433) and domestic mammals (741) was examined by the CF test for indications of 3 tickborne viruses, Crimean-Congo hemorrhagic fever (CCHF), Dugbe (DUG), and Tettnang (TET) (TABLE 1), which have never (CCHF, DUG) or only once (TET) been reported from Egypt. Dogs were from the Cairo pound; equines (donkey, horse, mule) from a large animal hospital in Cairo; all other mammals from abattoirs in the cities listed.

Antibodies to each of the 3 viruses were detected in sera from cattle in Wadi Natroun and Qena and from sheep and camels from Cairo. In addition, antibodies to CCHF virus were detected in sera of a human from Upper Egypt and a buffalo from Port Said; to DUG virus in sera from buffaloes from Cairo and Port Said, sheep from Alexandria, and dogs from Cairo; and to TET virus in sera from sheep from Alexandria. The percentages of positive reactions in all sera were 1.87 (CCHF), 2.98 (DUG), and 2.39 (TET).

Egypt is geographically situated between many CCHF foci in Eurasia and Africa, where 25 species and subspecies of ticks have been incriminated as CCHF virus vectors. Six of these vector species are common members of the Egyptian tick fauna (Hyalomma anatolicum anatolicum, H. marginatum rufipes, H. impeltatum, Rhipicephalus sanguineus, R. turanicus, and Boophilus annulatus). During their spring and fall passages, northward and southward migrating birds transport ticks through Egypt from within the African and Eurasian ranges of CCHF virus. There is an urgent need to determine the public health importance of CCHF virus in Egypt.

DUG virus has been isolated in Senegal, Nigeria, Central African Republic, Cameroun, Uganda, and Ethiopia from several species in the genera Amblyomma, Hyalomma, Rhipicephalus, and Boophilus. Of these, H. marginatum rufipes, H. impeltatum, and B. annulatus are common in Egypt, A. variegatum and A. lepidum are introduced on camels and cattle and by migrating birds, and H. truncatum is present in southeastern coastal area. DUG virus has also been isolated from febrile humans in Nigeria and Central African Republic and from cattle, sheep, and goats. Antibodies to the virus were found in human sera (3.5%) in Ibadan, Nigeria. The wide distribution of DUG virus in western and eastern Africa, its implication as a cause of human disease, and the possibility of introducing infected ticks by migrating birds and on imported sheep, camels and cattle, stimulated us to investigate Egyptian sera for antibodies against DUG virus.

TET virus was first isolated in 1970 from Ixodes ricinus in West Germany and from a pool (Art 1147) of Hyalomma dromedarii from Baragil, Egypt. The possible relationship of this virus with tickborne meningo-polyneuritis, erythema chronicum migrans, and Lyme arthritis has been suggested. The isolation of TET virus from the rural environment of Egypt, where many farmers live close to tick-infested domestic animals, prompted us to include TET virus in this seroepidemiological survey.

These results point to the need for more intensive epidemiological investigations of the risk to human health presented by tickborne viruses in Egypt.

(Medhat Darwish, Imam Z. Imam, Harry Hoogstraal)

Table 1. Complement-fixing antibodies against CCHF, Dugbe, and Tettngang viruses

in sera from Egypt

Sera	Locality	No.	CCHF virus		DUG virus		TET virus	
			No. positive	(%)	No. positive	(%)	No. positive	(%)
Human	Upper and Lower Egypt	433	1	(0.23)	0		0	
Donkey	Cairo	50	0		0		1	
Donkey	Giza	45	0		0		0	
Horse	Cairo	15	0		0		0	
Mule	Cairo	7	0		0		0	
Pig	Cairo	46	0		0		0	
Pig	Alexandria	5	0		0		0	
Goat	Cairo	17	0		0		0	
Goat	Matruh	16	0		0		0	
Cow	Cairo	43	0		0		0	
Cow	Alexandria	36	0		0		0	
Cow	Matruh	7	0		0		0	
Cow	Port Said	20	0		0		0	
Cow	Wadi Natroun	21	3	(14.28)	3	(14.28)	1	(4.76)
Cow	Qena	15	2	(13.33)	2	(13.33)	1	(6.6)
Buffalo	Cairo	48	0		1	(2.08)	0	
Buffalo	Alexandria	35	0		0		0	
Buffalo	Port Said	32	1	(3.12)	5	(15.63)	0	
Buffalo	Qena	16	0		0		0	
Camel	Cairo	34	3	(8.82)	3	(8.82)	3	(8.82)
Sheep	Cairo	66	12	(18.18)	12	(18.18)	21	(31.81)
Sheep	Alexandria	41	0		5	(12.19)	1	(2.43)
Sheep	Matruh	44	0		0		0	
Sheep	Port Said	10	0		0		0	
Sheep	Wadi Natroun	23	0		0		0	
Dog	Cairo	49	0		4	(8.16)	0	
Total:		1,174	22	(1.87)	35	(2.98)	28	(2.39)

# REPORT FROM THE ARBOVIRUS LABORATORY

## INSTITUT PASTEUR and ORSTOM

DAKAR - Sénégal

During the first half of 1977, virological and serological studies were carried out on samples from Senegal : Dakar, Bandia and Kedougou.

Moreover, serological studies were performed on sera from Gabon.

### I. - Virological studies

#### 1. - Human blood samples

10 samples from febrile children at the clinic in Bandia were processed for virus isolation without success.

#### 2. - Wild vertebrate samples

a) 31 blood samples collected from monkeys caught in Kedougou were inoculated into suckling mice without success.

b) 38 blood samples from rodents caught in Bandia were inoculated by the same technic ; 3 Bandia virus strains were recovered from a Tatera sp., a Mastomys sp. and a Taterillus.

#### 3. - Arthropods

1487 mosquitoes caught in Kedougou during rainy season in 1976 were pooled in 71 samples and inoculated into suckling mice. Many virus strains were isolated :

- 1 wild strain of yellow fever virus from a pool of Aedes furcifer-taylori.

- 8 strains of Zika virus : from Aedes luteocephalus (4) and Aedes furcifer-taylori (4).

- 3 strains of Bunyamwera virus : from Aedes vittatus (1) and Aedes dalzieli (2).

- 1 strain of Simbu virus from Aedes vittatus.

The isolation of a wild strain of yellow fever virus in Kedougou agrees with what serological studies, carried out in blood samples from humans and monkeys in Kedougou, had previously

pointed out.

## II. - Serological studies

### 1. - Human sera

#### a) From Senegal :

21 serum specimens from Dakar hospitals were examined for arbovirus antibodies by HI, CF and neutralization tests. No specific diagnosis was possible.

#### b) From Gabon :

75 serum specimens from 1 to 10 years old children were studied by the HI test.

31 % reacted with the yellow fever virus antigen while 10 % reacted with some group B arbovirus antigens.

These results very probably indicate a reaction to vaccination against yellow fever virus. A second study, after the rainy season in 1977, will be useful to point out the incidence of group B arboviruses within this population.

### 2. - Wild vertebrate samples

12 sera from monkeys caught in Kedougou were tested for HI and CF antibodies :

- 1 serum showed a recent infection with Zika virus (HI and CF tests positive).

- 6 sera had HI antibodies for chikungunya virus with 3 having CF antibodies therefore indicating a rather recent infection.

- 5 sera had HI and CF antibodies for group B arboviruses.

- In one case, the CF test was positive at a low level (1/8), with yellow fever antigen.

## III. - Experimental research work

Two works were carried out in the arbovirus laboratory and are to be published :

1. - "Long term infection of a cell culture from newborn mouse brain with the FNV strain of yellow fever virus" (H. Fleury, C. Adam and G. Heme).

A cell culture from brains of one day old mice was infected with a high multiplicity of the French Neurotropic strain of yellow fever virus (FNV) ; the infected cell culture produced

and released infectious FNV for more than 180 days post-inoculation with titers between  $10^{0.6}$  and  $10^{6.4}$  PFU/ml. The cell sheet exhibited some rare plaques of round cells with a slow centrifugal extension. The destruction of the cell sheet was not complete before 200 days post-inoculation.

2. - Concentration of a wild strain of yellow fever virus by PEG "precipitation" method and infection of Aedes aegypti by the digestive route (H. Fleury, C. Adam, M. Cornet and M. Valade).

A wild strain of yellow fever virus was cultivated in PS-2 cells and concentrated with 7.5 %, 10 % and 12.5 % PEG 6000. Concentrations obtained were between x 64 and x 100 (1.8 to 2.0 log 10) and titers between 7.1 and 7.9 PFU/ml. A suspension of this PEG concentrated virus was successfully used for infection of Aedes aegypti by the digestive route.

(J. Renaudet, H. Fleury and Y. Robin, Institut Pasteur  
M. Cornet and J.L. Camicas, ORSTOM, Dakar.)

(The above workers may be contacted through: Dr. Y. Robin, Directeur, Institut Pasteur de Dakar, 36 Avenue Pasteur, Boite Postal 220, Dakar, Senegal)

REPORT FROM THE WORLD HEALTH ORGANIZATION, ARBOVIRUS VECTOR  
RESEARCH UNIT, P.O. BOX 104, ENUGU, NIGERIA

The WHO, Arbovirus Vector Research Unit (AVRU) based in Enugu, Nigeria continues its vector ecology/arbovirology research program in collaboration with the Virus Research Laboratory, University of Ibadan, Ibadan, Nigeria.

A total of 456 pools of wild caught mosquitoes, 80% of which are represented in 15 Aedes species, (52% being Ae. africanus), have been sent to Ibadan for virus isolation attempts. Of 12 Flaviviruses isolated to date, from the study area at the Mamu River Forest Reserve, 10 have been identified by CF as follows: two isolates from pools of Ae. aegypti have been confirmed as dengue 1; and eight others from Ae. africanus as "Potiskum virus." Characterization and identification of the remaining isolates are pending (Table 1).

This is the first reported isolation of Potiskum virus (unregistered) from mosquitoes. Previously the virus was isolated in 1966 by the Ibadan laboratory from the town of Potiskum, Nigeria, from the Gambian giant rat, Cricetomys gambianus. Additional isolates in 1970 were made from the Multimammate rat, Mastomys natalensis and the Dark-bellied unstriped grass mouse, Arvicanthis niloticus from Kwara, Nigeria. Serologically, Potiskum is related to West Nile, Uganda S and Usutu viruses. None of these viruses have been isolated from Ae. africanus.

In addition, preliminary serological findings by CF tests from three of four sentinel green monkeys, Cercopithecus aethiops placed in a fresh water swamp forest canopy, shows antibody conversions to Bunyamwera, Zika and Potiskum. (Personal communications: Professor A. Fabiyi, Virus Research Laboratory, Ibadan).

Y. H. Bang, D. N. Bown, A. B. Knudsen, and B. Dobrokhotov.

Table 1: Flaviviruses isolated from mosquitoes pools collected at Mamu River Forest Reserve (May-October 1976).

Pool No.	Species	Pool size	Viruses No.	Locality	Method of Collection	Date Coll.	Ident.
46	<i>Ae. aegypti</i>	100	AR90388	Mamu River	Human-bait	27/5/76	Pending
65	<i>Ae. africanus</i>	83	AR90934	Swamp F.	Human-bait	15/6/76	Potiskum
81	<i>Ae. africanus</i>		AR90900	" "	" "	8/7/76	"
81	<i>Ae. africanus</i>	212	AR90994	" "	" "	8/7/76	"
81	<i>Ae. africanus</i>		AR90996	" "	" "	8/7/76	"
98	<i>Ae. africanus</i>	50	AR91295	" "	" "	21/7/76	"
98	<i>Ae. africanus</i>		AR91297	" "	" "	21/7/76	"
99	<i>Ae. africanus</i>	80	AR91300	" "	" "	21/7/76	"
99	<i>Ae. africanus</i>		AR91305	" "	" "	21/7/76	"
167	<i>Ae. aegypti</i>	46	AR92039	Clear fell	" "	16/9/76	dengue 1
167	<i>Ae. aegypti</i>		AR92040	forest	" "	16/7/76	"
200	<i>Ae. africanus</i>	5	AR92501	Swamp F.	Monkey-bait	27/10/76	Pending

REPORT FROM THE PASTEUR INSTITUTE OF BANGUI  
P.O. BOX 923, BANGUI, CENTRAL AFRICAN EMPIRE

During 1976 the Pasteur Institute of Bangui has continued to monitor the circulation of arbovirus in Central Africa.

Further attempts have been made to isolate virus strains from Aedes africanus and Aedes opok, captured in the gallery forest of Bozo, some 110 km north of Bangui. Following the isolation of six strains of MARIL virus during September, October and November 1974, seven strains of CHIKUNGUNYA virus from June to September 1975, and then five strains of BUNYAMWERA virus between October, November and December 1975, an epizootic of ZIKA virus was recorded during June and July 1976 when 20 strains were isolated. During this period also serologic conversion of this virus was observed in a sentry monkey (cercopithecus aethiops) kept in the forest gallery of Bozo. All virus isolations from mosquito populations have coincided with the rainy season or soon afterwards. The time period of 2 - 3 months for each series of virus isolation corresponds to the life span of a mosquito generation.

Among mosquitoes captured in 1975, ORUNGO virus from a pool of Anopheles (Gambiae complex) collected at Bangoran, and GOMOKA virus from Aedes argenteopunctatus collected at Dokouma, were isolated and identified for the first time in Central Africa.

In addition, a strain of C.H.F. CONGO virus was isolated from a human patient. The symptoms of the illness resembled those of malaria, with fever (39 - 39,7° C) of three days' duration. The illness resulted from laboratory contamination, the patient having handled a strain of Congo virus five days previously.

The isolation of virus from ticks has continued. A total of 31 strains (22 DUGBE, 4 THOGOTO, 3 JOS and 2 CONGO) have so far been identified. Similarly, from rodents 2 strains of Ippy and 3 strains of SEBOKELE virus have been recorded.

(J. Fabre)

REPORT FROM THE VETERINARY RESEARCH LABORATORY,  
SALISBURY, RHODESIA

There has been progress in several of the projects mentioned in the first report from this laboratory which appeared in the March, 1976, issue of the information exchange bulletin.

Rift Valley fever (RVF) and Mazoe virus in murids

A study of RVF in murids was completed and an article on the subject has been submitted for publication. Brains, spleens and livers of 2212 murids, 27 shrews and 7 dormice trapped in 7 areas of Rhodesia, were tested in 277 pools for the presence of RVF virus by inoculation of infant mice. There were no isolations of RVF, but 69 isolates were obtained of an unidentified virus, tentatively designated Mazoe virus. Sixteen out of 867 sera had low titre, 1:20 to 1:40, haemagglutination-inhibition (HAI) antibody activity against RVF antigen, but only one serum out of 1260 had neutralising (NT) activity at a minimal titre of 1:8. (The HAI results are revised slightly from those reported in 1976). The evidence suggests that murids fail to encounter infection in nature and are unlikely to play a role in circulation and dissemination of RVF virus.

Four out of seven widely distributed species of murid, Rhabdomys pumilio, Saccostomys campestris, Aethomys chrysophilus and Lemniscomys griselda, were shown to be capable of circulating levels of virus likely to be infective for mosquito vectors. No demonstrable viraemia was produced in members of 32-chromosome and 36-chromosome populations of the ubiquitous multimammate mouse, Praomys (Mastomys) natalensis. Results obtained in experiments with A. chrysophilus and 32-chromosome P. natalensis are presented in Figures 1 and 2.

Mazoe virus remains unidentified. Sixty-five of the isolates of this virus came from P. natalensis, two from Iatera leucogaster and one each from R. pumilio and Rattus rattus alexandrinus. In addition, 18 isolates have been obtained from pathological specimens from domestic animals, mainly aborted cattle and sheep foetuses.

Other arbovirus isolates.

The virus designated Nyabira in our first report, was identified conclusively as a new member of the Palyam serogroup of arboviruses and a paper reporting isolation of the virus was published (Swanepoel and Blackburn, 1976). Nyabira is the first member of the serogroup to be isolated from a vertebrate and a total of nine isolates have been obtained from aborted cattle foetuses. Isolates came from widely separated areas of Rhodesia and NT antibodies are widely distributed in cattle sera. Diagnostic NT antibody responses have been demonstrated in cattle which aborted. Pathogenicity experiments are in progress. The virus does not appear to be pathogenic for non-pregnant sheep and cattle and the viraemias demonstrated have been of minimal intensity, virus being

demonstrable in whole blood only. Replication of virus has been demonstrated in Aedes aegypti mosquitoes and Culicoides zuluensis midges inoculated with virus or fed infective blood meals.

An isolate tentatively designated Marandellas virus (strain 1063/74) in our previous report, was found to be an alphavirus in mouse brain and cell cultures examined electron-microscopically by Dr. F.A. Murphy of CDC, Atlanta. Preparation of potent antigen and serum made it possible for us to identify the isolate as Ndumu virus. The isolate is the first from a vertebrate and was obtained from the organs of a bovine which died from disease said to resemble theileriosis. The significance of Ndumu infection in cattle has not been established.

No further isolates have been obtained of the unidentified virus designated Gwebi (strain 1220/74) in our previous report. The virus came from an aborted cattle foetus and remains unidentified as yet.

#### Specificity of flavivirus antibodies in cattle sera.

It was mentioned in our last report that HAI antibodies to Wesslabron (WSL) virus are common in the sera of cattle in Rhodesia and although the pathogenicity of the virus for sheep is well documented, evidence is lacking that it produces disease in cattle. As part of the investigation of the role which WSL plays in cattle in Rhodesia, it was necessary to examine the specificity of antibodies to WSL virus in cattle sera. The known flaviviruses of southern Africa comprise WSL, Spondweni (SPD), Uautu (USU), Banzi (BAN) and West Nile (WN). As a preliminary to tests with cattle sera, the cross-reactivity of these viruses was studied in HAI, CF and NT tests with sera of infected guinea pigs. Yellow fever (YF) virus was included as an additional check on the specificity of antibodies to individual flaviviruses. All viruses were adapted to Vero cells to allow quantitative NT tests to be performed in microcultures. Relationships among the southern African viruses were found to conform to the general pattern described for flaviviruses in that the guinea pig sera were broadly cross-reactive in HAI tests, less cross-reactive in CF tests and were virtually monospecific in NT tests.

Flavivirus antibody cross-reactions observed in the sera of calves, heifers and ewes infected with WSL virus in pathogenicity experiments, are presented in Figures 3, 4 and 5. In all instances heterologous antibody activity was of lower titre and more transient than homologous WSL activity. It can be seen that the HAI antibodies induced by WSL infection were broadly cross-reactive while heterologous NT activity was confined mainly to YF and BAN viruses. Only the ewes showed heterologous CF activity and this was confined to YF and BAN viruses. The net impression from these experiments is that there is fairly close antigenic affinity between WSL, BAN and YF viruses; an affinity which has been described by other authors.

The next step was to test and interpret antibodies in field sera. Over a period of 56 months HAI antibodies to WSL virus were detected in 13,7 per cent. (1400/10213) of cattle sera submitted routinely to the

laboratory. A proportion of the sera submitted during 1974 were selected for intensive flavivirus antibody study. 207 HAI-positive sera from 73 herds were selected to confirm that the HAI responses were due to WSL infection. A further 112 HAI-negative sera were selected from 8 of the same HAI-positive herds to determine the full extent of WSL infection in HAI-positive herds. In the final category, 90 sera were selected from 8 WSL HAI-negative herds to determine whether or not, and to what extent WSL infection had taken place in such herds.

Only one of the selected sera had a CF titre and this was a 1:8 reaction with WSL antigen only. None of the sera in the two categories which lacked WSL HAI antibody, had HAI antibodies to any of the other flaviviruses. The HAI and NT titres to flaviviruses determined in the field sera are summarised in histogram form in Figures 6, 7 and 8. The proportions of sera reacting with the various antigens and viruses are shown in Tables I and II together with geometric mean titres ( $\pm$  S.E.). The cross-reactions observed in individual HAI-positive sera are analysed in Table III.

In 289 of the 409 selected sera, the WSL NT titres were higher than titres to other flaviviruses. The discrepancy was generally large, with WSL titres equal to or greater than 1:4096 being recorded while the highest titre recorded with another virus was 1:128 with WN virus in two sera. In only 41 sera were flavivirus NT titres equal to or greater than WSL titres recorded, and these were mostly low titres of 1:4 to 1:16 with BAN or YF viruses in sera which lacked WSL NT antibody.

The results left no doubt that WSL has been the dominant flavivirus in cattle in Rhodesia in recent years. Extrapolation from the results indicated that about half (49,4 per cent.) of the cattle in breeding herds had experienced WSL infection. Interpretation of titres recorded in field sera in relation to titres observed in pathogenicity experiments, indicated that about one-sixth (12,2 per cent.) of the cattle became infected within a year prior to sampling. Monitoring of pregnant cattle in the field during 1976 produced information which confirms the above estimates of the immune rate and challenge rate in cattle.

Relating serological evidence of recent WSL infection to the clinical histories attached to field specimens, failed to produce convincing evidence that WSL produced abortion or disease in cattle. The virus was not isolated from 797 aborted fetuses examined over 56 months, or from 518 other specimens from diseased animals. Yet numerous other isolates of arboviruses were obtained.

Taking into account the findings in this study and unpublished information from elsewhere, it was concluded that WSL infection is not a significant cause of abortion or other disease of cattle in Rhodesia, despite its wide occurrence. The WSL study is to be submitted as a thesis for a higher degree by one of us (NKB) and will be submitted for formal publication.

#### Intranuclear immunofluorescence in RVF infected cells.

Eosinophilic intranuclear filaments in Rift Valley Fever infected cells, described previously by other authors, were shown to fluoresce specifically in an indirect technique with antiserum to the virus.

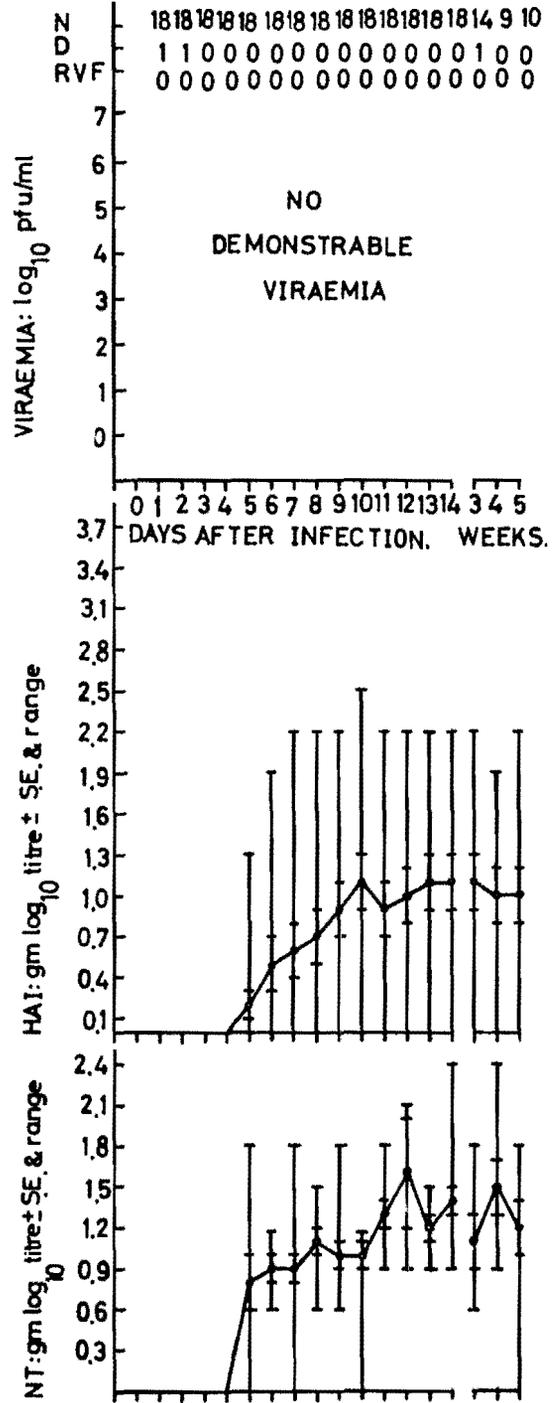
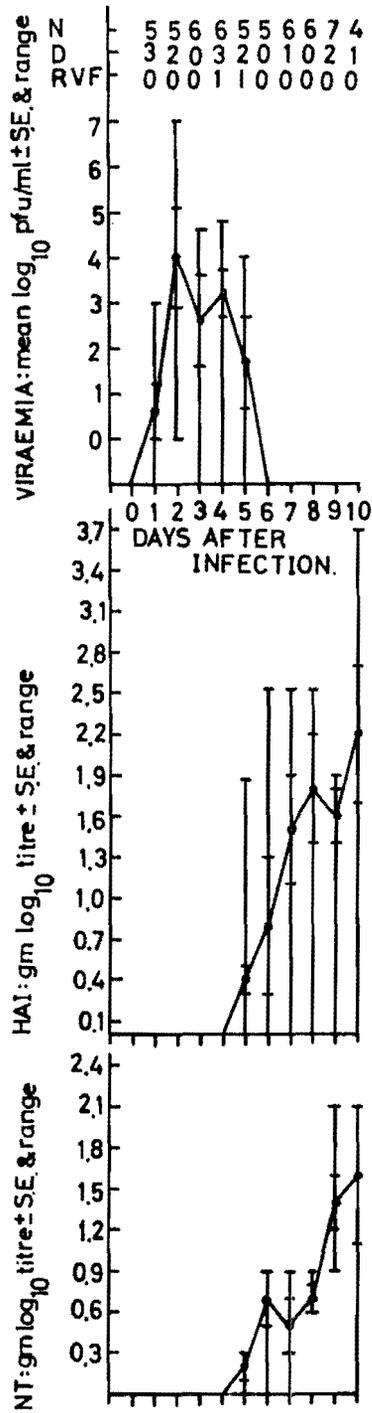
Actinomycin D failed to suppress development of the filaments or replication of the virus. We are not aware of the demonstration of virus-specified antigen in the nucleus with other Bunyaviruses and we are not certain whether this is a regular and obligatory feature of RVF replication. A note was published on the finding (Swanepoel and Blackburn, 1977).

#### References

- Swanepoel, R. and Blackburn, N.K. (1976) Vet Record, 99, 360.  
Swanepoel, R. and Blackburn, N.K. (1977) J. gen. Virol., 34, 557-561.

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Figures 1 and 2. RVF infection in *A. chrysophilus* and *P. natalensis*.  
 N=number killed for testing; D=total spontaneous deaths;  
 RVF=spontaneous deaths ascribed to RVF. Antibody curves show  
 geometric means.

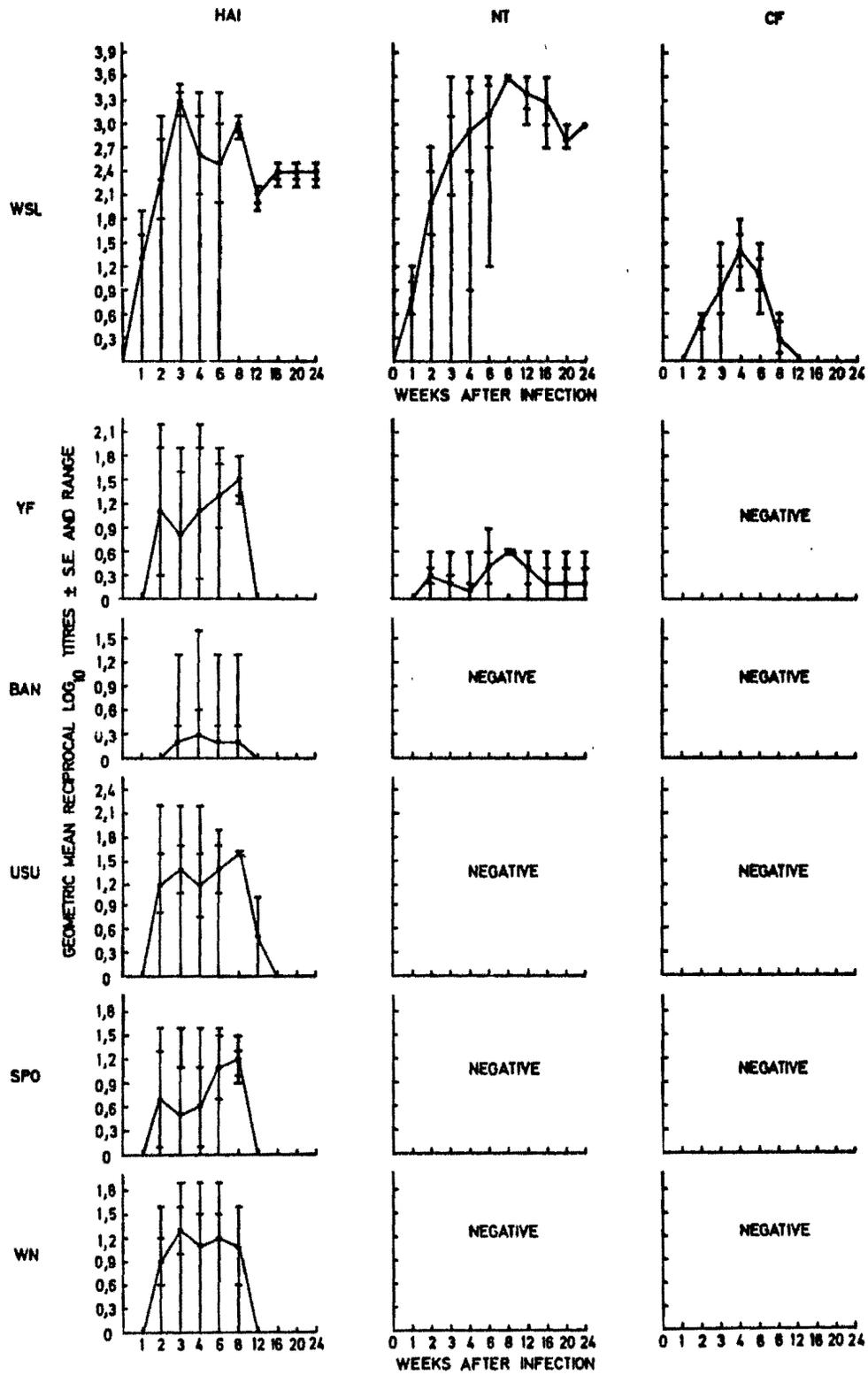


FIG 3.  
 CROSS-REACTIVITY FOR FLAVIVIRUSES OF ANTIBODIES INDUCED IN SIX CALVES  
 BY INFECTION WITH WSL VIRUS

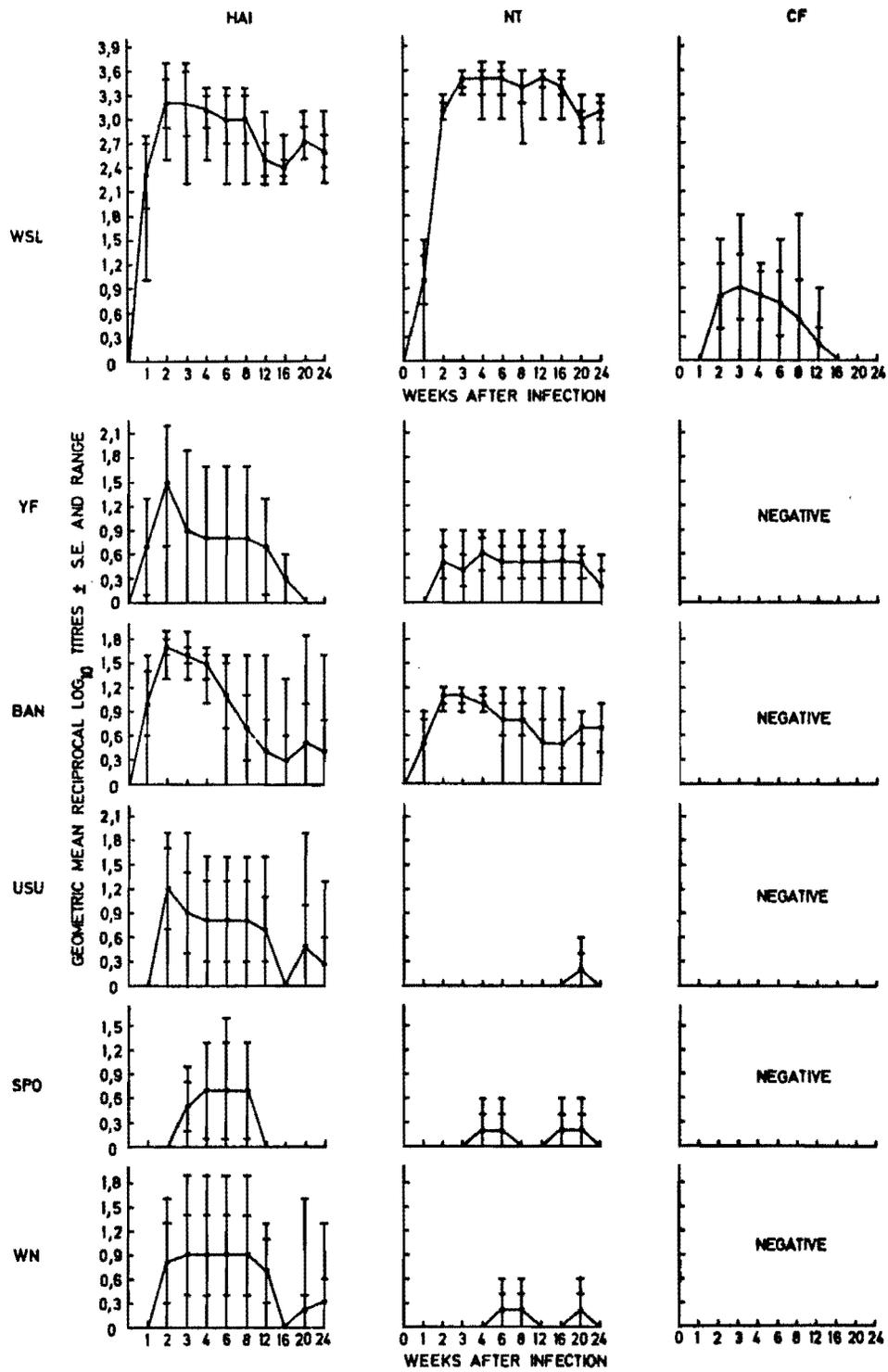


FIG 4.  
 CROSS-REACTIVITY FOR FLAVIVIRUSES OF ANTIBODIES INDUCED IN FOUR CATTLE  
 BY INFECTION WITH WSL VIRUS

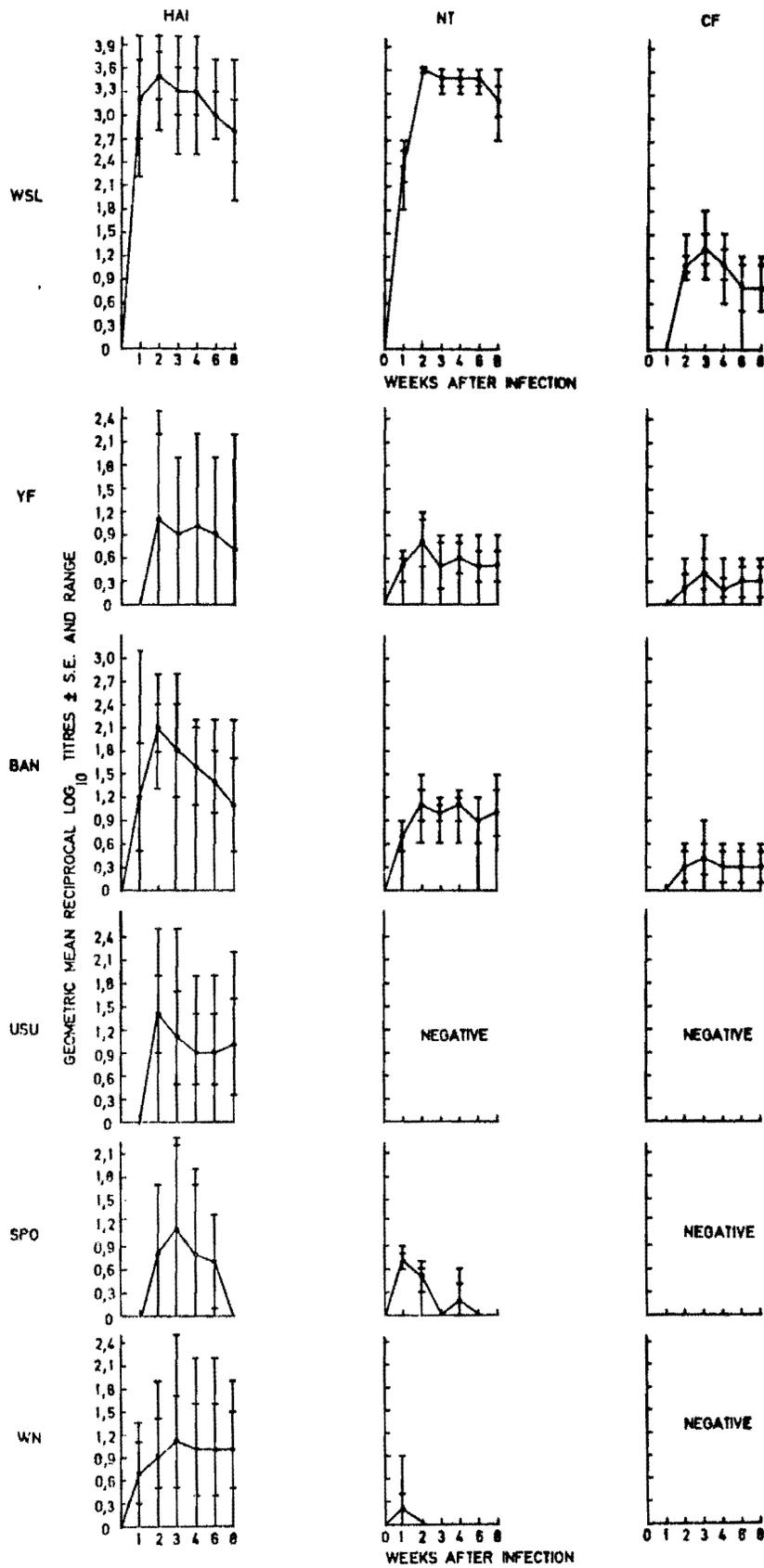


FIG 5.  
CROSS-REACTIVITY FOR FLAVIVIRUSES OF ANTIBODIES INDUCED IN FOUR SHEEP  
BY INFECTION WITH WSL VIRUS

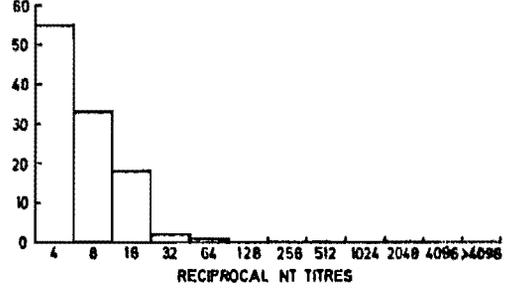
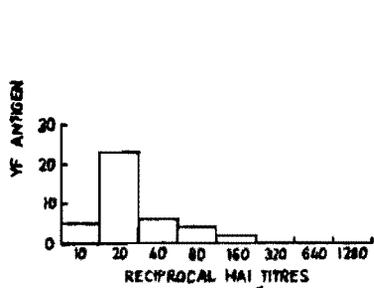
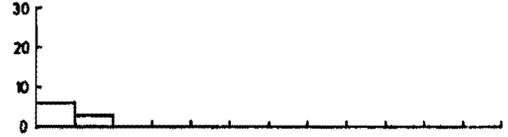
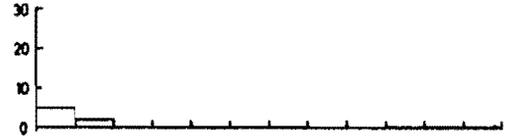
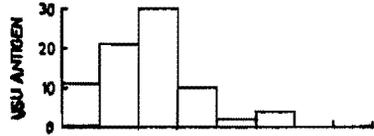
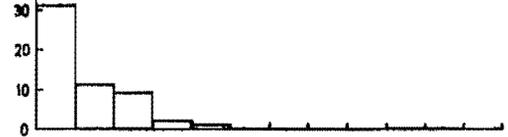
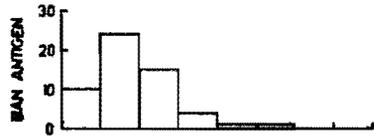
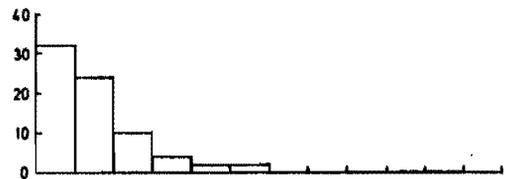
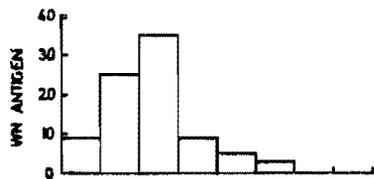
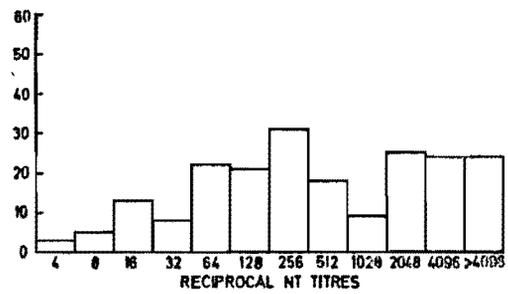
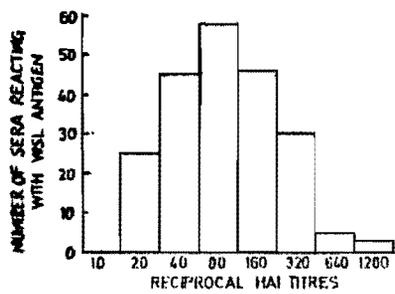


FIG 6. TITRES OF FIELD SERA AGAINST FLAVIVIRUSES  
a) WSL HAI-positive sera

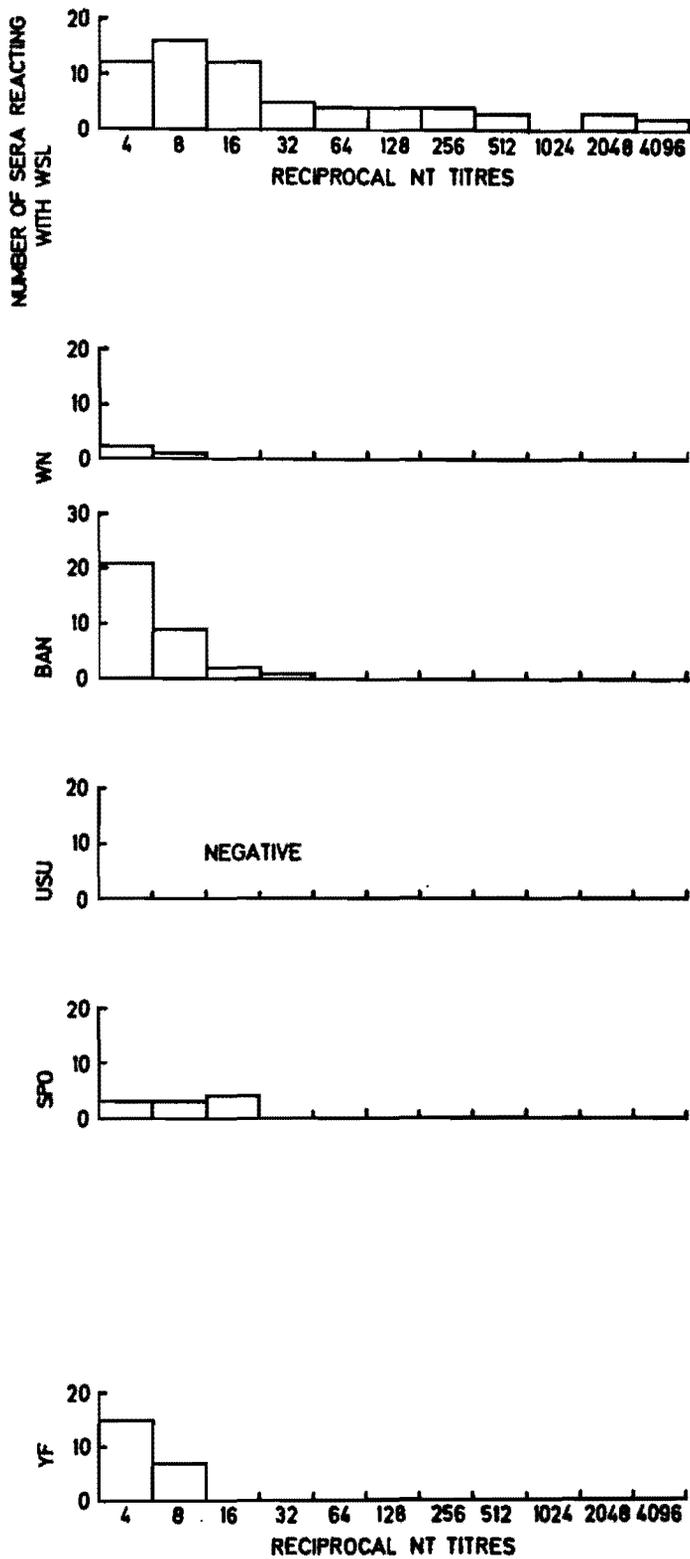


FIG 7.

NT TITRES OF FIELD SERA AGAINST FLAVIVIRUSES

b) WSL HAI-negative sera from WSL HAI-positive herds. None of the sera were HAI-positive with any virus

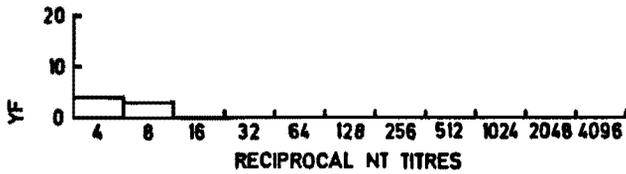
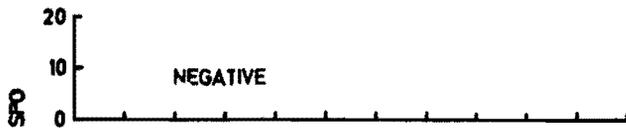
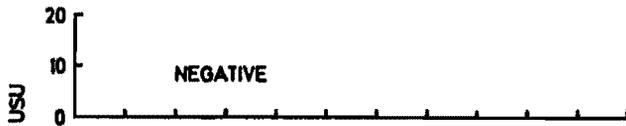
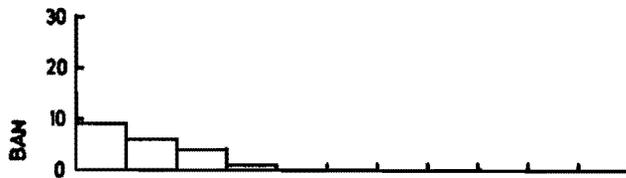
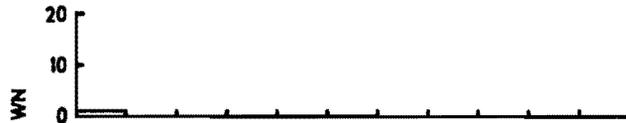
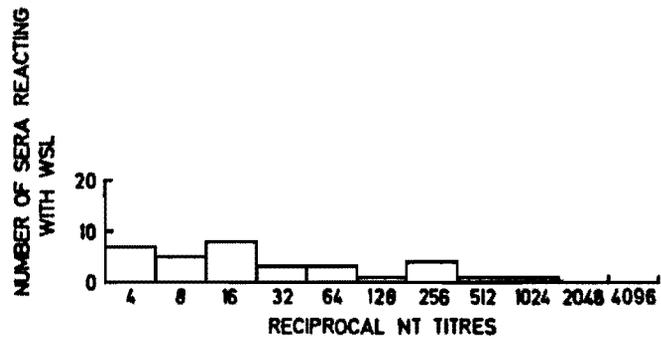


FIG 8.

NT TITRES OF FIELD SERA AGAINST FLAVIVIRUSES

c) sera from WSL HAI-negative herds. None of the sera were HAI-positive with any virus.

Table I. Flavivirus antibody reactions of 207 WSL HAI-positive sera.

Antigen/ virus	Sera reacting in HAI tests:			Sera reacting in NT tests:		
	No. of sera reacting	% sera reacting	Reciprocal of geometric mean HAI titre $\pm$ S.E.	No. of sera reacting	% sera reacting	Reciprocal of geometric mean NT titre $\pm$ S.E.
WSL	207	100	91,2 $\pm$ 1,1	205	99,0	333,1 $\pm$ 1,1
WN	86	41,5	4,4 $\pm$ 1,1	71	34,3	2,0 $\pm$ 1,1
BAN	55	26,5	2,4 $\pm$ 1,1	54	26,1	1,6 $\pm$ 1,1
USU	78	37,7	3,8 $\pm$ 1,1	7	3,4	1,0 $\pm$ 0,1
SPO	50	24,2	2,0 $\pm$ 1,1	10	4,8	1,1 $\pm$ 1,0
YF	45	21,7	2,0 $\pm$ 1,1	107	51,7	2,6 $\pm$ 1,1
Nil	-	-	N/A	2	1,0	N/A

Table II. Flavivirus antibody reactions of WSL HAI-negative sera.

Virus	112 sera from WSL HAI- positive herds			90 sera from WSL HAI- negative herds		
	No. of sera reacting	% sera reacting	Reciprocal of geometric mean NT titre $\pm$ S.E.	No. of sera reacting	% sera reacting	Reciprocal of geometric mean NT titre $\pm$ S.E.
WSL	65	58,0	6,9 $\pm$ 1,2	32	35,6	3,2 $\pm$ 1,2
WN	2	1,8	1,0 $\pm$ 0,1	1	1,1	0,1 $\pm$ 0,1
BAN	33	29,5	1,6 $\pm$ 1,1	21	23,3	1,5 $\pm$ 1,1
USU	-	-	N/A	-	-	N/A
SPO	10	8,9	1,2 $\pm$ 1,1	-	-	N/A
YF	22	19,6	1,4 $\pm$ 1,1	7	7,8	1,1 $\pm$ 1,0
Nil	34	30,4	N/A	42	46,7	N/A

TABLE III. CROSS REACTIONS IN HAI-POSITIVE FIELD SERA.

No. of sera reacting in HAI tests with the following antigens:	No. of sera reacting in NT tests with the following viruses:																		HAI Totals				
	NIL	WSL ONLY	WSL WN	WSL BAN	WSL USU	WSL YF	WSL BAN BAN	WSL USU USU	WSL SPO SPO	WSL YF YF	WSL YF YF	WSL BAN BAN	WSL USU USU	WSL SPO SPO	WSL YF YF	WSL YF YF	WSL BAN BAN	WSL USU USU			WSL SPO SPO	WSL YF YF	
WSL only	-	42	2	2	1	33	6	-	-	4	5	-	-	-	6	1	-	-	-	-	1	103	103
WSL WN	-	3	1	-	-	1	-	-	-	2	-	-	-	-	1	-	-	-	-	-	-	8	24
WSL BAN	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	3	
WSL USU	-	1	1	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	4	
WSL SPO	-	1	-	-	-	3	-	-	-	2	-	-	-	-	1	-	-	-	1	-	-	8	
WSL YF	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
WSL WN BAN	-	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	3	21
WSL WN USU	-	6	2	1	-	-	-	-	-	3	1	-	-	-	1	-	-	-	-	-	-	14	
WSL WN SPO	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	
WSL USU YF	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	
WSL WN BAN USU	-	1	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	4	14
WSL WN BAN SPO	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	
WSL WN USU SPO	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	
WSL WN USU YF	-	2	-	-	-	2	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	6	
WSL WN BAN USU SPO	-	1	-	-	-	2	-	-	-	1	-	-	-	-	4	-	-	-	-	-	1	9	19
WSL WN BAN USU YF	-	2	1	-	-	-	-	-	-	1	-	-	-	-	4	-	-	-	-	-	1	9	
WSL WN USU SPO YF	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
WSL WN BAN USU SPO YF	2	5	2	-	-	2	3	-	1	-	3	1	-	2	1	1	1	-	-	1	1	26	26
NT TOTALS	2	69	11	3	1	47	9	1	1	17	11	1	1	2	21	2	1	1	1	1	4	207	
	2	69	62				40					29					5						

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REPORT FROM THE DEPARTMENT OF EPIDEMIOLOGY, SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF SÃO PAULO, BRAZIL.

During late January-July period attempts were made to determine Aedes scapularis and Aedes serratus host feeding patterns by utilization of the precipitin test. The specimens were collected at several research stations of the Ribera Valley, São Paulo State, Brazil, where a break of encephalitis occurred. Collections were made with human bait and so, only positive reactions to others blood sources were computed, as an attempt to establish some association between human host attraction and others previously utilized for blood meal.

From 1099 tested specimens of Aedes serratus, 51 reacted positively to antisera with the next results:

Host	N	%
Marsupial ( <u>Didelphis</u> )	14	27,4
Bird	4	7,8
Rodent	31	60,8
Cat	2	3,9
Total	51	99,9

With Aedes scapularis, 120 mosquitoes were tested and only 3 reacted positively to antisera other than human. One positive result each was obtained, with marsupial, cat and dog.

By these preliminary data it seems that A. serratus attracted by human bait may have obtained former feedings, with some frequency, on rodents and marsupials. In relation to A. scapularis the available data are still insufficient for to rise any hypothesis.

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REPORT FROM THE EVANDRO CHAGAS INSTITUTE, FSESP  
BRAZILIAN MINISTRY OF HEALTH  
BELEM , BRAZIL

1. YELLOW FEVER IN PARA STATE , 1977

Five cases of yellow fever ( YF ) were diagnosed in Para State during the first semester of 1977 . Four of them were fatal , with confirmation by liver histopathology . The other case survived, being confirmed by virus isolation and serology . The five cases were as follows :

Case 1. (MAR 582 ) . A.B. , male, 42 y.o. , native from Belem, but living in the Maraba County for about 30 years . He died on the May 6, in a hospital of Belem, to where it had been removed on the May 3 . His disease lasted about 6 days . No virus could be isolated from blood, liver, heart, kidney and lung collected 11 hours after death . Serum obtained from post-mortem blood were used to the following biochemical determinations : SGOT ( 5.000 units/ 100 cc ), SGPT ( 5.100 units / 100 cc ), bilirubin ( 4,9 mg / 100 cc ) .

Leptospira agglutination tests were negative in his serum nor HBs Ag could be detected . He was probably infected either at Fazenda S. Raimundo ( S. João do Araguaia county ) or at Fazenda Sta. Maria ( Maraba county ) .

Case 2. RC. , male, 22 y.o. Died on May 22 , in the FSESP hospital of Maraba . He was native from Ceara State, but was working in the Fazenda Mineira , Itupiranga, for the past 2 months prior to the onset of illness .

Case 3. F.A.R. , male, 32 y.o. He died on May 25, in the Clinica Manoel Mendes, Maraba . No virus could be isolated from a liver fragment taken 14 hs after his death . The place of infection is a mystery . He was working in Itaporanga, Goias State, as a tractor driver . He left Itaporanga on May 9, arriving in Maraba on the 14 . Two days later he took a shot of YF vaccine and on May 18 he became sick . His relatives denied that he had contact with the forest after his arrival to the town of Maraba . No Aedes aegypti is known to exist in this town .

Case 4. J.B.A.S., male, 46 y.o. Born in the State of Bahia, but he was working as a wood - cutter a few Km south of Km 100 of the Altamira - Itaituba section of the Trans Amazon highway when he got sick ( June 13 ). He died on June 20, in the FSESP hospital of Altamira .

Case 5. ( ALT 13617 ) M.A.L., male, 36 y.o. YF virus was isolated from a blood sample taken on June 14, when he was on the 2 nd day of illness ( fever, headache, vomiting and myalgia ). During the 10 days prior to onset of illness he was working as a carpenter at the Km 92 of the Altamira - Itaituba section of the Trans Amazon highway . He referred that had taken YF vaccine in 1974 . No HI or CF antibodies were found on the acute serum sample ( taken June 14 ) to group B viruses ( YF, SLE, Ilheus and Bussuquara ). However, high HI and CF antibody titers to these viruses ( including to the isolate - H 324213 ) on convalescent serum taken 2 months later ( August 15 ), were found.

#### General epidemiology

The examination of the records from the FSESP hospital in Maraba, where many patients from Maraba, S. João do Araguaia and Itupiranga counties are admitted, revealed the occurrence of 8 deaths from, "hepatitis" between January and May 1977 . Laboratory tests and autopsy were not performed . All were male and worked in agriculture or had some contact with the jungle . The ages varied from 7 to 52 . In addition, 2 other fatal cases of "hepatitis" occurred in the Clinica Manoel Mendes, Maraba, early in June . These 2 men worked in the Fazenda Santa Fé ( Km 60 of the Maraba - Castanhal road - BR 150 ).

It is conceivable that some of these 10 fatalities were due to Y.F.

Maraba and nearby counties are intersected by the Trans Amazon highway . There are some 21.000 colonists in the area which have direct support from the Government . As a rule , they are vaccinated against YF . In addition , it is estimated that about 20.000 persons are settled in the rural area , engaged in agricultural and cattle farming activities . Most of these individuals work for private companies .

Serum was collected from 328 persons of rural areas, for serological studies . Six of these individuals were febrile and their blood were inoculated into mice with negative results . The results of the serology are awaited .

Blood samples were taken for serological studies from 435 persons living in the areas adjacent to the place where case 4 was working . The results are awaited .

Two fatal cases of "hepatitis" occurred in the FSESP hospital of Altamira, during the first half of 1977 ( 4 in 1976 , in the same period ) . It should be noted that a patient which was working about 70 Km from case 4 developed hemorrhagic manifestations and died June 23, in a private hospital of the town . No specimens were collected for laboratory examinations .

#### Ecological studies .

Vertebrates and arthropods were collected in the areas where cases 1 and 2 were probably infected . Only arthropods were captured in the other suspected places of virus circulation .

No virus could be isolated up to now .

The arthropods collected and examined are listed in Table 1 . It should be noted the scarcity of mosquitoes , probably due to low rainfall .

The vertebrates captured were as follows :

1. Fazenda S. Raimundo ( S. João do Araguaia ) : 8 rodents ( 3 Prochimys , 2 Oxymycterus , 2 Zygodontomys , 1 Oryzomys ) 11 marsupials ( 9 Philander , 1 Didelphis , 1 Monodelphis ) , 2 Bradypus tridactylus and 5 jacuraru .

2. Fazenda Mineira ( Itupiranga ) : 12 monkeys ( 8 Chiropotes satanas , 3 Alouatta b. belzebul and 1 Cebus sp ) 16 rodents ( 5 Proechimys , 6 Zygodontomys , 2 Oryzomys , 2 Coendou , 1 Rattus alexandrinus ) , 5 marsupials ( 4 Didelphis , 1 Marmosa ) , 3 carnivora ( 2 Potos flavus , 1 Nasua ) , 4 edentates ( 3 Choleopus didactylus and 1 Dasypus oitocintus ) .

As soon as the cases were diagnosed , YF vaccination was intensified by SUCAM .

## 2. PROBABLE LABORATORY INFECTION WITH CARAPARU VIRUS

On March 6, 1977 J.F.S.T.R. ( BEL 6444 ), a 26 y.o. male felt sick , with fever, headache myalgia, pain in the eyes and malaise . The symptms were thought at first to be due to influenza . His temperature on the following day was 37º 8 C and he persisted febrile for 2 more days , with maximum temperature recorded of 38º 4 C . On the 2 nd day of illness his WBC were 5.800per cubic mm and a throat washing and blood were collected. The washing was inoculated into eggs,tissue culture and mice with negative results . From the blood, however , a virus was isolated, subsequently identified as Caraparu .

J.F.S.T.R. has been working since 1973 in arbovirus serology .

HI test performed with 3 serum samples against Group C antigens revealed the following results :

<u>Sera</u>	<u>ANTIGENS</u>						
	MAR	ORI	APEU	CAR	MUR	ITQ	NEP
Pre ( Aug 13,74 )	0	0	0	0	0	0	0
Acute ( March 7,77 )	0	0	0	0	0	0	0
Post ( Aug 29, 77 )	0	0	1=20	1=40	0	0	0
Group C	1=640	1=640	1=320	1=320	1=640	1=640	1=640

## 3. EXPERIMENTAL TRANSMISSION WITH OROPOUCHE VIRUS

Successful transmission of Oropouche virus from hamster to hamster by C. paraensis has been demonstrated in the laboratory , whereas, Cx.quinquefasciatus were inefficient in transmitting the agent under similar conditions . These findings together with the fact that no virus can be demonstrated in the throat of viremic patients, suggests that C. paraensis may play a role as vectors during urban epidemics .

F.P. Pinheiro, Amélia P.A.T. Rosa, Jorge F.T. Rosa, Otávio F.P. Oliva, Ronald<sup>o</sup> do Freitas, D. Roberts, A. Hoch, M.L.C. Gomes .

Tabela 1. Arthropods collected by human bait for attempted virus isolation in the places were proven or suspected cases of YF occurred . May - June de 1977. Para State.

Arthropods ( Genus)	Fazendas: S.Raimundo 1 e Sta.Maria 2	Fazendg : Mineira 3	Fazenda Sta. Fé 4	Altamira	TOTAL
<u>Aedes</u>	91/82 (7)	208/205 (20)	1/0 (0)		300/287 (27)
<u>Culex</u>	61/53 (5)	92/89 (9)			153/142 (14)
<u>Haemagogus</u>	18/18 (2)	41/40 (5)		33/33 (4)	92/91 (11)
<u>Mansonia</u>	146/144 (9)	8/8 (2)			154/152 (11)
<u>Coquillettidia</u>	15/10 (1)	44/44 (1)			59/54 (5)
<u>Psorophora</u>	71/69 (7)	157/154 (13)			228/223 (20)
<u>Limatus</u>	10/10 (3)	23/23 (2)			33/33 (5)
<u>Sabethes</u>	2/0 (0)	101/92 (7)		23/23 (11)	126/115 (18)
<u>Trichoprosopon</u>	8/6 (1)	59/56 (5)			67/62 (6)
<u>Wyeomyia</u>	37/35 (2)	182/177 (8)			219/212 (10)
<u>Anopheles</u>	522/522 (28)	22/22 (8)		3/3 (0)	547/547 (36)
<u>Phoniomyia</u>		31/31 (2)			31/31 (2)
<u>Chagasia</u>		11/11 (2)			11/11 (2)
TOTAL	981/949 (65)	979/952 (89)	1/0 (0)	59/59 (15)	2.020/1960 (167)
Nº Hours of colletion	152	81	3,5	75	311,5
Mosquitoes / Man / Hour	6,4	12,2	0,2	0,7	6,4

Numerator : nº insects identified . Denominator : nº insects inoculated . ( ) nº pools inoculated.

1 - Município de São João do Araguaia KM 42

2 - Município de Marabá KM 4

3 - Município de Itupiranga, KM 6 from the Agrovila Costa e Silva

4 - BR 150 , Est. Marabá - Castanhal ( Pa ) KM 60.

REPORT FROM GORGAS MEMORIAL LABORATORY  
PANAMA CITY, PANAMA

St. Louis encephalitis virus

An outbreak of St. Louis encephalitis (SLE) virus activity occurred this year on Altos de Majé Island in Bayano Lake which was formed after the closure of the hydroelectric dam in Eastern Panama. Peak activity was noted in February and March although virus was detected as late as June.

Activity was first recognized by recovery of the virus from mosquito pools collected at Majé Station (some 60 km. east of Panama City), as part of a long-term study of the changing ecology of arboviruses in the Bayano. In February and March, 8 of 104 pools of Mansonia indubitans from 3 consecutive biweekly collections yielded SLE virus in Vero cell cultures. Again in June another burst of activity was seen when 2 of 53 pools of the same species from 2 consecutive collections were SLE-positive.

Conversions in sentinels (plaque neutralizing antibody) were also observed. All 3 of the sentinel rhesus monkeys at Majé converted between January and March bleedings; the sentinel black spider monkey converted in March. Among sentinel chickens, 1 of 4 exposed from March to May and 2 of 6 from May to July, became positive. Sentinel hamsters proved good indicators of SLE activity: 1 of 20 exposed January-February, 11 of 26 in February-March and 2 of 20 in March-May developed antibody.

SLE virus was previously isolated in Majé at the beginning of studies there (August 1973) when a pool of Haemagogus lucifer mosquitoes was positive. Between August and September 1973, 2 of 4 sentinel rhesus converted. And indeed in that same period we observed SLE virus activity in several areas, from Panama City to Majé: virus was isolated from cormorants in the City and on Pacheca Island in Panama Bay and from mosquitoes collected in horse-baited traps during a study of the eastern equine encephalitis outbreak in horses east of the City (see Information Exchange #25). Serologic studies indicated that SLE virus was also present in Majé Station in December 1973-January 1974 (1 of 4 sentinel chickens converted) and not again until June-July 1976 (2 of 21 hamsters positive).

For several years we have carried out various types of studies aimed at delineating the probably complex natural cycles of SLE virus in Panama where human infections have been demonstrated by antibody studies and isolation, but no case of encephalitis due to this virus has ever been recognized. Thus, monkeys and sloths have been shown to be frequently infected in forested areas. (Info. Exchanges #23, #30). Studies on laboratory-infected sloths pointed to the likely role of both species of sloths as amplifying hosts in the forest cycle (manuscript in preparation). Although sick and dead cormorants yielded SLE isolates in 1973, cormorants

infected experimentally in the laboratory did not die (Info. Exchange #30). During 1977 a survey of fledgling cormorants from Pacheca Island showed no SLE virus and no viral antibodies. Finally, various mosquitoes were assessed as potential vectors of SLE virus in Panama (manuscript in preparation).

In the most recent burst of activity it is of interest that Mansonia indubitans is clearly involved. This species which was almost absent from Altos de Majé before the Bayano dam was closed in early 1976 has flourished since then, particularly in 1977. The immature stages live attached to the roots of Pistia stratiotes (water lettuce) which now abounds in the newly formed lake. The females of this species bite both day and night in the forest and have a preference for the canopy, but will also attack man on the forest floor. They have been shown to feed both on mammals and birds.

Although an assessment of the geographic distribution of SLE activity on the Isthmus of Panama has not been made, most of our information about its presence has come from east of the Panama Canal. It was of interest to discover that among 70 horses from the central and western provinces of Panama (all west of the Canal) sampled in 1977, 26 (37%) had neutralizing antibody ( $\geq 1:8$ ) to SLE virus.

#### Triatomines as vectors

Continuing studies to assess the potential of Triatomines as vectors of arboviruses, colonized Rhodnius neglectus were fed on viremic night monkeys ( $\geq 6.0$  logs/ml) infected with a wild yellow fever virus strain. One group of bugs was punctured through the abdomen with a fine needle on the day of the blood meal and the other was not. One of four punctured Triatoma had 2.6 logs of virus/bug, 94 days after virus ingestion, while no YF virus could be detected in non-punctured bugs 10 or 15 days after the blood meal.

A group of wild-caught Rhodnius pallescens (which were free of trypanosomes) were allowed to feed on viremic mice infected with Venezuelan equine encephalitis virus (3880 strain). At one month 3.7 logs of virus were found in one of 3 bugs tested.

(Pedro Galindo and staff, Gorgas Memorial Laboratory, P.O. Box 2016, Balboa Heights, Canal Zone)

REPORT FROM THE SAN JUAN LABORATORIES  
CENTER FOR DISEASE CONTROL  
GPO BOX 4532, SAN JUAN  
PUERTO RICO 00936

Dengue in Jamaica, 1977

In May 1977, Dr. Dorothy King, Head of the Department of Microbiology, University of the West Indies, Jamaica, sent 14 pairs of sera from cases which she had diagnosed serologically as dengue. We confirmed the diagnosis by complement fixation (CF) and hemagglutination inhibition (HI) in every case, and noted at the time that two of the cases had serological pictures more suggestive of a DEN-1 than a DEN-2 infection. The date of onset of the earliest confirmed case was February 26. Subsequent plaque reduction neutralization tests (PRNT) in LLC-MK<sub>2</sub> cells showed a monotypic conversion to DEN-1 in one case. Three strains of virus were isolated from acute sera by inoculation into male Aedes aegypti, and two of these were typed by CF as DEN-1 at the San Juan Laboratories (SJL); all three were subsequently confirmed as DEN-1 by the Walter Reed Army Institute of Research (WRAIR). These three cases had onset in May.

The Jamaica Ministry of Health reported that the epidemic began in Kingston, reached its peak there in the week ending July 9, and then declined. Approximately 10% of the population was involved, i.e., around 60,000 cases. Other parts of the island were affected later. All ages were involved, but there was no evidence of hemorrhagic symptoms or shock. Control was by means of ground ultra-low-volume (ULV) and aerial spraying with malathion. The first cycle of ground spraying was completed on July 13 in the Kingston area. The international port and tourist areas of Montego Bay, Ocho Rios, and Port Antonio were aerial sprayed.

This is the first time that DEN-1 has been isolated in the Western Hemisphere, although there is serological evidence of its possible occurrence in Panama in the early years of this century. Therefore, the epidemic can be expected to spread widely throughout the Caribbean. Suspected imported cases have been reported from Trinidad, Dominica, and the United States (see below).

Dengue in Trinidad, Dominica, and Bermuda, 1977

The Caribbean Epidemiology Research Centre (CAREC) sent specimens from people who fell ill with typical symptoms of dengue after returning from Jamaica to Trinidad and the island of Dominica. CAREC had diagnosed some of these serologically as dengue cases. Several of the Trinidad and two of the Dominica cases were confirmed serologically at SJL as compatible with dengue infection. One of the Dominica cases appears to be indigenous, since the patient had not travelled outside the island for 3 months before onset. A flavivirus was isolated in mosquitoes at SJL from another Dominica case.

Four paired sera received from suspect cases from Barbados were totally negative for CF or HI antibodies to DEN-1, -2, -3, -4, St. Louis encephalitis, and yellow fever. Two travellers who fell ill after returning to Bermuda from Jamaica had broad spectrum serological conversions to dengue.

Imported Dengue in the USA, 1977

Suspected cases of dengue in travellers returning from Jamaica have been reported from 15 states and the District of Columbia. These include seven of the ten states in which A. aegypti, the dengue vector, is known to occur. A strain of virus was isolated at SJL and WRAIR from a case in Baton Rouge, Louisiana, and typed as DEN-1 by WRAIR. Serological conversions compatible with a diagnosis of dengue have been found so far in cases from Florida, Indiana, Maryland, Mississippi, New York, North Carolina, Ohio, Oregon, Virginia, and the District of Columbia.

Dengue in French Guiana, 1977

Dr. J. P. Digoutte, Director of the Institut Pasteur in Cayenne, reported to the SJL an outbreak of dengue in Cayenne in February, March, and April. He sent us two strains of virus which he had adapted to mouse brain and provisionally typed as DEN-2. We confirmed his identification by CF on material taken after one further mouse passage.

Continuing Dengue in Puerto Rico, 1977

Dengue has reached epidemic proportions once more in Puerto Rico. Number of suspected cases reported:

<u>Epidemic Week No.</u>	<u>1975</u>	<u>1977</u>
1	9	13
2	32	19
3	37	29
4	63	32
5	59	59
6	94	106
7	152	spray 264
8	214	spray NA*
9	99	NA

\*Not available at time of writing

In 1975, case reports began increasing in November (week No. 1 ended November 2); air spraying was begun in week No. 7 and continued in week No. 8, after which reports declined. In 1977, reports began increasing in July (week No. 1 ended July 16); ground ULV spraying was begun in week No. 7 and is being continued, so that we hope to see a peak soon. Most of the cases are being reported from the greater San Juan metropolitan area, but the number of municipalities out on the island reporting cases of dengue rose from four in week No. 6 to twenty-eight in week No. 7 this year. No cases with severe hemorrhagic symptoms have been seen, but four cases were investigated in adults in which the platelet count dropped below 100,000.

Of 55 serum pairs tested by CF and HI from cases with onset in July 1977, 76% showed conversions for dengue, much higher than the rate of 22% for the 1975-76 epidemic. Six strains of flaviviruses were isolated by mosquito inoculation from the acute sera of cases from the San Juan metropolitan area, with onsets in July and August, and these are being adapted for typing. None of the patients had any history of travel to Jamaica, but some of their serological pictures are different from those of previous Puerto Rican patients, and there may be more than one type of dengue currently in circulation.

Larval surveys for A. aegypti carried out in July 1977 showed that house indices ranged from 2-88% in 77/79 municipalities surveyed; 74 of these had indices of 5% or greater, and 59 had 20% or greater. Eight new, truck-mounted, ULV spray machines were purchased by the Puerto Rico Health Department and put into service together with one other large and three smaller machines, to spray 96% malathion. Two cycles of spraying at 5-day intervals were completed by the end of August in the Bayamón area of western San Juan, where most of the cases were concentrated at that time. It is recognized that adulticiding alone is not the answer to the A. aegypti menace, and clean-up and source-reduction campaigns are being mounted islandwide. It is hoped that the health departments of other Caribbean countries will take notice of the present situation and increase source-reduction efforts in their jurisdictions.

(J. P. Woodall, R. H. López-Correa, C. G. Moore, G. E. Sather,  
E. Ruiz-Tibén, G. Kuno)

REPORT OF THE DEPARTMENT OF EQUINE ENCEPHALITIS,  
INSTITUTO NACIONAL DE INVESTIGACIONES PECUARIAS, MEXICO, D.F.

Experimental inoculation of cattle with Venezuelan Equine Encephalomyelitis  
(VEE) virus, TC-83 strain.

Due to the fact that in serologic surveys done in México it is common to find high percentages of cattle sera with HI antibodies against VEE virus, it has been postulated that the antibodies may be due either to field virus or vaccinal virus. The last possibility may occur because in México there is an annual vaccination campaign in equines and thus the possibility that the virus might be transmitted to cattle through mosquitoes.

This study was designed to determine the serologic response of cattle to VEE virus, TC-83 strain.

In the first experiment, 5 Hereford cattle of two years of age and free of HI antibodies were inoculated with TC-83 strain as follows: one animal with  $10^5$  LD<sub>50</sub>/sm/ic/ml; two animals with  $10^4$  and two animals remained as controls. They were bled on days 0, 1, 2, 3, 4, 5, 6, 10, 17 and 29 and the sera were tested by the HI test with TC-83 antigen.

In the second experiment, the same five animals were reinoculated 36 days after the first inoculation; two animals with  $10^6$  LD<sub>50</sub>/sm/ic/ml, two with  $10^5$  and one with  $10^4$ . The animals were bled 15 days later to check HI antibodies.

In a third experiment, 16 Indo-Brazil cattle of two years of age were distributed in 4 groups of 4 animals each. In group I, one animal received  $10^8$  LD<sub>50</sub>/sm/ic/ml and three animals  $10^{7.3}$ , group II received  $10^7$ , group III,  $10^6$  and group IV was used as control. Blood was collected at 0, 2, 3, 4, 5, 6, 7, 15, 30 and 60 days after inoculation and the sera were tested by the HI test.

None of the animals of experiments 1 and 2 had antibodies. The animals inoculated with  $10^8$ ,  $10^{7.3}$  and  $10^{7.0}$  LD<sub>50</sub>/sm/ic/ml had HI titers reaching 1:40 only in two animals at 30 days post-inoculation decreasing to 1:20 at 60 days.

These results show that the TC-83 strain does not elicit high HI antibody levels in cattle and they suggest that the high titers found in cattle in field conditions are produced as a response to epizootic or enzootic virus rather than vaccinal virus.

( M. Gallo de la Torre, A. Morilla-González )

(Complete address: Department of Equine Encephalitis, Instituto Nacional de Investigaciones Pecuarias, Palo Alto, Mexico 10, D.F., Mexico)

REPORT FROM THE BUREAU OF LABORATORIES  
 TEXAS DEPARTMENT OF HEALTH, 1100 W. 49TH STREET  
 AUSTIN, TEXAS 78756

The following is a report on the status of our Arbovirus activities. The period extends from 1-02-77 to 6-29-77.

MOSQUITOES

There have been 796 pools of mosquitoes tested, totaling 4883 mosquitoes. Of these, 23 pools have been positive for isolation of Hart Park, as shown in the following table.

Virus-positive mosquito pools, April-June, 1977

<u>Locality</u>	<u>Collection Period</u>	<u>Species</u>	<u>No. Isolations</u>	<u>Virus</u>
Dallas	4/26-29	C. quinque	1	Hart Park
	5/16-20	C. restuans	2	Hart Park
		C. quinque	3	Hart Park
	5/23-26	C. restuans	1	Hart Park
		C. tarsalis	2	Hart Park
	6/7	C. quinque	2	Hart Park
Fort Worth	5/23	C. restuans	2	Hart Park
		C. quinque	1	Hart Park
	5/31	C. quinque	1	Hart Park
		C. restuans	2	Hart Park
	6/06	An. punctipennis	1	Hart Park
		C. restuans	1	Hart Park
		C. quinque	2	Hart Park
Montgomery Co.	5/23	An. quadrimaculatus	1	Hart Park
Cameron Co.	5/31	C. quinque	1	Hart Park

NESTLING BIRD BLOODS FOR ISOLATION

108 wild birds bloods have been submitted for virus isolation. None has yielded an arbovirus isolate.

WILD BIRD BLOODS FOR SEROLOGY

186 wild bird bloods have been tested for arbovirus antibody. Out of 66 specimens received from San Antonio, 1 was positive for EEE, with an HI titer of 1:20. This was submitted in May, 1977, and was collected in the early part of that month.

During the early part of the year (February), specimens received from Dallas yielded 8 positives by HI for SLE. Titers were 1:10 or greater.

Wild birds tested for arbovirus HI antibodies, Jan-Jun, 1977

<u>Locality</u>	<u>Collection Period</u>	<u>No. Tested</u>	<u>No. Positive</u>	<u>Titers</u>
Dallas	1/25	13	0	
	1/26-31	39	8 (SLE)	<u>&gt;1:10</u>
	2/2-4	8	0	
	2/7	8	0	
	2/23	9	0	
	6/14	13	0	
	6/13-14	30	0	
San Antonio	5/4	26	0	
	5/12	20	1 (EEE)	1:20
	5/27	6	0	
	6/7	9	0	
	6/10	5	0	
		186	9	

SENTINEL FLOCKS

Three specimens have been positive for SLE of 583 tested, at titers of >1:20. Two were collected at Dallas on 5/02/77, and the other at Dallas on 6/08/77.

Sentinel chickens tested for arbovirus HI antibodies, April-June, 1977

<u>Locality</u>	<u>Collection Period</u>	<u>No. Tested</u>	<u>No. Positive</u>	<u>Titers</u>
Dallas	4/4	100	0	
	5/2	99	2 (SLE)	<u>&gt;1:20</u>
	5/17	98		
	5/26	2		
	6/6	38		
	6/8	50	1 (SLE)	<u>&gt;1:20</u>
	6/14	100		
Lubbock	6/7	87	0	
		583	3	

(Charles E. Sweet and Lois Leffingwell)

REPORT FROM  
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES  
OFFICE OF LABORATORY SERVICES  
POST OFFICE BOX 210  
JACKSONVILLE, FLORIDA 32201

During the period January through August 1977, no laboratory confirmed cases of SLE has been detected in Florida. As in the past, the State Public Health Surveillance for SLE has included the provision of virus diagnostic service to the medical community and the testing of animal, both avian and mammalian, sera for HI antibodies against a select battery of arbovirus antigens. Mosquito surveillance was limited to virus isolation attempts of Culex nigrapalpus in suckling mice.

Laboratories performing the tests were the Central Virology Laboratory in Jacksonville under Ms. Elsie Buff and the Epidemiology Research Center in Tampa under Dr. Flora Mae Wellings.

A total of 1,026 human sera from suspect cases of CNS disease and/or FUO were tested with only seven patients having constant low level Group B antibodies. One case of Dengue fever was detected, details to be provided later in this report.

Two of 20 horses tested yielded titers against EEE(1) and WEE(1). Four horses yielded VEE titers, apparently vaccine associated. Seven rodent sera were negative.

There were 113 bird and 52 sentinel chicken sera tested with negative findings.

A total of 124 pools of Culex nigrapalpus inoculated into suckling mice yielded no virus isolations.

DENGUE

On 13 July, a 21-year-old white male reported to the student medical service of Tampa University in Tampa, Florida. He stated that he thought he might have Dengue fever. He and his younger brother had been visiting their father in Jamaica and returned to Baton Rouge, Louisiana on 3 July. His brother became ill on 4 July, whereas, he became ill on 6 July. Symptoms included headache, stiff neck, lethargy, a maculopapular eruption on hands and feet, cough, chest pain, generalized muscle pain and weakness, and enlarged maxillary, axillary and inguinal glands. His fever reached 105°F and he stated that he had passed out twice.

Sera obtained on 13, 21, and 26 July were tested for hemagglutination-inhibition antibody when received. Results were comparable to those obtained when all three were included in a single test. Titers are shown below.

<u>Antigen</u>	<u>7-13-77</u>	<u>SERUM</u>	
		<u>7-21-77</u>	<u>7-26-77</u>
SLE	1:20	1:80	1:160
Dengue 2	1:10	1:80	1:160
Dengue 1	1:40	1:160	1:320

Additionally, examination of acute and convalescent sera failed to indicate the presence of antibody against EE, VE and Calif (BFS-283) hemagglutinins.

Aliquots of the three sera have been sent to the San Juan Laboratory for confirmation.

Mosquito abatement procedures were initiated on 13 July in and around the home of the individual and at Tampa University. No secondary cases have been forthcoming.

(N. J. Schneider, E. E. Buff and F. M. Wellings)

REPORT FROM THE VIRAL DISEASES DIVISION (VDD), BUREAU OF EPIDEMIOLOGY

CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

30333

Surveillance for Human Arboviral Infections - United States, 1977

St. Louis Encephalitis (SLE) - A total of 15 laboratory documented cases of SLE infection have been reported at the end of August. The first 2 SLE cases, reported by Dr. J. Luby's Laboratory in Dallas, Texas, involved Dallas residents who had onset of illness on June 10 and June 15 respectively. We have not been able to identify cases with an earlier onset ever reported in the United States.

Despite the early reports from Dallas no further cases developed and the subsequent reports have all involved cases from other States with onset in July. The largest cluster of these cases includes 2 confirmed and 3 presumptive cases of SLE from Slidell, Louisiana, located near the northeast shore of Lake Pontchartrain, and 1 other confirmed case from Bogalusa, 30 miles north of Slidell. Four confirmed cases were reported from Illinois, 2 from Jackson County in the southwest corner and 2 from LaSalle and DuPage counties in the northern part of the State. One confirmed case each was reported from Alabama and Ohio and 1 presumptive case from Memphis, Tennessee.

Western Equine Encephalitis (WEE) - Evidence of WEE in horses was widespread in the western United States and by the end of July the Veterinary Services Laboratories, U. S. Department of Agriculture, had serologic documentation of WEE in horses from 14 States. A total of 12 human cases of WEE have been reported, all but 2 of them with onset of illness in July.

Five of the cases were from the Dakotas: 3 from North Dakota and 2 from South Dakota. Two cases were reported from Colorado where more than 100 confirmed and presumptive equine cases of WEE have been identified, most from the irrigated eastern slope of Colorado, north and east of Denver.

In late June, the Vector-borne Diseases Division, Bureau of Laboratories, CDC isolated WEE virus from blood of nestling house sparrows collected in Fort Collins, Colorado. Mosquito surveillance was then initiated in various areas of eastern Colorado with over 200 isolations of WEE virus and 4 of SLE from pools of Culex tarsalis collected in Larimer, Weld, Boulder, Pueblo, Fremont, Morgan and Logan Counties. Occasional isolations were made from Aedes vexans.

The other cases of WEE include 2 patients from eastern and western Montana, 2 from the high plains of northwest Texas and 1 from Dona Ana County on New Mexico's southern border.

California Encephalitis - Four cases of California Encephalitis were reported by the beginning of September. Three of the cases were in children residing in adjoining counties in central Illinois and 1 was reported in a child from Missouri.

Dengue - Periodic assessment of mosquito prevalence conducted by the Vector-borne Disease Division, Bureau of Tropical Diseases, CDC, has shown Aedes aegypti to be prevalent in the 10 southeastern States. To prevent the establishment of dengue in areas of the southeastern United States with populations of Aedes aegypti, the CDC has attempted to monitor imported dengue in returning U.S. residents. When the recent epidemic of dengue type 1 in Jamaica was detected, surveillance and control activities were intensified. All state health departments were alerted to the problem and requested to report any returning travelers from Jamaica with dengue-like illness. Vector control officers from the southeast states were invited to attend a special workshop conducted by the Bureau of Tropical Diseases to coordinate their response to possible introductions of dengue. The United States quarantine stations were alerted, airplane and ship disinfection was intensified, and a travel

advisory memorandum was sent to all travel agencies, airlines, and ship's offices alerting them of the situation.

Suspect dengue cases were investigated in returning residents from 21 States and the District of Columbia involving a total of over 50 individuals. Suspect cases were reported from 8 of the 10 southeastern States with Aedes aegypti populations. Although these suspected cases were followed by surveillance, no evidence of secondary transmission was found.

At the end of August testing, mostly by the San Juan Laboratories, CDC, was positive for 12 of the suspect cases, negative for 5 and pending for the others. The rate of imported cases had declined sharply and only 6 suspect cases were identified in persons leaving Jamaica after early August.

(Karl Kappus, David Morens, David Nelson, and Lawrence Schonberger)

Intracerebral Development of Infectious Virus, Complement-Fixing, and  
Hemagglutinating Antigens of Four Types of Dengue Virus in Suckling Mice

Infected suckling mouse brains (ISMB) are used routinely for the production of complement-fixing and hemagglutinating antigens, and for the preparation of seed stocks of dengue viruses. The ISMB are usually harvested when 10% of the inoculated mice are dead, and the rest sick or moribund. The present investigations were carried out to determine the best time to harvest the ISMB for the preparation of CF and HA antigens and for obtaining maximum virus titers. Previous work by Calisher and Maness (1) on four arboviruses other than dengue seemed to indicate that an earlier harvest time might be feasible.

Each dengue virus type was inoculated into 40-80 litters of ten mice each. Inoculations were performed IC with 0.02 ml of virus suspension containing approximately 1000 SMLD<sub>50</sub>.

At twelve-hour intervals after inoculation, groups of 25 mice were collected randomly and frozen at -55 C for later use.

When all mice for a particular virus had been collected, they were thawed and harvested by aspiration through the unopened skull.

CF and HA antigens were prepared from each pool using the sucrose-acetone extraction method of Clark & Casals (2). Infectivity titrations of each pool were performed in two-day old suckling mice using 0.02 ml IC.

Results for all four dengue types are shown in Tables 1-4. CF titers are expressed as the optimal antigen dilution. Infectivity titers for the four types reached high levels before any deaths occurred and usually

before signs of illness were apparent. HA antigen appeared earlier than CF activity. Although both HA and CF titers frequently reached high levels before any deaths occurred, especially so for Den-2, the normal collection time seemed to give as good results as did an earlier one.

(Dane W. Sanderlin and W. Adrian Chappell)

References:

1. Calisher, C. H. and K. S. C. Maness. 1970. Development of four arboviruses in mice and application to rapid test procedures. *Appl. Microbiol.* 20:398-404.
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Table 1. Dengue type 1. Relationship between time of inoculation and signs of infection to CF, HA, and infectivity titers.

Hours post inoculation	Symptoms of illness <sup>a</sup>	CF <sup>b</sup>	HA <sup>b</sup>	Infectivity <sup>c</sup>
24	0	0	0	0
36	0	0	0	0
48	0	0	0	0
60	0	0	0	0
72	0	0	0	1.6
84	0	0	0	1.5
96	0	0	0	2.4
108	0	0	0	2.8
120	0	0	0	3.0
132	0	0	20	4.2
144	0	0	20	3.6
156	ND	ND	ND	ND
168	0	4	40	4.2
180	0	4	80	4.7
192	0	4	320	4.7
204	0	4	160	4.4
216	S	4	160	4.7
228 <sup>d</sup>	S, Pa, M, D	4	160	4.2
240	S, D, M, C	8	160	4.2
252	M, D, C	8	320	4.0
264	M, D, C	8	160	3.2

<sup>a</sup>S, sick; Pa, paralyzed; M, moribund; D, dead; C, cannibalized; ND, not done.

<sup>b</sup>CF and HA titers expressed as the reciprocal of highest dilution.

<sup>c</sup>Infectivity titers expressed as log SMLD<sub>50</sub> per 0.02 ml IC.

<sup>d</sup>Hour at which approximately 10% of the mice were dead.

Table 2. Dengue type 2. Relationship between time of inoculation and signs of illness to CF, HA, and infectivity titers.

Hours post inoculation	Symptoms of illness <sup>a</sup>	CF <sup>b</sup>	HA <sup>b</sup>	Infectivity <sup>c</sup>
12	0	0	0	0
24	0	0	0	0
36	0	0	0	1.2
48	0	0	0	2.7
60	0	0	0	3.0
72	0	0	0	4.5
84	0	4	0	4.8
96	0	4	80	5.1
108	T	8	160	6.2
120	S	16	160	5.8
132	S	64	320	6.2
144	S, Pa, D	32	320	6.3
156 <sup>d</sup>	M, D, C	32	160	5.8

<sup>a</sup>Symbols; T, tremor; S, sick; Pa, paralyzed; M, moribund; D, dead; ND, not done.

<sup>b</sup>CF and HA titers expressed as reciprocal of highest dilution.

<sup>c</sup>Infectivity titers expressed as log SMLD<sub>50</sub> per 0.02 ml IC.

<sup>d</sup>Hour at which approximately 10% of the mice were dead.

Table 3. Dengue type 3. Relationship between time of inoculation and signs of illness to CF, HA, and infectivity titers.

Hours post inoculation	Symptoms of illness <sup>a</sup>	CF <sup>b</sup>	HA <sup>b</sup>	Infectivity <sup>c</sup>
12	-	-	-	-
24	-	-	-	-
36	-	-	-	-
48	-	-	-	1.0
60	-	-	-	1.0
72	-	-	-	1.3
84	-	-	-	1.6
96	-	-	-	2.3
108	-	-	-	2.6
120	-	-	-	3.4
132	-	-	-	3.3
144	-	-	-	3.5
156	T	-	40	3.4
168	ND	ND	ND	ND
180	T	4	160	4.2
192	ND	ND	ND	ND
204	S, Pa, T	4	320	4.5
223 <sup>d</sup>	C, M, S	4	320	5.2

<sup>a</sup>T, tremor; S, sick; Pa, paralyzed, M, moribund; C, cannibalized; ND, not done.

<sup>b</sup>CF and HA titers expressed as reciprocal of highest dilution.

<sup>c</sup>Infectivity titers expressed as log SMLD<sub>50</sub> per 0.02 ml IC.

<sup>d</sup>Hour at which approximately 10% of the mice were dead.

Table 4. Dengue type 4. Relationship between time of inoculation and signs of illness to CF, HA, and infectivity titers.

Hours post inoculation	Symptoms of illness <sup>a</sup>	CF <sup>b</sup>	HA <sup>b</sup>	Infectivity <sup>c</sup>
12	0	0	0	0
24	0	0	0	1.6
36	0	0	0	1.9
48	0	0	0	3.0
60	0	0	0	4.5
72	0	0	40	5.2
84	0	4	160	5.8
96	S, T	32	160	6.4
108	D, S, M	32	320	6.5
120 <sup>d</sup>	D, S, M, C	32	1280	6.3
132	C, D, M	32	320	6.7

<sup>a</sup>T, tremor; S, sick; M, moribund; D, dead; C, cannibalized.

<sup>b</sup>CF and HA titers expressed as reciprocal of highest dilution.

<sup>c</sup>Infectivity titers expressed as log SMLD<sub>50</sub> per 0.02 ml IC.

<sup>d</sup>Hour at which approximately 10% of the mice were dead.

REPORT FROM THE MEMPHIS AND SHELBY COUNTY HEALTH DEPARTMENT  
814 JEFFERSON AVENUE  
MEMPHIS, TENNESSEE 38105

Beginning in late April, 1977, the Insect Vector Control Division of the Memphis and Shelby County Health Department maintained continuous daily surveillance of SLE virus activity in the community. This annual surveillance program comprises antibody-detecting HI serum testing of wild sparrows and sentinel chicken flocks in our own laboratory, and testing of adult *Culex pipiens* for SLE virus isolation.

The results of our 1977 SLE Surveillance Program, through August 12, 1977, are tabulated below:

COLLECTING AREA	SPECIES	AGE	TEST PERFORMED	NO. TESTED	NO. POSITIVE	% POSITIVE
Memphis	Sparrow	Adult	HI	378	15	3.96
Memphis	Sparrow	Juvenile	HI	901	12	1.33
Memphis	Sparrow	Nestling	HI	89	3	3.36
Memphis	Chicken	(Varied)	HI	527	28	5.31
Memphis	Pigeon	Unknown	HI	3	0	-
Memphis	Starling	Unknown	HI	1	0	-

Following the intensified viral activity peak in June, the percentage of positive avian sera has remained below the general danger threshold level of 4-5%. These data suggest that SLE virus activity in the Memphis area is currently low and probably does not present a threat to the human population at the present time.

(James Hamm)

We have been investigating mechanisms of genetic resistance to mortality from flavivirus encephalitis in congenic mice. Early work on this phenomenon was done by Dr. Webster and colleagues about 40 years ago. More recently Dr. Koprowski and his colleagues have looked at various aspects of the problem.

In the past, the role of the immune response had not been examined in depth. We are investigating the contribution of immunity and other host factors to the phenotypic expression of genetic resistance. Our previous studies have shown that congenic adult C3H/He and C3H/RV mice are equally susceptible to infection with Banzi virus (flavivirus) by parenteral inoculation. Encephalitis develops in both strains, but mortality is high in C3H/He mice and low in C3H/RV mice. Immunodeficient C3H/RV mice, however, incur high mortality from Banzi infection (J. Infect. Dis. 134:158, 166, 1976).

Some of our recent work related to in vivo studies with adoptive immunity is briefly summarized below.

Adult C3H/He mice were inoculated subcutaneously with  $2.0 \log_{10} \text{TCID}_{50}$  of Banzi virus and 24 hours later were adoptively immunized with  $2 \times 10^8$  live Banzi virus-primed C3H/RV spleen cells. Cells transferred by 5 days post-priming prevented mortality in 67% of infected recipients while cells transferred at 7 days postpriming or later usually protected 100 percent of infected recipients. Recipients were not protected by  $2 \times 10^8$  nonimmune C3H/RV spleen cells or by heat-killed spleen cells from optimally immune C3H/RV mice. Protection after transfer was abrogated by pretreating immune spleen cells with rabbit antimouse brain serum (anti-T cell serum) and complement but not by pretreating cells with normal rabbit serum and complement. By postinfection day 8, titers of Banzi virus in brains of adoptively immunized C3H/He mice were less than  $3.0 \log_{10} \text{TCID}_{50}/0.1 \text{ gm}$  whereas titers in mice given nonimmune donor spleen cells exceeded  $7.0 \log_{10} \text{TCID}_{50}/0.1 \text{ gm}$ . Live virus-primed C3H/He spleen cells also protected C3H/He recipients against lethal Banzi virus infection. Adoptive transfer was not effective, however, with donor cells harvested before postpriming day 7. Furthermore, dose-response experiments indicated that immune C3H/RV spleen cells were more effective than equivalent numbers of immune C3H/He spleen cells in protecting infected C3H/He recipients from mortality. These studies indicate that in experimental Banzi virus infection: 1) cell-mediated immunity is an important host defense; 2) immune T cells are required for adoptive transfer of cell-mediated immunity; and 3) immune C3H/RV spleen cells are, on a cell for cell basis, more efficient than immune C3H/He spleen cells at conferring adoptive immunity.

(Drs. Robert O. Jacoby and Pravin Bhatt)

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

Arbovirus Surveillance 1977

During the current arbovirus season to date, 1,558 pools totaling 109,382 mosquitoes collected statewide have been tested. A total of 22 virus isolates have been obtained, and 18 of these have been identified: 16 California (CAL) and 2 Eastern equine encephalitis (EEE) (Table 1). Thirty sentinel pheasants and 19 sentinel chickens are being exposed in Oswego and Erie counties respectively. No EEE or St. Louis encephalitis (SLE) antibody conversions and no EEE outbreaks in horses have been detected as of August 26.

Sera of 99 patients with central nervous system infections and 15 with fever of unknown origin were tested for arbovirus antibodies (EEE, Western equine encephalitis, SLE, Powassan, and CAL). None of these patients had serologic evidence confirming a current arbovirus infection, but in 2 patients evidence was obtained of CAL infection at some time in the past.

(Sunthorn Srihongse, Margaret A. Grayson, and Rudolf Deibel)

(Complete address: State of New York Department of Health, Laboratories of Virology, Division of Laboratories and Research, Tower Building, Empire State Plaza, Albany, New York 12201)

Table 1

Arboviruses Isolated from Mosquitoes Collected in New York State  
June - July 1977

Species	No. Tested		No. Isolates			County
	Pools	Specimens	EEE	CAL	Unid.	
<u>Aedes aurifer</u>	31	3,045				
<u>A. canadensis</u>	175	12,994		2		Warren and Saratoga
<u>A. cantator</u>	19	1,844				
<u>A. communis</u>	89	5,162		5		Warren
<u>A. cinereus</u>	42	3,098		4	1	Warren
<u>A. stimulans</u>	127	7,581			1	Schuyler
<u>A. triseriatus</u>	47	539				
<u>A. vexans</u>	234	15,195			1	Suffolk
<u>A. spp.</u>	67	3,748		1		Warren
<u>Anopheles spp.</u>	121	9,048		2	1	Chemung
<u>Coquillettidia perturbans</u>	484	41,660		2		Warren
<u>Culiseta spp.</u>	58	3,171	2			Oswego
<u>Culex spp.</u>	61	2,263				
Other	3	34				
Total	1,558	109,382	2	16	4	

Report from: THE NATIONAL ARBOVIRUS REFERENCE SERVICE\*

Department of Medical Microbiology,

University of Toronto, Toronto, Ontario.

A SIMPLE METHOD FOR THE INACTIVATION OF ST. LOUIS ENCEPHALITIS  
VIRUS PREPARATIONS FOR IMMUNOFLUORESCENT MICROSCOPY

The immunofluorescence (ImFl) technique has proven to be a valuable method for the rapid and specific diagnosis of viral diseases. However, the possibility of working with infectious antigens poses a relevant bio-hazard. Some methods of fixation and inactivation have restricted use because they may destroy the antigen and, thus, diminish the effectiveness of the microscopy. Other methods are hazardous themselves, e.g. heating of the preparation in acetone.

We compared different methods of treatment with respect to their potency for inactivation of St. Louis Encephalitis (SLE) virus in infected cells prepared for ImFl microscopy. The experiments were carried out as follows: -

The cells, BHK-21, were grown in 200ml plastic flasks. After the formation of confluent monolayers the growth medium (medium BHK-21 with 10% fetal calf serum) was changed to maintenance medium (MEM + 3% fetal calf serum), and virus was inoculated. When advanced cytopathic effect was determined the cells were trypsinized. After one washing in graduated centrifuge tubes the cells were resuspended in Bacto-FA buffer (Difco) with 0.1ml of packed cells resuspended in 2.0ml of buffer. Control,

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\* A Laboratory Operating in Conjunction with the Laboratory Centre for Disease Control - Department of National Health and Welfare, Canada.

uninfected, monolayers, were handled in the same manner. The suspensions derived were dropped on glass slides, using dropping tip pipettes, placing in separate smears on each slide both infected and control suspensions containing approximately  $2.0 \times 10^6$  cells per ml. After drying at room temperature the slides were treated by different methods (Table 1). Treated slides were stored at  $-70^\circ\text{C}$  prior to staining for ImFl.

Three slides from each treatment were examined for residual infectivity. Cells were scraped off the slide into 0.5ml of phosphate buffered saline containing 0.75% bovalbumin and 50 ug per ml of gentamicin. The suspensions were subjected to one freeze-thaw cycle and 0.02ml aliquots were injected intracerebrally into 2-5 day old suckling mice. The material from each sample was injected into eight animals. Infected mice were monitored for survival for 15 days.

As can be seen from Table 1, treatment with acetone alone for 15 to 60 min was not sufficient for virus inactivation. Of the methods used, treatment with acetone for 30min with the subsequent heating of the preparations in a 0.3% solution of beta-propiolactone (BPL) at  $56^\circ\text{C}$  for 40 or 60min invariably provided full inactivation of viral infectivity for the suckling mice. Analogous results were achieved with SLE preparations from infected VERO cells.

Preparations were processed by the indirect staining technique for ImFl using commercially obtained fluorescein-labelled anti-human conjugate (Behring Diagnostics) and SLE immune human sera obtained during the 1975 outbreak in Ontario. Examination of smears by ImFl revealed strong fluorescing in both control and BPL treated preparations with no discernable decrease in the intensity of fluorescence in BPL treated preparations.

(A. Yabrov, H. Artsob and L. Spence)

TABLE I

INFLUENCE OF DIFFERENT TREATMENTS ON THE INFECTIVITY  
OF SLE VIRUS IN THE PREPARATIONS FOR ImFl.

Type of treatment	No. of survivors	No. of animals injected
Not treated	0**	8
Acetone 15 min	0	8
Acetone 30 min	4	8
Acetone 60 min	4	8
Acetone 30 min + BPL* 120 min, 37°C	7	8
Acetone 30 min +BPL 40 min, 56°C	8	8
Acetone 30 min + BPL 60 min, 56°C	8	8
Acetone 30 min + heating (dry) 56°C, 30 min	6	8
Acetone 30 min + heating (dry) 56°C, 60 min	6	8
Acetone 30 min + 18.5% formalin (60 min at room temperature)	7	8
18.5% formalin (60 min at room temperature)	7	8

\* BPL - 0.3% solution of betapropiolactone in phosphate buffer.  
37°C - incubator; 56°C - water bath

\*\* The numbers represent an average of 4-5 independent experiments  
for each treatment

REPORT FROM THE LABORATORY FOR ARBOVIRUS RESEARCH AND SURVEILLANCE, THE  
UNIVERSITY OF NOTRE DAME (UNDLARS)

An arboviral encephalitis surveillance program was begun in the late spring of 1977 for the State of Indiana. The surveillance program was initiated by the Indiana State Board of Health (ISBH) in response to various requests generated by the 1975 epidemic of St. Louis encephalitis in Indiana and other midwestern states.

The surveillance program is a joint operation between ISBH and the University of Notre Dame's Laboratory for Arbovirus Research and Surveillance (UNDLARS), a part of the Vector Biology Laboratory. The latter facility has been expanding its arbovirus work since the fall of 1974 and is establishing a P3 level facility. Financial support from the state is strictly limited to surveillance at this time.

Four 2-man teams from ISBH have been mist netting house (English) sparrows and starlings from mid-June to late August. Captured birds are banded, bled (< 1.0ml drawn), and released. Whole blood samples are sent to UNDLARS within 48 hours of drawing for serological screening. Serum samples are screened for SLE, EEE, and WEE by hemagglutination-inhibition; positive HI samples are being confirmed using virus neutralization tests.

Through late August, 38 HI positive birds, all house sparrows, had been detected (Table 1). These positive birds were noted in testing a total of 1837 serum samples. Included in these positives were several "recaptures" which gave significant seroconversion timing information as recapture occurred in the same site for each bird as its initial capture and banding. All HI positives have been for SLE only.

Collections will continue through September, 1977, at a moderate rate, and during the winter, birds captured in population movement studies will also be bled and tested. A complete report of this season's results will appear in the next issue of Arthropod-borne Virus Information Exchange.

Additional funds have been obtained for the next two years to initiate a virus isolation program to run concurrent with the avian serology program. Surveillance for La Crosse virus, and other arboviruses that may occur in Indiana, will be included with continued surveillance for SLE, WEE, and EEE.

(Paul R. Grimstad, UNDLARS, and Michael J. Sinsko, ISBH)

(Complete address; University of Notre Dame, Department of Biology,  
Laboratory for Arbovirus Research and Surveillance, Notre Dame, Indiana  
46556)

Figure 1. Counties in Indiana where SLE seropositive house sparrows were collected.

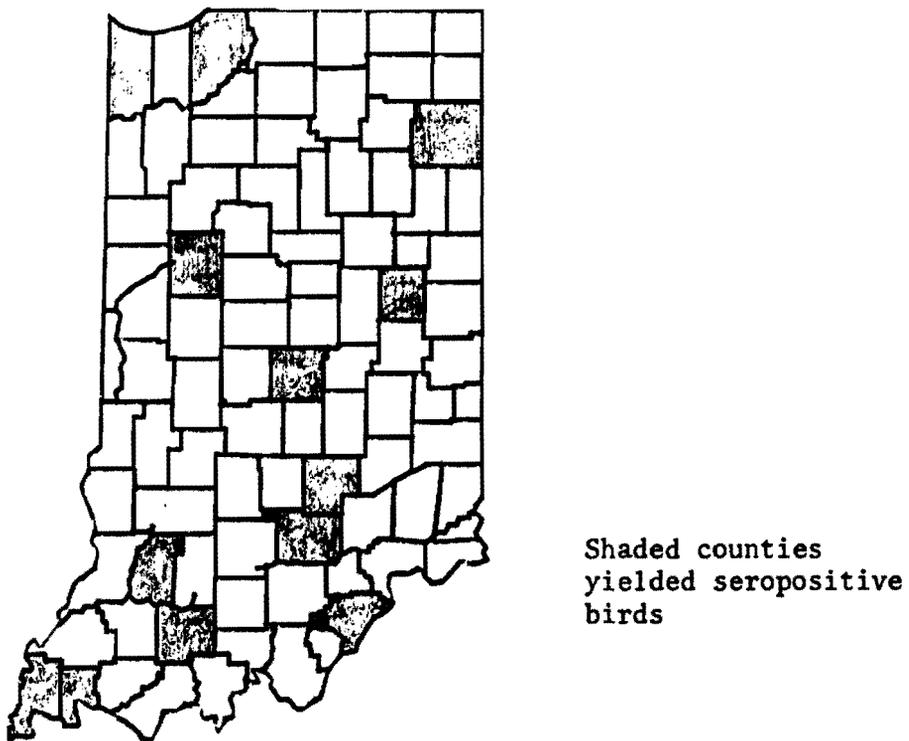


Table 1. Counties in Indiana where HI positive sparrows have been detected

COUNTY COLLECTED IN	AGE OF BIRD TESTED		
	ADULT ('76)*	ADULT ('77)	JUVENILE
ALLEN	4		
BARTHOLOMEW		1	5
CLARK	1		
DAVISS	2		3
DELAWARE	1	2	1
DUBOIS		1	1
JACKSON	1	2	4
LAKE		1	
LAPORTE			1
MARION	2	1	
POSEY			1
TIPPECANOE	1		
VANDEBURGH			2

\*Assumed to be last year's infection based on HI titers  $\leq 40$ ; '77 adults had titers  $\geq 80$ . All HI titers  $\geq 20$  were considered positive.

REPORT FROM THE STATE OF ILLINOIS  
DEPARTMENT OF PUBLIC HEALTH, DIVISION OF LABORATORIES  
2121 WEST TAYLOR STREET  
CHICAGO, ILLINOIS 60612

Studies of arbovirus activity in Illinois this year reflected low levels of virus transmission. Extensive bird serology studies were conducted on a repetitive basis in Cook County and central and southern Illinois and are presented in Table 1. Higher SLE antibody percentages were found in the southern part of the state than in more northerly areas. At this time, four human SLE cases have been confirmed: 2 in the southern region (5/F, 30/M), 1 in the central (4/M), and 1 adjacent to Cook County (42/M). The initial case (5/F) had an onset of June 16, approximately 5 weeks earlier than the first case of 1976.

SLE virus has been isolated from 2 pools of mosquitoes. The first was from a pool of 16 Culex salinarius collected in southeastern Illinois on June 3. The second was from a pool of 16 Culex pipiens complex collected on July 26 in Cook County. Approximately 16,500 mosquitoes in 450 pools have been tested for virus. These were predominantly Culex mosquitoes. Over 1600 bird bloods have also been tested for virus without an isolation. The vast majority of these were nestling and fledgling house sparrows.

Thus far, 4 human LaCrosse encephalitis infections have been confirmed in Illinois. All were children ages 4 to 6 years from a two-county area in north central Illinois where human cases appear annually.

(Dr. Gary G. Clark, and Mr. Harvey L. Pretula)

Table 1. Illinois Birds Tested for HI Antibodies to SLE, WEE, and EEE Viruses by Region, Date of Collection and Age.

REGION	Number (percent) with SLE HI titer $\geq 20$ /No. tested											
	20 Mar-30 Apr		1 May-28 May		29 May-2 Jul		3 Jul-29 Jul		31 Jul-20 Aug		TOTALS	
	Juv. All ages		Juv. All ages		Juv. All ages		Juv. All ages		Juv. All ages		Juv. All ages	
Cook County	0/8	0/36	0/11	0/46	2/370	4/525	0/340	1/401	0/70	0/80	2/799	5/1058
					(0.5)	(0.8)		(0.2)			(0.2)	(0.5)
Central Illinois			0/11	0/24	1/123	5/186	0/138	0/225*	3/222	3/269	4/494	8/704
				(0.8)	(2.7)			(1.4)	(1.1)	(0.8)	(1.1)	
Southern Illinois	0/1	1/20	2/141	14/320	6/330	30/580	2/456	2/641	3/313	3/417	13/1241	50/1978
		(5.0)	(1.4)	(4.4)	(1.8)	(5.2)	(0.5)	(0.3)	(1.0)	(0.7)	(1.0)	(2.5)
Totals	0/9	1/56	2/163	14/390	9/825	30/1291	2/934	3/1267	6/605	6/766	19/2534	63/3770
		(1.8)	(1.2)	(3.6)	(1.1)	(3.0)	(0.2)	(0.2)	(1.0)	(0.8)	(0.7)	(1.7)

\* 1 Adult House Sparrow with Antibodies to WEE.

REPORT FROM THE ZOOSES RESEARCH UNIT, DEPARTMENT OF PREVENTIVE  
MEDICINE, UNIVERSITY OF WISCONSIN, MADISON 53706.

Transmission of La Crosse (LAC) virus through eggs and saliva from venereally infected colonized *Aedes triseriatus*. During previous studies with colonized *Aedes triseriatus* LAC was detected in bursal contents of 52% of females dissected within 24 hours after induced mating with infected males. However, disseminated viral antigen was detected by FA in only 4.5% of non-lower-genital tract tissues of females dissected 3 to 21 days post-mating. Transmission through eggs and by bite to vertebrate was observed, but rates were not determined.

Subsequent studies have been conducted with similar F<sub>3</sub> generation mosquitoes and methods, except that blood meals (not given<sup>3</sup> to females dissected in the past study) were provided on mice at 12 day intervals for stimulation of egg production and detection of transmission ability.

High rates of disseminated infection and transmission of virus through saliva to mice (37 to 60%) were observed in several trials in which venereally infected colonized females had been given first blood meals on mice 5 to 7 days prior to mating with infected males. In another trial where the first blood meal was not provided until several days after mating the rate of disseminated infection was only 2%, in 1 of 52 surviving females.

In studies of transmission of virus through eggs of venereally infected females LAC virus was not found (by inoculation into mice of pools of eggs collected from 16 females) in first ovarian cycle eggs collected on ovicloths provided each female through day 6 post-mating, but virus was present in progeny reared from second ovarian cycle eggs deposited 7 through 14 days post-mating, from 4 of 9 females surviving and depositing eggs for study. The filial infection rate in progeny of these 4 females with LAC virus was 64% (179/279).

The absence of LAC virus in eggs from the first ovarian cycle and later appearance with disseminated infection and transmission in saliva indicates that transfer through eggs of venereally infected females involves transovarial transmission (rather than just transovum transfer of virus directly from bursal contents to eggs within the lower genital tract).

Studies of venereal transmission with field collected male *Aedes triseriatus* naturally infected with LAC virus. Collections of larvae were begun in April, 1977 from basal tree-hole oviposition sites in western Wisconsin which were found to contain larvae infected with LAC virus during 1976 or 1977. Collected larvae were reared to adults in the laboratory. Upon emergence, adults were separated into individual containers and held for detection of LAC virus from natural transovarial transmission.

Virus was detected in adult males by two methods: (1) testing triturated midlegs from one side of adult mosquitoes for the presence of virus in tissue culture or suckling mice and (2) pooling remnants of dissected males previously mated, and screening for virus in suckling mice followed, if positive by detection of individuals by FA technique.

Seven transovarially infected males were detected, three of which survived for mating to negative field-collected females (as demonstrated before mating by lack of transmission to suckling mice) by induced insemination. These 3 females were each held for approximately 30 days following mating and allowed to feed on suckling mice three times in transmission attempts. None of the three females transmitted virus and upon inoculation after death into suckling mice, none was shown to contain LAC virus. Their progeny, however, are being studied for possible LAC virus by inoculation into suckling mice. Reproductive tracts of these naturally infected males were dissected and triturated for plaque titration in Vero cell tissue culture and simultaneous intracranial inoculation of suckling mice.

The 1977 isolation rate of transovarially infected males from the five tree-holes previously found positive during 1976 was 0.49% (3/612). The rates from the six tree-holes positive during 1977 was 1.76% (7/397). Of the seven infected males found this year, three were from the only tree-hole found positive during both 1976 and 1977. The rate of trans-ovarial transmission in male A. triseriatus during 1977 was similar to that of females reared from the same larvae collections.

(Wayne Thompson and Laura Kramer)

Eastern equine encephalitis virus (EEEV) in birds

Studies on the susceptibility of House Sparrows (Passer domesticus) and Red-winged Blackbirds (Agelaius phoeniceus) to EEEV have shown that sparrows can survive an infecting dose of 70 Vero cell pfu and have a neutralizing antibody titer of 5-10 (2 birds) at 230 days. Attempts at detecting persistent virus in tissues of these birds by explants and co-cultivation with Vero cells have been negative.

Three groups of blackbirds in different physiological conditions were compared for their susceptibility to infection by 400 Vero cell pfu of EEEV. We artificially controlled day length to rapidly induce gonadal development. Two of 7 males survived in group I (minimal gonadal development); 0 of 5 males survived in group II (near maximal gonadal development); 0 of 5 females and 0 of 6 males survived in group III (natural gonadal development-caught April 25, 1977). The majority of the birds died by  $\leq 42$  hours and had viremia titers of at least  $10^{10.5}$  SMicLD<sub>50</sub>/0.1 cc at 17 hours post inoculation.

Oral and cloacal swabs were taken 17-112 hours post-inoculation. Five of the six birds tested had virus in their cloacal swabs, while one of two birds had virus in its oral swab. A 3-drop orthotolidine test indicated that three out of ten oral swab samples had occult blood. Six of eleven swab samples were positive for virus by suckling mouse inoculation. The test for occult blood has not been sufficiently sensitive to rule out contamination from occult viremic blood in the swab samples.

Oral inoculation of Red-winged Blackbirds under mosquito free conditions with  $10^{3.9}$  SMicLD<sub>50</sub> of EEEV resulted in 3 of 4 birds having a detectable viremia by 22 hours. Viremia titers were 1.6, 6.3, and 8.5 log<sub>10</sub> SMicLD<sub>50</sub>/0.1 cc blood. One contact control bird showed signs of illness on day 4 and died on day 5. Histological sections of this bird's brain showed glial cell proliferation and satellitosis, yet no virus was recovered from the blood from day 1-5. We are now trying to isolate EEEV from the throat and cloacal swabs and organ suspensions of this bird.

Laboratory and field studies of Powassan and other boreal viruses.

Ten snowshoe hares (Lepus americanus) without detectable Powassan virus (POWV) neutralizing antibody were inoculated with 0.5 ml of  $10^3$  TCLD<sub>50</sub>/0.5 ml of POWV by both the intramuscular and subcutaneous routes. These hares were bled daily for 9 days, every

2-3 days for 17 days, and monthly thereafter. These samples were tested to determine the magnitude and duration of both viremia (via suckling mouse titrations) and immune response to POWV (via serum neutralization tests in cell culture). The results are presented in Tables 1 and 2. These results indicate that hares can be infected, circulate virus, and develop high levels of neutralizing antibody. The maximum observed viremias are probably adequate to infect ticks.

Snowshoe hares were trapped near Rochester, Alberta, Canada (115° West longitude; 54° North latitude). Blood samples were taken, and serum was tested for POWV neutralizing antibody. Serum diluted 1:4 from 231 of 1343 hares tested neutralized POWV. This is further evidence for infection of hares in the field.

A blood sample taken from a white-footed deer mouse (Peromyscus maniculatus) trapped near Fort McKay, Alberta, Canada (111° West longitude; 57° North latitude) yielded an agent which is lethal for suckling mice when inoculated intracerebrally. Re-isolation has been successful. The agent passed a 22 nanometer filter and is chloroform sensitive. Unextracted antigen did not agglutinate chicken erythrocytes at room temperature at pH 7.2. Investigation on this agent is continuing.

#### Ecologic investigation of Aedes triseriatus breeding sites.

Studies concentrated on the community relationships and micro-environment of water-filled tree holes in southwestern Wisconsin. These tree holes are the sites of oviposition and larval development for the mosquito Aedes triseriatus Say, the principal vector of the La Crosse encephalitis virus (LAC).

Environmental measurements and water samples were collected every three weeks for two field seasons from tree holes within the LACV endemic area. Comparison samples were taken for part of one season from tree holes outside the endemic area. Water chemistry tests, microfauna identifications and population counts were performed for each sample.

Three microenvironmental characteristics of these tree holes were found to correlate with their general community structure: low dissolved oxygen levels (1 - 3ppm) low pH (5-7), and high levels of organic matter. The protozoan communities were found to be 1) unexpectedly stable throughout their active season, and 2) simple both in species composition and in trophic structure. Virtually all protozoans found were filter or detritus feeders. Most insect larvae found were also detritus and filter feeders; few active carnivores were found.

Artificial laboratory "tree hole" communities were inoculated with a variety of LACV doses, then sub-samples were taken at intervals and injected into suckling mice to determine whether LACV can infect or persist in protozoans. So far these experiments have yielded negative results.

There appear to be distinct correlations between microenvironmental conditions in a tree hole and the number of adult A. triseriatus which emerge from it. Definite conclusions must await completion of population studies on the insect.

(L. W. Turtinen, R. L. Zarnke, S. A. Wolf, C. Seymour, T. M. Yuill)

Table 1. Geometric mean of Powassan viremia in 10 snowshoe hares following Powassan inoculation.

	Days Post-Inoculation					
	0	1	2	3	4	5
Viremia*	∅	1.49	2.49	0.63	0.12	∅

\* Expressed as the log<sub>10</sub> suckling mouse intracerebral LD<sub>50</sub> per 0.03 ml of snowshoe hare blood.

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Table 2. Geometric mean of Powassan antibody response in 10 hares following Powassan inoculation.

	Days Post-Inoculation																	
	1	2	3	4	5	6	7	9	11	13	15	18	25	57	88	139	166	204
Antibody titer*	∅	∅	∅	1.0	2.2	8.5	7.4	21.7	24.7	24.6	162.8	217.5	35.6	43.7	32.6	48.2	6.2	4.0

\* Expressed as reciprocal of serum dilution which neutralized 100 LD<sub>50</sub> of Powassan.

REPORT FROM THE VECTOR-BORNE DISEASES DIVISION  
CENTER FOR DISEASE CONTROL  
P.O. BOX 2087  
FORT COLLINS, COLORADO 80522

Recent Observation on Human Colorado Tick Fever in  
Colorado - Surveillance, Risk of Exposure, and Morbidity Factors

In 1976, 220 cases of CTF were officially reported to the Colorado State Health Department. This total was lower than the previous year (261); but higher than the previous 5 and 16 year averages (180 and 143 cases respectively). Based on surveillance of campers in 1975 and 1976, fewer than 10% of expected cases are detected and reported; this suggests that from 2,000 to 3,000 human CTF cases occur annually in Colorado. In our experience many cases first sought medical assistance at great distances from their source of exposure (i.e., Connecticut, New Mexico, Arizona, etc.); physicians in nonendemic regions of the country are less likely to suspect or test for CTF virus infection.

Risk of Exposure to CTF:

Prospective studies of the risk of CTF virus exposure of campers in two eastern slope areas of the Colorado Rocky Mountains were conducted in 1975 and 1976. Each participant in the study was requested to submit ticks found on their person or clothing and to fill out and return forms by mail on illnesses acquired within 7 days of leaving the campground. Since no human specimens were requested as part of the study, evaluation for CTF illness was on clinical and epidemiological grounds only.

From May 21 through July 15, 1975, and in June 1976, campers in two study sites were recruited. Information obtained included:  
(a) number of days occupying the campsite, (b) number of persons noting

ticks on their person or biting them, and (c) histories of illness compatible with CTF within 7 days of leaving the campsite. Table 1 presents information obtained from campers recruited in Moraine Campground, Rocky Mountain National Park, 40 miles southwest of Fort Collins and individual campers at Cherokee Christian Ranch, Livermore, Colorado, 40 miles northwest of Fort Collins.

Risk of tick exposure and consequent CTF-like illness is illustrated by the 4,387 camper days surveyed in these two parks. Nineteen to twenty-three percent of ticks submitted from campers in these two areas were positive for CTF virus. A total of 139 tick exposures were reported, reflecting a rate of 3.2 known exposures per 100 camper days. Forty exposures resulted in known tick bites, for an approximate rate of 1 bite per 100 camper days. Projecting a 20% tick infection rate, one case of CTF would be expected for every 500 camper days; a projected number of nine cases would thus be expected in these two population groups. A total of thirteen CTF compatible or confirmed illnesses were reported within 7 days after leaving the study sites--a rate of one case per 313 camper days. In our experience, approximately 10% of persons with proven CTF cases were not aware of tick exposure and only half reported tick bites. This could account for the disparity between observed and projected cases.

In summary, follow-up in Cherokee and Moraine parks suggests that the risk of developing CTF for an individual camper is low (one chance per 300 to 400 camper days). That only 150-250 cases are detected each year in Colorado suggests that many infections are not confirmed by laboratory methods or are not reported. For example, in 1973 an

estimated 31,275 persons used Moraine Park for 5 days or more in April, May, and June, a total of 156,375 camper days. At a rate of one case of CTF per 400 camper days, a total of 391 cases may have resulted from use of this park alone. In 1975 and 1976 one CTF-compatible illness was detected per 316 camper days in Moraine Park study groups. Ticks submitted from two of these cases were positive for CTF virus, and one case was also confirmed as CTF by testing of patient's specimens.

#### Morbidity Factors:

In 1973-74, 43% of 222 confirmed cases were in males 20 to 60 years of age. Sixty-eight percent of patients consulted a physician, 20% were hospitalized, and 70% of patients over age 30 required more than 3 weeks to fully recover from their illness. The economic impact of CTF in humans may be estimated by considering physician costs in 68%, hospitalization in 20%, plus the 4-5 workdays missed by the 43% of males 20-60 years of age, and decreased work efficiency for more than 3 weeks in over two-thirds of older adult patients.

Leukopenia appears to be present in a high proportion of cases. A WBC of  $\leq 4,500$  was observed in 67% of cases studied in 1973-74 and in 82% of 50 patients studied in 1975-1976. Thrombocytopenia has been noted previously but this parameter has not been measured in most cases brought to our attention. Preliminary results on patients studied intensively by our laboratory suggests depressed platelet counts may be a universal response to CTF virus infection in humans. One CTF associated death with a probable consumption coagulopathy was confirmed

by our laboratory in 1971, and Eklund, in 1959, reported a fatality in a 4-year-old child with a bleeding diathesis. Morbidity resulting from effects of consumptive coagulopathy may be more frequent than has been reported but a fatal outcome appears to be rare according to our experience over the past 15 years.

(Jack D. Poland)

Table 1

Risk of tick exposure and CTF-like illness among campers in two Rocky Mountain eastern slope campgrounds May-July 1975 and June 1976.

Year	Campground	No. of Campers	Camper Days	CTF-like Illness*	Tick exposure		Ticks submitted	
					Total	No. Bites	No.	Pos-CTF
1975	Moraine	230	713	3	30	13	19	21%
	Cherokee	343	2058	6	56	19	13	23%
1976	Moraine	238	1184	3	30	4	16	19%
	Cherokee	72	432	1	23	4	36	19%
Totals		883	4387	13	139	40	84	20%

\* Occurring  $\leq$  7 days after leaving the study site.

REPORT FROM THE UNIVERSITY OF BRITISH COLUMBIA, FACULTY OF MEDICINE  
DIVISION OF MEDICAL MICROBIOLOGY, 2075 WESBROOK PLACE, VANCOUVER, B.C.  
CANADA V6T 1W5

REPORT FROM THE UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, CANADA, V6T 1W5.

St. Louis encephalitis virus, which was isolated from a fatal human case in eastern Canada during 1975, has been transmitted by wild-caught arctic Aedes communis mosquitoes after 13 days of extrinsic incubation at 13°C when 300 mouse LD<sub>50</sub> were fed to or injected intrathoracically into mosquitoes. Northway virus, which was isolated from A. hexodontus mosquitoes collected at Inuvik, N.W.T. during July 1976, replicated in arctic A. communis mosquitoes after 13 or more days of extrinsic incubation at 13 and at 23°C following intrathoracic injection of 30 plaque forming units (PFU) and antigen was detected in virus-positive mosquitoes by indirect immunoperoxidase and indirect immunofluorescence tests. After propagation of Northway virus in BHK21 tissue cultures, enveloped virions 89 nm diameter were observed budding into intracytoplasmic vesicles, in thin sections of cell pellets.

Mouse neutralizing antibodies to the snowshoe hare subtype of California encephalitis virus (Marsh Lake 23 strain) were found in sera from 6 of 23 arctic ground squirrels (Citellus undulatus) collected in the western Canadian arctic during summer 1977 including 3 of 8 at Fort Simpson, 2 of 6 at Hay River, and 1 of 9 at Inuvik, Northwest Territories. Of 192 mosquito pools comprising about 10,000 mosquitoes collected in southern Northwest Territories during June 1977, which have been processed to date, no virus has been recovered.

(D. M. MCLEAN)

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

Studies of Quaranfil group Viruses

The C5502 Johnston Atoll type virus isolated from Ornithodoros capensis ticks collected at Upolu Cay, Australia in 1966, belongs to the Quaranfil virus group as shown by cross CF tests. Cross immunity studies done in 1967 showed that mice vaccinated im with C5502 virus were immune to subsequent challenge ic with the homologous virus and the Johnston Atoll virus. Mice vaccinated im with the C5502 Johnston Atoll virus and challenged with Quaranfil virus by ic inoculation showed a mortality of 5/6. One sickened and recovered. If the mice were immunized by im inoculation and subsequent ic inoculation with the C5502 virus they were immune when challenged with the Quaranfil virus by ic inoculation.

In recent studies, six mice given Quaranfil virus by sc inoculation and subsequently challenged with the homologous virus given by ic inoculation, all became nervous and irritable after the usual incubation period but soon recovered. The six control mice died. Six mice immunized with Quaranfil virus by sc inoculation were challenged with LCM virus by ic inoculation. They sickened, similar to those receiving the Quaranfil virus following prior sc immunization and challenge with the homologous virus by ic inoculation. One mouse died but the other five recovered. The six mice that had been given the Quaranfil virus by both sc and ic inoculation were bled for serology studied. The serum pool was negative for LCM antibody by the indirect FA test. The Quaranfil virus was inoculated into Vero cell cultures. The cells harvested on the 3rd day were negative for LCM by the direct FA test. The symptoms observed in adult mice infected with Quaranfil virus by the ic route are similar to those in mice infected with LCM virus. Mice immunized with LCM virus by sc or sc and ic inoculation did not exhibit immunity to Quaranfil virus when the Quaranfil virus was given by ic inoculation.

The one way cross immunity observed between the Quaranfil virus and LCM virus suggests that the EGAR1113 strain of Quaranfil virus may be related to LCM virus group in a manner similar to Tacaribe virus. There are some studies which show cross immunity between members of the Tacaribe group of viruses, yet they are very different according to serological tests.

A review of the work done on EGAR1113 in Egypt shows that this virus multiplies in Ornithodoros and Argas ticks and is infectious for chickens. Pigeons showed antibody to the virus at the place the virus was obtained from ticks. With the long history of house mice in North Africa one can assume that LCM virus is present in Egypt. It has been reported that LCM virus multiplies in tick cell cultures so it is not unlikely that the virus might become adapted to ticks parasitizing birds. From my field studies of the Tacaribe group of viruses in South America I believe that they are variants of LCM virus obtained by natural passage in various species of rodents. I found house mice living in the camps of the field workers in Argentina and where workers were infected with Junin virus. We also know

that both LCM virus and Junin virus produce infections in man in the natural foci of Junin virus in Argentina. The recent outbreaks of LCM virus in children in Germany and the United States from exposure to pet hamsters carrying LCM virus shows how readily this virus becomes adapted to small mammals. There are many instances of infection of laboratory animals by LCM virus where wild house mice gain entrance into the animal quarters. Special genetic strains of mice used for maintenance of tumor cells were found to be infected with LCM virus and there are examples of laboratory contamination of cell cultures with LCM virus.

Cross immunity studies comparing Quarantil virus with LCM virus should be done using hyperimmune sera.

(Harold N. Johnson, Rockefeller Foundation, Retired)

#### Studies of Hughes group Viruses

Several variants of the Hughes group viruses have been isolated in our studies of bird tick viruses. The Punta Salinas virus isolated from ticks collected in Peru was passed in chick embryos to determine its growth pattern following amniotic and allantoic sac inoculation. The virus multiplied in chicken embryos inoculated by both routes, possibly better following amniotic sac inoculation. The titer obtained in the amniotic and allantoic fluids was low and no hemagglutination was observed. The Hughes virus was subpassed in chicken embryos by the allantoic sac route of inoculation and after three passages the titer was increasing. This was not high enough to expect hemagglutination by tests with guinea pig red blood cells and further passage will be done. These studies were initiated because in studies of a variant of Newcastle virus isolated in India and adapted to infant mice the virus did not produce hemagglutination when the virus obtained from infected brain tissue was used as the antigen. Subpassage in chicken embryos by the allantoic sac route resulted in an increase in titer and hemagglutination was produced by the infected allantoic fluid.

(Harold N. Johnson, Rockefeller Foundation, Retired)

#### Arbovirus Surveillance in California

This preliminary report for the 1977 season notes the return of some WEE virus activity after several very low years. There have been 6 WEE isolates from Culex tarsalis collected May 24 through June 30 in the Needles area of San Bernardino County and adjacent Arizona. As usual, Turlock and Hart Park viruses have also been prevalent. Seroconversion during April/May for SLE PRNT antibody was shown in 1 of 30 chickens near El Centro, Imperial County. No equine or human cases have been detected thus far. There have been 9 cases of Colorado tick fever documented thus far in 1977.

(R. W. Emmons, California State Department of Health; and W. C. Reeves, J. L. Hardy, University of California School of Public Health)

REPORT FROM THE DEPARTMENT OF BACTERIOLOGY  
UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA 95616

Studies were undertaken to develop an in vitro plaque assay for dengue virus using BHK-21 cells and a semisolid gum overlay. Dengue-2 (NGC strain) kindly provided by Dr. Walter Brandt was used in these studies. BHK-21 clone 15 cells were kindly provided by Dr. Joel Dalrymple. The tragacanth gum used was obtained from Fischer Scientific Co. and required substantial preparation before use. (The procedure used will gladly be supplied upon request.)

The stock virus which grew to low titer in BHK cells was adapted to BHK cells by serial passage at an m.o.i. of 0.01. Dengue titers increased with each successive passage reaching  $10^7$  PFU/ml by passage number 6. Throughout the passage history, plaque size was heterogeneous. No significant shift to larger or smaller plaques was noted. Growth curves of dengue on BHK cells revealed a nearly linear release of virus into the tissue culture fluid between 12 and 30 hours post infection with titers increasing from  $10^2$  PFU/ml at 12 hours to  $10^5$  PFU/ml at 30 hours. Titers obtained at 48 to 60 hours post infection were generally  $10^6$  PFU/ml.

Dengue grown in BHK cells was tested for biostability under a variety of conditions. The results of these studies are summarized below.

a) Multiple freezing and thawing.

3 times -70° C to 25° C                      no reduction in titer

b) Storage at 37° C and 4° C

<u>Temperature</u>	<u>Time</u>	<u>% Surviving</u>
4	24 hr	100 %
37	24 hr	0.008 %
4	48 hr	60 %
37	48 hr	<0.001 %

c) Effect of pH on storage at 4° for 12 hours.

<u>pH</u>	<u>titer</u>
8.0	$1 \times 10^4$
7.5	$8 \times 10^3$
7.0	$6 \times 10^3$
6.5	$3 \times 10^3$

Thus under appropriate conditions dengue virus is a relatively stable agent. It is important to realize that the results reported here apply only to NGC strain dengue-2. Other serotypes and strains may differ significantly in their biostability.

(J. S. Manning, J. K. Collins)

REPORT FROM THE PACIFIC RESEARCH SECTION, NATIONAL INSTITUTE OF ALLERGY  
AND INFECTIOUS DISEASES, P.O. BOX 1680  
HONOLULU, HAWAII 96806

Reported virus isolations from patients who die of dengue hemorrhagic fever or dengue shock syndrome are very rare - especially from tissues other than blood. We should like to report the recovery of dengue viruses from the liver at autopsy of 4 such patients in the hope that it will encourage others to seek similar data.

In each instance, virus was isolated by the inoculation of organ suspensions into mosquitoes (male Aedes albopictus or Toxorhynchites amboinensis). The mosquitoes subsequently were examined for virus infection by a fluorescent antibody technique and viruses typed by a complement fixation technique using infected mosquitoes as a source of antigen.

Case 1. Dengue type 2 virus was isolated from the liver and spleen of a Vietnamese child who died either 3 or 5 days after onset of illness. No virus was recovered from heart blood obtained at the same time. The blood contained plaque reduction neutralizing antibody against dengue type 2 virus at a titer of >1:1280.

Case 2. Dengue type 3 virus was isolated from the liver of a Burmese child who died 5 days after onset of illness. No virus was recovered from spleen, mesenteric lymph node, brain, or heart blood obtained at the same time, but virus was recovered from a serum specimen obtained 2 days before death.

Case 3. Dengue type 3 virus was isolated from the liver of a Burmese child who died 4 days after onset of illness. No virus was recovered from spleen, mesenteric lymph node, brain, or heart blood obtained at the same time.

Case 4. Dengue type 2 virus was isolated from the liver, spleen, and midbrain of a Burmese child who died 5 days after onset of illness. No virus was recovered from serum taken at time of death.

The time of autopsy varied from 1 to 22 hours after the time of death. None of the last 3 patients received infusions of plasma or blood prior to death. Information in this respect is not available for the first patient.

These virus positive cases were from a group of 20 patients studied in a similar manner. However, with the exception of one patient from whose serum dengue virus was recovered prior to death, it could not be determined if the other 16 patients actually had dengue infections.

(Leon Rosen, Pacific Research Section; Mi Mi Khin, Department of Medical Research, Rangoon, Burma and Tin U, Rangoon Children's Hospital, Burma)

REPORT FROM THE ARBOVIRUS LABORATORY  
 INSTITUT PASTEUR - NOUMEA - NEW CALEDONIA

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From March 1975 an epidemic of dengue fever was spreading over New Caledonia and other South Pacific islands. Here in Noumea we have studied sera coming from New Caledonia, Loyalty islands, New Hebrides, Wallie and Futuna and Solomon Islands.

In Noumea we have obtained 88 strains of dengue 1 virus between april 1975 and december 1976.

The present paper concern data obtained during the period between december 1976 and may 1977.

1° - Serological studies

Sera from patient suffering from dengue like fever were studied by IH test against dengue 1 antigens. So during the period 1 december 1976 - 31 th may 1977, 710 sera were studied. Among them 182 (23,63 %) were positive.

The monthly distribution of the cases appears as follow

Month	December	January	February	March	April	Mai	TOTAL
Confirmed cases	5	8	43	50	51	25	182
Suspected cases	28	29	129	228	170	126	710

1.1. Sex distribution

	total	Men %	total	Women %
cases with a IH positive serology	93	51,1	89	49,9
suspected cases	372		338	47,6

Men/Women ratio is 52/48 in New Celedonia. So the difference is not significant.

## 1.2. Age distribution

The Age of 584 patients is known

Age (years)	confirmed cases		suspected cases	
	Total	%	Total	%
0 - 1	1	0,66	31	5,3
5 - 9	2	1,32	31	5,3
10 - 19	34	22,51	104	17,8
20 - 29	29	19,20	122	20,8
30 - 39	32	21,19	143	24,5
40 - 49	22	14,56	70	12
50 - 59	17	11,25	44	7,5
+ de 60	14	9,27	39	6,7
	<u>151</u>		<u>584</u>	

Having regard to serology people between 10 and 39 years old have been particularly hit by dengue fever.

## 1.3. Geographic distribution

	confirmed cases		suspected cases	
	Total	%	Total	%
Noumea	159	87,36	648	91,26
La Foa	4	4,19	14	1,97
Koné	0	0	3	0,42
La Tontouta	0	0	3	0,42
Bourail	0	0	3	0,42
Kouaoua/Canala	3	1,65	12	1,69
Thio	4	2,19	8	1,13
Ouvea	12	6,59	19	2,68
	<u>182</u>		<u>710</u>	

In addition to Noumea, the main town, the only focus of some importance has been the loyalty island of Ouvea.

## 1.4. Ethnic distribution

665 persons are concerned by these data

	Confirmed cases		Suspected cases		Ethnic distribution in	
	Total	%	Total	%	Noumea	New Caledonia (Noumea excepted)
European	114	67,45	458	68,67	55,09 %	38,09 %
Melanesian	47	27,81	174	26,16	17,94 %	41,72 %
Polyneesian	3	1,77	9	1,35	14,43 %	11,97 %
Vietnamese	3	1,77	8	1,20	2,73 %	1,45 %
Indonesians	2	1,18	16	2,40	4,89 %	3,83 %
	<u>169</u>		<u>665</u>			

Dengue fever has been found among European and Melanesians people in a bigger proportion than foreseeable if considering ethnic distribution in New Caledonia.

2° - Virus isolation

2.1. Human blood samples

262 blood specimens collected from febrile patients were inoculated IC to suckling mice, 15 strains of virus were isolated, one of them after a blind passage (N° 1436). Incubation period in mice before paralysis was situated from 10 to 17 days.

Dengue viruses isolation from human blood samples, after inoculation to suckling mice, compared with results of IH tests on first and second sera of the same patients, appears on table I.

TABLE I

Code N°	Sex	Age	Race	First serum		Second serum	
				days after onset of fever	reciprocal of IH title	days after onset of fever	reciprocal of IH title
1384	M	35	European	5	80	30	80
1397	M	33	European	3	10	8	5 120
1434	F	28	Melanesian	unknown	40		
1435	M	30	Melanesian	unknown	10	20	10 420
1447	F	42	European	unknown	10	28	80
1467	F	20	European	unknown	10		
1476	F	22	European	5	10	11	160
1534	M	?	European	5	10		
1557	F	34	European	3	10		
1558	M	15	European	3	10	32	40
1607	F	40	European	3	40	15	5 120
1615	M	25	Melanesian	unknown	20	38	20
1659	M	42	Melanesian	unknown	80	19	1 280
1665	F	53	Melanesian	unknown	10	10	2 560
1673	M	43	European	4	80	22	1 280

112 first sera were inoculated intra thoracically to breeding *Aedes aegypti*. After 12 days thorax and abdomen of these mosquitoes were grinded and inoculated suckling mice, for attempts of isolation. With heads prints were made on slides and processed for virus research by direct immunofluorescent reaction (Gubler and Rosen technique).

3 dengue 1 strains have been isolated by this method table II. With this method isolation from sera with high rate of antibodies is possible (case n° 1414 had IH antibodies with 1/40960 rate).

TABLE II

Dengue virus isolated from human blood samples  
by intra thoracic inoculation to *Aedes aegypti*

Code N°	Sex	Age	RACE	First serum		Second serum	
				days after on- set of fever	reciprocal of :IH title	days after on- set of fever	reciprocal of :IH title
1414	M	?	European	-	40 960	-	-
1421	M	25	Melanesian	1	40	-	
1457	F	?	European	?	1 280	-	

81

### 2.2. Isolation from culicidae

One strain of virus was isolated from *Aedes vigilax*, this strain is not yet identified, but it is not related to dengue virus. *Aedes vigilax* is not classically a dengue virus vector.

G. LE GONIDEC - P. FAURAN

QUEENSLAND INSTITUTE OF MEDICAL RESEARCH AND  
DEPARTMENT OF SOCIAL AND PREVENTIVE MEDICINE,  
UNIVERSITY OF QUEENSLAND

The following summary of work in 1976-77 is extracted from the Annual Report of the QIMR. The complete report will be available, in limited numbers, on request to the Librarian of the Institute after November 1977.

" Epidemiology of arboviruses

The unit continued its long-term surveillance of arbovirus infection in northern and western Queensland. Less evidence of infection with the alphavirus Sindbis or flaviviruses related to Murray Valley encephalitis was found in 1976-77 in sentinel chickens held near Charleville, in south-west Queensland, than in any year since the mid-1960's. In contrast, both virus groups were active in the Flinders River basin of north Queensland. No obvious explanation for this pattern is evident in rainfall patterns, but further analysis is needed.

Much effort in this year went to identification of a large number of virus strains isolated in the Institute's field programme or submitted by collaborating scientists to the Institute as a WHO Collaborating Centre for Arbovirus Reference and Research. These studies continue to be rewarding in "new" viruses (e.g. the strain MI19334 isolated from Ixodes uriae from Macquarie Island) or in suggesting "new" host-virus associations (e.g. several isolations of Alfuy virus from Culex pullus.) This comment applies particularly to a series of over 100 virus strains submitted by scientists at CSIRO Division of Animal Health, Long Pocket Laboratories, which included at least five viruses not previously isolated in Australia and gave many new host and geographical records.

Serological tests of arbovirus infection in man are now offered by several public health or diagnostic laboratories, but the Institute continued to offer support as a reference laboratory. In the year under review it confirmed the first recognized case of overt Murray Valley encephalitis at a centre in Cape York Peninsula where subclinical infection with MVE virus has been frequent for at least twenty years. It also confirmed the occurrence of dengue over a wide area of New Guinea.

Waterbird populations in south-west Queensland, particularly at Lake Bullawarra, formed the main topic of study during the year. Numbers of waterbirds using the area were compared between two successive wet seasons and attempts were made to relate the considerable differences observed to climatic variation. Associated serological and virus isolation studies are incomplete but suggest little arbovirus activity in this area in the summer of 1977.

Three egret rookeries on the east coast, at Brisbane, Murwillumbah and Grafton were visited early in 1977. Blood obtained from birds in two of these showed no antibodies to arboviruses. Four Cattle Egrets and two Large Egrets were obtained from the Brisbane rookery and used in studies of experimental infection with Murray Valley encephalitis virus. Transient low-titre viraemia was followed by high-titre antibody persisting for at least several months.

Kowanyama was visited in January for the purposes of bird survey work.

(R.L. Doherty, J.G. Carley, D.J. Gravatt, Cheryl Filippich).

### Entomology

Mosquito biology and systematics. Type specimens were examined in overseas museums and institutions investigating aspects of mosquito biology and control were visited. Taxonomic studies were concerned principally with Culex, Aedes and Coquillettidia. A new undescribed Aedes was recognised from the Northern Territory. Eighteen species identified from the Lake Bullawarra study area included one new record for Queensland.

A mosquito problem at Gladstone was found to be due to Culex sitiens and Anopheles hilli and associated with a later stage of ecological changes in impounded tidal areas than problems investigated there previously. An hilli was recorded for the first time breeding on a coral cay, and Aedes aegypti was identified from Cloncurry and Clermont.

(Elizabeth N. Marks).

Mosquito ecology and mosquito-virus relationships. Much of the year was spent in analysing field data in the studies of Culex annulirostris, the mosquito vector of Murray Valley encephalitis. Nine manuscripts dealing with taxonomic, ecological and virological aspects are nearing completion. Additional progress was made in completing an experimental virus study of Aedes aegypti and with an analysis of feeding patterns of biting midges at Kowanyama.

New field projects were commenced to collect blood engorged Cx annulirostris and other mosquitoes in a study of natural feeding patterns at Charleville, and to complement an ornithological study relating to Murray Valley encephalitis at Lake Bullawarra, near Thargomindah.

Quarantine duties at the International Terminal involving surveillance for the vector of dengue fever, Ae aegypti, continued.

(B.H. Kay).

### Arbovirus immunology

An investigation has begun into cell mediated immunity to Ross River virus in the mouse. This work so far suggests that immune lymphocytes do not replicate when re-exposed to the virus and that exposure of spleen cells to viable virus possibly results in the death of a population of B lymphocytes. This could be the reason why detectable antibody production appears to lag 24 - 48 hours behind the appearance of cytotoxic lymphocytes.

(J.G. Aaskov).

### Laboratory studies of arboviruses

Structure and genetics of orbiviruses. Orbiviruses include pathogenic agents of man (Colorado tick fever), domestic animals (bluetongue of sheep and African horse-sickness) and native animals (epizootic haemorrhagic disease of deer). None of these diseases are known in Australia, but a number of orbiviruses have been isolated from mosquitoes and biting midges in the arbovirus isolation programme conducted by the Institute.

The orbiviruses are divided into groups depending on their serological reactions; the Australian viruses are classified into five groups. Of these, Eubenangee and Corriparta group viruses have also been found in Africa, and D'Aguilar virus is a member of the Palyam group found also in India and Africa; the Wallal and Warrego group viruses have so far been found only in Australia. The serological groups are composed of large numbers of viruses or virus types, and the antigenic diversity within these groups forms the basis of our studies of orbiviruses.

We are able to analyse the ribonucleic acid from orbiviruses by gel electrophoresis. The pattern of separation depends on the sizes of the RNA fragments and the orbiviruses show a characteristic pattern of ten pieces of RNA. Each of these RNA pieces is the genetic code for virus proteins synthesized in infected cells. Analysis of the RNA patterns of virus isolations reveal a diversity, even within a serological group, not suggested by normal serological tests. The pattern of RNA segments of viruses within serological groups can to some extent be correlated with the known antigenic properties of the proteins; for example, the RNA segments coding for proteins on the surface of the viruses are recognisably distinct in the gel electrophoresis pattern. The proteins on the surface would be involved in the neutralization of virus by antibody produced to them in infected animals.

In cells infected with two orbiviruses it is possible that genetic information could be exchanged and a hybrid arise with proteins derived from both parent viruses. Our work with temperature-sensitive mutants shows that recombination between related viruses occurs with high frequency. Isolation of a recombinant virus with nine pieces of RNA from one virus and one from another shows that exchange of RNA segments is possible in mixed infections. Antiserum to the recombinant virus neutralizes both parent viruses and suggests that the recombinant has surface proteins derived from both viruses. These are also indications that such interchange is possible between unrelated viruses. These results suggest that exchange of genes in mixed infections between orbiviruses may be an important mechanism in the evolution of new strains of orbiviruses.

(B.M. Gorman, P.J. Walker, Jill Taylor).

Biochemistry of viruses. When viewed in the electron microscope, Leanyer virus appears to be similar to viruses such as Ross River virus (alphavirus) and Murray Valley encephalitis virus (flavivirus). Other properties suggested that it could not be clearly defined as belonging to either group. After purification of the virus and comparison of its proteins with the known structure of alphaviruses and flaviviruses, no definite answer is possible. Using methods which yield pure reference viruses, the preparation of Leanyer virus yielded eight proteins. Some of these may be cellular contamination but the molecular weights of the three major proteins are not consistent with the structure of alphaviruses or flaviviruses.

(M.H. Symons).

### Acarology

The Acari (mites and ticks) are of actual or potential medical importance in three ways: tissue damage (e.g. scabies), blood loss (e.g. ticks) and vectorship of disease organisms (e.g. scrub typhus). For all that, it remains true that, even at the level of mechanical collection and description, much useful and relevant basic research has still to be done on the classification of the mite parasites of Australian vertebrates.

In the current year, the identification of mites collected on three expeditions in areas of widely differing habitat in Queensland (Bamaga, Kowanyama and Cherbourg) were finalised, providing much useful data for three papers.

The first (and largest) is least advanced but will treat the laelapine parasites of rodents. It will incorporate an important circum-Australian collection received from the Institute of Medical and Veterinary Science, Adelaide, and the accent this year has been on the illustration of material from "old endemic" rodents.

The second, on the blood-sucking rhinonyssine nasal mites of birds, was drafted. This brings together many previously unknown life stages, interesting extensions in geographical range and new host-records, specially in Ptilonyssus and Tinaminyssus, the commonest genera in passerine and non-passerine birds, respectively.

Four new Australian species of chiggers (larval trombiculid mites) were figured and described in the third paper (genera Guntheria and Trombicula). These were augmented with significant new host and distributional records of 36 known Australian species from nine genera, including specimens of the vector of scrub typhus (Leptotrombidium deliense) from Bamaga.

Work in the two other directions was completed. The available Oriental-Australian paramegistid material from snakes and lizards was prepared for publication. Only one genus (Ophiomegistus) is involved. It comprises five known species from Malaya, the Philippines, New Guinea and Australia. Further records of these, including the previously unknown males of two, were detailed. A new species was described from New Guinea.

Part 4 in the series "Oriental Mesostigmata (Acari)" was written. It includes 15 species from Malaysia and New Guinea, largely rhinonyssines from birds and spinturnicids from bats. "

(R. Domrow).

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REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF  
WESTERN AUSTRALIA, PERTH, WESTERN AUSTRALIA, AUSTRALIA

SUMMARY OF CURRENT VIRUS ISOLATIONS FROM MOSQUITOES OF THE  
KIMBERLEY OF NORTH-WEST  
AUSTRALIA

A. Virus Isolations

Table 1 shows the number of virus isolates from 1,000 pools comprising five mosquito species. Over the four year period the isolation rate has been maintained near 20%.

Table 2 shows the identification of 104 of the 197 isolates from the five vectors. It is interesting to note the first isolation of a strain of MVE from *Culex fatigans*. *Culex annulirostris* continues to be not only the dominant mosquito of North-West Australia, but to yield the greatest number of viruses.

Table 3 records the isolations by field trips. It is of interest to see the difference between the wet and dry season isolation rate. The rate for the dry season is still maintained at about 30%.

Table 4 shows the rainfall figures over this collection period for the town of Kununurra at the main study site. The particularly heavy rainfall in the 1973-74 wet season preceded an outbreak of MVE in South-Eastern Australia and was associated with significant MVE antibody conversion in sentinel cattle herds in North-West Australia.

B. Mosquito captures in North-West Australia (see Table 5)

The majority of mosquito captures were made by chicken-baited drum traps, although human biting catches, guinea-pig baited traps and a truck trap were also used to a limited extent.

Field trips to the study site were generally of a month's duration and timed to coincide with the beginning (November/December) and end (April) of each wet season. The one exception was a field trip made in June/July, 1976.

Most mosquito trapping was carried out in the Kununurra-Diversion Dam area around the lake and adjacent swamps created by the Diversion Dam. More limited trapping was also carried out in the estuaries, lower Ord River region and in the Irrigation Area to the immediate north of Kununurra.

*Culex annulirostris* was found to be numerically dominant in all areas, at all times of the year and by all capture methods. It also appears to be the most widespread species, having been captured regularly at all trap sites used.

(N.F. Stanley, A. Wright, S. Anderson, K.H.Chan, D. Britten).

Table 1.

Virus Isolations from Kimberley Mosquitoes

*Mosquito species from which viruses have been isolated (May, 1972 - November, 1976)*

Mosquito species	No. pools tested	No. Isolates	%
<i>Culex annulirostris</i>	774	154	20
<i>Aedeomyia catasticta</i>	111	37	33
<i>Aedes normanensis</i>	16	3	19
<i>Aedes tremulus</i>	6	1	17
<i>Culex fatigans</i>	96	2	2
TOTAL	1,003	197	19.6

Table 2

## Virus Isolations from Kimberley Mosquitoes

Current identification of 104 of the 197 isolates from 5 vectors referred to in Table

VECTOR	MVE	Kunjin	Sindbis	Koongol	Wongal	Non-HA Koongol gp to be typed	Corriparta	Rhabdov	Non-HA to be typed	Total
<i>Culex annulirostris</i>	18	20	10	3	8	17	1	2	14	93
<i>Aedeomyia catasticta</i>							6	1		7
<i>Aedes tremulus</i>		1								1
<i>Aedes normanensis</i>			2							2
<i>Culex fatigans</i>	1									1
Total	19	21	12	3	8	17	7	3	14	104

Table 3

## Virus Isolation from Kimberley Mosquitoes

Trip No.	Period of Mosquito Collection	Total pools processed for virus isolation	No. viruses isolated	No. viruses characterized or partially characterized	GROUPS						Non-HA to be typed		
					B		A	K'gol		Corriparta		UNGPD	
					MVE	Kunjin	Sindbis	Koongol	Wongal	To be typed		Corriparta	Rhabdovirus
1	May-June 1972 D	33	7	7	3	1	1	2					
2	Nov. 72 - Mar. 73 W	70	1	1						1			
3	Apr-May 1973 D	112	37	37	2	10		3	6	11	2	2	1
4	Nov-Dec 1973 W	63	5	5							4	1	
5	Mar-Apr 1974 D	207	50	49	12	10	11			6			10
6	Nov-Dec 1974 W	99	19	5	2								3
Totals		584	119	104	19	21	12	3	8	17	7	3	14

D = Dry Season

W = Wet Season

Table 4  
Kununurra Rainfall Figures  
(in mm)

MONTH	71/72	72/73	73/74	74/75	75/76	1962-1975 averages
OCTOBER	80	53	56	6	179	31
NOVEMBER	17	73	291	99	94	71
DECEMBER	159	66	174	102	141	98
JANUARY	96	156	255	166	183	201
FEBRUARY	154	102	300	223	348	221
MARCH	245	251	318	143	236	163
APRIL	71	58	80	53	nil	24
MAY	nil	nil	15	nil	N.A.	15
TOTAL	822	759	1,489	792	1,181	824

Table 5  
Ord River Mosquito Captures, 1972 - 1977

Species	No. Captured	% Total
<i>Culex (Culex) annulirostris</i>	64,322	83.63
<i>Aedeomyia catasticta</i>	5,137	6.68
<i>Culex (Culex) fatigans</i>	4,568	5.94
<i>Aedes (Ochlerotatus)</i> <i>normanensis</i>	871	1.13
<i>Anopheles (Cellia) annulipes</i>	411	0.53
<i>Mansonia (Coquillettidia)</i> <i>xanthogaster</i>	285	0.37
<i>Culex (Culiciomyia) pullus</i>	231	0.30
<i>Aedes (Finlaya) notoscriptus</i>	207	0.27
<i>Aedes (Macleaya) tremulus</i>	159	0.21
<i>Aedes (Ochlerotatus) vigilax</i>	136	0.18
Other species (eighteen)	581	0.76
TOTAL	76,908	

REPORT FROM THE VIROLOGY DEPARTMENT, NAMRU-2, JAKARTA DETACHMENT  
JAKARTA, INDONESIA

ENCEPHALOPATHY ASSOCIATED WITH DENGUE INFECTION

Rather strict criteria for the clinical diagnosis of dengue hemorrhagic fever have been outlined by the WHO Technical Advisory Group<sup>(1)</sup> and are used by most physicians working with this disease. These include fever of 2 to 7 days duration, various hemorrhagic manifestations including at least a positive tourniquet test, hepatomegaly, thrombocytopenia and hemoconcentration. In our experience, however, strict adherence to these criteria may result in many dengue infections going undiagnosed. The majority of undiagnosed dengue patients suffer from mild forms of the disease, but a significant number may have severe and even fatal infections. Included in this latter group are patients presenting with signs of encephalopathy with or without the signs suggestive of dengue hemorrhagic fever. Clinically, patients with encephalitic signs may range in severity from very mild with only decreased alertness and/or convulsions to very severe with all of the clinical signs and symptoms compatible with acute encephalitis. Here we present 2 cases representing the mild and severe forms of encephalopathy with and without the signs associated with dengue hemorrhagic fever.

Patient R, a one-year-old well nourished Indonesian male admitted on 20 May, 1976, with a history of 3 days of fever and constipation for 4 days. Three hours prior to admission he had general convulsions 3 times at intervals of 15 minutes. The seizure lasted for approximately one hour. On admission the patient was somnolent and dyspnoeic with a body temperature of 39°C. Respiration rate was 60 per minute, pulse 160, funduscopic examination was normal, heart and lungs were normal, the liver was enlarged to  $\frac{1}{4}$  of the distance between the costal margin and the mid-line and the lumbar puncture was normal with NaCl 725 Mg%, chloride 440 Mg%, glucose 66 Mg% and total protein 64 Mg%. Both upper and lower extremities were spastic. The remainder of the physical examination was not remarkable. Laboratory examination showed a hematocrit of 35% (within normal limits for Indonesian children), hemoglobin of 11.8 gm%, platelets of 309,000 per cmm and leucocytes of 11,800 per cmm. The tourniquet test was positive and later that morning petechiae were observed on the right hand. The working diagnosis was encephalitis. Treatment consisted of Intra-venous fluid drip of half strength normal saline in 5% glucose at 16 drops per minute; procaine penicillin 400,000 U.I. I.M., chloromycetin 4 x 200 mg I.V., hydrocortisone 3 x 25 mg I.M. and phenobarbital 3 x 12.5 mg.

The patient's clinical condition continued to deteriorate and on 21/5/76 he was still semicomatose with spastic tetraparesis. Rales were heard in both lungs. He still had petechiae on his right hand. On 22/5/76, X-ray showed bronchopneumonia duplex. He was still semicomatose with spastic tetraparesis. At 1200 he had an apneic attack and died at 1205.

A blood sample taken on 20/5/76 had no detectable dengue HI antibody when tested with 8 units of dengue type 1 antigen. Dengue was confirmed, however, by isolation of dengue type 3<sup>2</sup> virus from this serum by intrathoracic inoculation of mosquitoes.

Patient H, a 3-year-old well nourished Indonesian female admitted on 4 February, 1977, with a history of 3 days of low grade fever. Eighteen hours prior to admission she had general convulsions which lasted for about 40 minutes. She subsequently had 2 more attacks, the last one an hour and a half before admission. There was no history of trauma and this was her first experience with convulsions. On admission, she was somnolent, had a temperature of 37.2, blood pressure of 90/70 and pulse rate of 140 per minute. The heart and lungs were normal, liver was not palpable and the lumbar puncture was normal with NaCl 725 Mg%, chloride 440 Mg%, glucose 64 Mg% and total protein 60 Mg%. The hemoglobin was 12 gm%, hematocrit 40% and platelets 284,000 per cmm. The tourniquet test was not done, but petechiae were observed on her right arm. The working diagnosis was encephalopathy. She was treated with procaine penicillin 400,000 U.I., I.M., Chloramphenicol 4 x 175 mg I.V., cortisone 3 x 50 mg I.M. and intra-venous fluid drip with Lactated Ringers solution.

The next day the patient went into profound shock with undetectable blood pressure and a weak pulse of 168 per minute. She was still semicomatose and her temperature was 38.2. She had cold extremities and cold clammy skin. Periodic examination of hemoglobin and hematocrit on this day showed constant values of 12.5 gm% and 40-42% respectively. The platelet count decreased, however, from 258,000 to 125,000 per cmm. The shock was overcome by intra-venous push with Lactated Ringers solution. On the third day of hospitalization the patient showed remarkable improvement with a strong pulse and blood pressure, but the platelet count continued to decrease to a low of 31,000 per cmm on the fourth day of hospitalization. At that time she was mentally alert and continued to improve. There were no other hemorrhagic manifestations. She was discharged on 11 February with no neurological sequelae.

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<sup>2</sup> The virus was typed by Dr. L. Rosen, Pacific Research Section, Honolulu, Hawaii

Serology showed a significant rise in dengue HI antibody between the acute and convalescent sera with titers of 1:80 and 1:2560 using 8 units of dengue type 1 antigen. Dengue type 2 virus was isolated from the acute serum.

These two patients, both virologically confirmed as dengue, represent the severe and mild forms of encephalopathy. The first showed none of the signs and symptoms normally associated with dengue hemorrhagic fever whereas the second was rather typical severe DHF/DSS, but with encephalitic symptoms. In addition to these 2 patients, we have serologically confirmed as group B arbovirus infections, many other patients with a wide range of encephalitic symptoms. It is highly probable that these were also dengue infections since the only other group B arbovirus in Jakarta, Japanese B encephalitis, is not known to be maintained in an urban environment in the absence of swine and mosquitoes of the Culex tritaeniorhynchus complex. This virus has only been isolated from West Jakarta where pig farms and rice fields provide the ecological requirements for Japanese B encephalitis virus transmission.

(D. J. Gubler, Sumarmo and H. Wulur)

#### References

1. Technical guides for diagnosis, treatment, surveillance, prevention and control of dengue hemorrhagic fever, World Health Organization, 1975.

(Mailing address from the United States: Dr. D. J. Gubler, NAMRU Jakarta, Department of State, Washington, D.C. 20520)

REPORT FROM THE VIROLOGY DEPARTMENT OF  
SCHOOL OF TROPICAL MEDICINE  
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Haemagglutination-inhibiting antibodies against Gr.A and Gr.B viruses in sera of certain animals.

Blood samples of 100 cattle, 97 buffaloes, and 100 pigs were collected from a slaughter house in Calcutta between September 1973 and March 1974. It may be mentioned here that an epidemic of Japanese encephalities (JE) broke out in the state of West Bengal in 1973. All the animals belonged to the three States of India, namely Uttar Pradesh, West Bengal and Haryana. The sera of these animals were tested for the presence of haemagglutination inhibiting (HI) antibodies against Chikungunya (Chik), Dengue type 2 (D2), JE and West Nile (WN) virus antigens.

HI titers are shown in the following table:

<u>ANIMAL</u>	<u>NO.OF SERA</u>	<u>NO. POSITIVE IN DILUTION OF 1 IN 20 OR ABOVE AGAINST ANTIGENS.</u>					<u>ONE OR MORE GR.B</u>
		<u>CHIK</u>	<u>D2</u>	<u>JE</u>	<u>WN</u>		
CATTLE	100	6(6%)	4(4%)	19(19%)	16(16%)	19(19%)	
BUFFALO	97	10(10.3%)	0	19(19.5%)	11(11.3%)	20(20.6%)	
PIG	100	8(8%)	11(11%)	24(24%)	23(23%)	24(24%)	

(S.K.Chakravarty, M.Soman, K.K.Mukherjee, J.K.Sarkar,  
M.S.Chakravarty).

Vector Potential of *Phlebotomus papatasi* to Phlebotomus Fever Viruses

Experimental studies on laboratory reared *P. papatasi* have been undertaken to understand the rate of engorgement, infection threshold, persistence, multiplication and transmission of SF-Naples and SF-Sicilian viruses.

In vitro and in vivo methods were employed to infect the sandflies. In in vitro the females were allowed to feed on defibrinised chick blood containing 4-5 dex of virus through freshly prepared chick-skin membrane. In in vivo suckling or young viraemic hamsters circulating 5-6 dex of virus were exposed overnight to the females for feeding. The females were processed individually and in pools from the day of engorgement up to 13th day post-feeding. The observations are summarized as under:-

The rate of engorgement in in vivo and in vitro was 80 per cent, and 40-50 per cent respectively. It was also found that the persistence of virus was probably dependant on either mode of engorgement or the source of infection, as the females which had in vitro blood meal could not retain the virus more than a day, where as, female fed on viraemic host could retain the virus up to 10 days.

The females having no traces of blood in the abdomen were also processed and were found positive for the virus. This indicates that though the females could not get engorged they might have probed the viraemic host and got infected. The males released along with the females while feeding, were found positive for the virus. It is difficult to speculate at this stage the source of infection in males.

The females infected with SF-Naples could retain the virus up to 10 days while those infected with SF-Sicilian could retain and transmit the virus by bite to suckling hamsters up to 5 days post feeding.

The detail studies are in progress.

(M.K. Goverdhan and G.B. Modi)

REPORT FROM THE VIROLOGY DEPARTMENT, US ARMY MEDICAL COMPONENT,  
ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES, BANGKOK, THAILAND  
A SPECIAL OVERSEAS ACTIVITY OF THE WALTER REED ARMY INSTITUTE OF RESEARCH  
(FORMERLY SEATO MEDICAL RESEARCH LABORATORY, BANGKOK, THAILAND)

Isolation of dengue viruses from patients with hemorrhagic fever has been attempted in Bangkok, Thailand, intermittently over the past 14 years. Compilation of the isolates showed that dengue 2 was prevalent each year isolation was attempted and that approximately 50% of the isolates were of this serotype. Until 1976 the remaining half consisted of approximately equal numbers of dengue 1 and 3. Dengue 4 was isolated during the years 1962-1965 but made up less than five percent of the total isolations. This serotype was not isolated again until 1975. In 1976 it largely replaced dengue 1 and 3.

In Bangkok, dengue, which is a seasonal illness, occurred much later than usual in 1976, and the disease continued at a high incidence into 1977. It is interesting to speculate that the replacement of dengue 1 and 3 by dengue 4 may relate to the changing incidence of the disease. A similar shift in incidence occurred during 1977 throughout the kingdom of Thailand. Attempts are being made to document the dengue serotypes that are prevalent in provinces other than Bangkok.

From 1973-1976 isolations were attempted from patients with hemorrhagic fever hospitalized at the Children's Hospital of Bangkok. These patients were seen and graded in a consistent manner using the following criteria of Suchitra Nimmannitya, et al., (Am. J. Trop. Med. Hyg., 18: 954-951, 1969):

- Grade 1: Fever accompanied by non-specific constitutional symptoms. A positive tourniquet test was the only manifestation of hemorrhage.
- Grade 2: Fever accompanied by skin hemorrhage or other bleeding, such as from the nose or gums.
- Grade 3: Circulatory failure manifested by a rapid, weak pulse, narrowing of the pulse pressure (less than or equal to 20 mm.Hg.), or hypotension.
- Grade 4: Blood pressure and pulse are undetectable.

From 631 patients there were 103 dengue viruses isolated (16.3%). Of these, 92 have been identified by plaque reduction neutralization tests employing LLC-MK2 cells and standardized monkey anti-dengue 1, 2, 3 and 4 sera. Each case was classified as either a primary or a

secondary dengue infection according to the criteria of Winter, et al., (Am. J. Trop. Med. Hyg., 17: 590-599, 1968). Patients with convalescent hemmagglutination inhibition titers of 1:640 or greater to two or more dengue antigens were considered to have secondary infection. Those with titers of less than 1:640 were assumed to have primary infection. There were 86 cases which could be accurately graded and in which the primary or secondary nature of the infection could be determined. Of these, 13 (15.1%) were dengue 1, 45 (52.3%) were dengue 2, 20 (23.3%) were dengue 3, and 8 (9.3%) were dengue 4. There were 21 patients with serological evidence of primary dengue infection. The majority of the primary infections caused mild hemorrhagic disease; however, shock did occur in primary infections caused by each serotype. There was no significant difference between dengue types with regard to the severity of primary infection; however, the number of patients examined was small.

Dengue viruses were isolated from 65 patients with secondary infections. Dengue 2 was isolated from 63.6% of the secondary cases. There was a significant difference between the proportion of dengue isolated from primary and secondary dengue cases (Chi square = 14.1625, p 0.001). Shock observed in secondary dengue patients did not appear to be associated with a particular dengue serotype.

Submitted by Robert McNair Scott, Ananda Nisalak and Surapee Seridhoranakul, SEATO Medical Research Laboratory and Suchitra Nimmannitya, Children's Hospital, Bangkok, Thailand.

August 1977

(NOTE: At the present time the mailing address of Dr. Robert McNair Scott from the United States remains Department of Virology, United States Army Medical Component, Southeast Asia Treaty Organization, APO San Francisco 96346)

TABLE 1

DENGUE VIRUS ISOLATION FROM HEMORRHAGIC FEVER PATIENTS  
IN BANGKOK 1962-1976

Year	Dengue Serotype				Total
	1 No. (%)	2 No. (%)	3 No. (%)	4 No. (%)	
1962	14 (35.0)	16 (40.0)	9 (22.5)	1 (2.5)	40
1963	6 (20.7)	7 (24.1)	16 (55.2)	0	29
1964	23 (25.0)	46 (50.0)	20 (21.7)	3 (3.3)	92
1965	0	5 (83.3)	0	1 (16.7)	6
1969	1 (20.0)	1 (20.0)	3 (60.0)	0	5
1972	0	14 (100)	0	0	14
1973	5 (22.7)	13 (59.1)	4 (18.2)	0	22
1974	7 (22.6)	14 (45.2)	10 (32.3)	0	31
1975	3 (13.6)	12 (54.5)	6 (27.3)	1 (4.6)	22
1976	0	8 (47.1)	2 (11.8)	7 (41.2)	17
Total	59 (21.3)	135 (48.7)	70 (25.3)	13 (4.7)	277

TABLE 2

DENGUE TYPES ISOLATED BY SEVERITY OF DISEASE  
PRIMARY CASES 1973-1976

Dengue Type	Dengue Hemorrhagic Fever		Total
	Without Shock* No. (% of Type)	With Shock** No. (% of Type)	
1	6 (85.7)	1 (14.3)	7
2	2 (66.7)	1 (33.3)	3
3	7 (87.5)	1 (12.5)	8
4	1 (33.3)	2 (66.7)	3
Total	16 (76.2)	5 (23.8)	21

\* Grade 1 and 2

\*\* Grade 3 and 4

TABLE 3

DENGUE TYPES ISOLATED BY SEVERITY OF DISEASE  
SECONDARY CASES 1973-1976

Dengue Type	Dengue Hemorrhagic Fever		Total
	Without Shock* No. (% of Type)	With Shock** No. (% of Type)	
1	3 (50)	3 (50)	6
2	20 (47.6)	22 (52.4)	42
3	8 (66.7)	4 (33.3)	12
4	3 (60)	2 (40)	5
Total	34 (52.3)	31 (47.7)	65

\* Grade 1 and 2

\*\* Grade 3 and 4

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

The purpose of **this** communication is to stress the occurrence of dengue myocarditis with acute or delayed onset following an influenza-like illness which may lead into chronic cardiac disease with or without heart failure.

Serological evidence strongly points to the cause and effect relationship between dengue and myocarditis. The significance of this preliminary report is to create awareness among medical and public health **practitioners** that dengue virus should be included in the differential diagnosis of myocarditis following a history of influenza-like fever.

SEROLOGICAL PROCEDURES USED:

Neutralization test (NT) in Vero cell line cultures was utilized for the detection of infection due to any of the 6 serotypes of Coxsackie B viruses.

Hemagglutination-inhibition test (HI) by the microtiter method was used for the detection of infection due to dengue and chikungunya viruses.

ANTIBODY TITERS USED AS INDEXES OF SIGNIFICANCE:

Serological confirmation of the specific viral etiology of an infection was made if the increase in antibody titer is equal to or greater than four-fold between the acute or early and convalescent or late serum samples.

In cases where only the late serum sample is available for testing, Table I shows the base line antibody titers used. For dengue infections, antidengue HI titer of at least 1:640 or greater was used instead of at least 1:1280 which is the lower limit of significance among Filipinos because of two reasons: firstly, majority of cases tested gave blood samples after a month or longer from the onset of illness, in which case, a minimum of two-fold decline in antibody titer is expected, and secondly, a recent survey (1972 to 1975) on HI antibodies against dengue covering an extensive area of the Philippines shows that 99.06% of 2,503 serum samples have an HI titer of 1:320 or less (1).

For chikungunya, an HI antibody titer of at least 1:20 or greater was considered based on our observations since 1970 to date that HI test against chikungunya antigen consistently yielded less than 1:20 results.

For infections due to any of the six serotypes of Coxsackie B viruses, an NT antibody titer of at least 1:40 or greater was considered based on the results of the serums of apparently healthy normal Filipinos.

#### ISOLATION OF VIRUSES:

Almost all the viral myocarditis patients went to the hospitals for consultation due to prolonged ailments of the heart following a history of an influenza-like illness. Such a condition precluded attempts at dengue virus isolation from the blood of patients.

#### RESULTS:

Preliminary results show that of 46 cases of myocarditis or other symptoms referable to the heart 20% (9 cases) were positive for arbovirus B infection, most probably dengue, 26.1% (12 cases) were caused by Coxsackie B viruses, none was attributable to chikungunya virus, and 53.9% (25 cases) were negative to all the viral antigens (Table 2). No dual infection was observed.

The six serotypes of Coxsackie B viruses in the order of their frequency among the 12 positive cases due to Coxsackie B viruses are shown in Table 3. Serotypes 3 and 4 were the two most commonly occurring, and serotype 5 was not involved in any of the cases. These frequencies did not parallel the frequencies of isolations of these viruses among normal Filipino children.

One of the two control groups studied was 10 cases of rheumatic carditis with 5 of them showing NT antibody titer of at least 1:40 to equal or greater than 1:160 to one serotype of Coxsackie B virus and the 5 others had uniformly less than 1:20 against all the six serotypes. As shown in Table 4, none of the rheumatic carditis cases was found with significant HI antibody titer against dengue or chikungunya viruses. HI antidengue titers ranged from 1:10 to 1:160 while antichikungunya was uniformly negative (less than 1:20).

The nine cases of myocarditis that were serologically confirmed as arboviral in etiology, most probably dengue, are detailed in Table 5. Two of them were referred as Stokes Adams and 7 was viral myocarditis. Only one was at the acute stage while the rest were cardiac disorders as a sequel to "dengue-like" but not chikungunya fevers. These latter cases confirm the report of Obeyeskere and Hermon that arboviral heart disease can lead to unexplained chronic cardiac disorder with or without heart failure.

From these preliminary observations relating dengue but not chikungunya virus to heart disease among Filipinos in addition to the already established enteroviral etiology, notably the six serotypes of Coxsackie B virus, it is of paramount importance to be aware of such a cause and effect relationship since dengue fevers are so common in the Philippines. Even with dengue virus added to the Coxsackie B viruses as being responsible locally for heart disease, undoubtedly half of the case of myocarditis remain undiagnosed. The role of other viruses known to cause cardiac complications and are widespread in occurrence in the Philippines such as measles, mumps, poliomyelitis, varicella, and influenza, should be studied further.

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Submitted by : Veronica F. Egan, Marieta R. Maaba, Jessie Mabel Gasmen and Elena F. Almagro.

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Table 1

Antibody Titers Used as Indexes of Significance for  
Single Serum Serology

Virus	Serological Test	Reciprocal of Antibody Titer
Dengue 2	HI	$\geq 640$
Chikungunya	HI	$\geq 20$
Coxsackie Bs	NT	$\geq 40$

Table 2

Viral Etiology of 46 Cases of Myocarditis

Virus	Total Number Examined	Number Positive	Percent Positive
Dengue 2	46	9	20
Coxsackie Bs	46	12	26.1
Chikungunya	46	0	0
Total % Positives	46	21	46.1
Total % Negatives	46	25	53.9

Table 3

Frequency of Coxsackie B Serotypes in Twelve Cases of Serologically Confirmed Coxsackie B Myocarditis.

Coxsackie B Serotype	No. of Positive Cases
3	4
4	4
1	2
2	1
6	1
5	0

Table 4

Serological Responses of Ten Cases Rheumatic Carditis

Virus Antigen	Number Examined	Number Positive
Dengue 2	10	0
Chikungunya	10	0
Coxsackie Bs	10	5

Table 5

HI Antidengue 2 Titers of the 9 cases of  
Dengue Myocarditis

Case Identification	Clinical Impression	Age Years/Sex	Days from Onset of Illness	Reciprocal of HI Antidengue 2 Titer
9027-1	VM*	20 - F	46	640
9909-1	VM	1.5-M	94	1280
9907-1	VM	80 - F	90	1280
9474-1	SA**	11 - M	2	640
-2			18	1280
-3			29	640
9342-1	VM	3 - M	83	640
-2			94	320
9900-1	SA	14 - F	3487	640
-2			3501	640
9919-1	VM	8 - F	217	320
			230	1280
10144-1	VM	8 - F	188	640
-2			202	320
10132-1	VM	2 - F	187	640

\* - Viral myocarditis

\*\* - Stokes Adams

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE  
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1. Infectivity titration of dengue viruses by focus counting and its application to neutralization tests

Focus counting on BHK21 cells either by indirect immunofluorescent or by peroxidase-anti-peroxidase technique has been used in the rapid infectivity titration of all 4 types of dengue viruses (DEN-1,2,3, and 4) in this laboratory as reported. Both methods gave essentially the same focus counting unit (FFU) titers which were comparable to those obtained by suckling mouse intracerebral (SMIC) LD<sub>50</sub> titration. The titers were also similar to those obtained by plaque counting (PFU) on LLC-MK<sub>2</sub> cells with the exception of DEN-2 (Table 1).

The focus counting method was applied to assay neutralizing antibody against dengue viruses. Serially diluted rabbit immune sera were mixed with an equal volume of virus specimen diluted to give about 200 FFU/0.1 ml. The mixture was incubated at 28°C for 2 hr and then inoculated to BHK21 cells to form foci (0.1 ml/well on Lab-Tek 8-chamber slide). Percent reduction of focus count was plotted on a probit chart against the logarithm of serum dilution, which gave straight lines. From these lines we can estimate the 50 % focus reduction titer of the serum, i. e. the serum dilution which reduced the focus count to 50 % of the control. Table 2 shows neutralizing titers of anti-dengue sera obtained by this method (FRNT) and 50 % plaque reduction titers (PRNT) obtained on LLC-MK<sub>2</sub> cells. These two kinds of titers were almost the same except that the FRNT titer of anti-DEN-2 serum is lower than the PRNT titer. This might be related with the relative insensitivity of LLC-MK<sub>2</sub> cells to DEN-2 virus compared with BHK21 cells.

The focus reduction neutralization test (FRNT) was applied to several human sera obtained from healthy individuals with possible previous dengue infection(s). Some of the results are shown in Table 3. In general the FRNT is more sensitive than HI test. Exceptions were sometimes observed with sera of the secondary type antibody pattern (No. 19 or 23). Also the FRNT is more specific than HI test to detect long-lasting, type-specific antibody. Serum No. 25 was obtained from a person exposed to dengue 30 years before, and the person of serum No. 3 was presumably exposed to dengue virus type 4 during his laboratory experiment without any obvious symptoms.

Table 1. Relative infectivity (log/ml) of dengue viruses

Viruses (strain)	BHK21 FFU	LLC-MK <sub>2</sub> PFU	SMIC LD <sub>50</sub>
DEN-1 (Hawaii)	6.15	6.55	6.76
DEN-2 (New Guinea B)	8.32	7.06	8.31
DEN-3 (H-87)	5.70	5.81	6.70
DEN-4 (H-241)	6.32	6.17	6.42

Table 2. Neutralizing titer of anti-dengue sera

Antisera	50 % FRNT on BHK21	50 % PRNT on LLC-MK <sub>2</sub>
Anti-DEN-1	940	620
Anti-DEN-2	820	2500
Anti-DEN-3	1100	1380
Anti-DEN-4	190	320

Table 3. Hemagglutination-inhibition and focus reduction neutralization tests on human sera

Serum No.	Nationality	Age	HI				FRNT			
			DEN-1	DEN-2	DEN-3	DEN-4	DEN-1	DEN-2	DEN-3	DEN-4
1	Thai	40	20	40	20	80	18	960	16	64
2	Thai	39	40	40	40	80	270	150	420	350
3	Korea	34	<20	<20	<20	<20	<10	<10	<10	105
9	Thai	28	40	80	80	80	55	230	330	205
13	Thai	40	160	160	80	80	770	1450	580	72
17	Burma	32	160	320	80	20	460	1150	250	100
18	Japan	28	80	<20	<20	<20	5400	<10	<10	<10
19	Japan	36	80	20	20	40	460	15	<10	80
23	Philippin	29	1280	320	160	80	76	820	230	125
25	Japan	54	<20	<20	<20	<20	200	10	32	<10

## 2. Isolation of a sensitive cell clone to dengue and chikungunya viruses from Singh's *Aedes albopictus* cells

Twenty clones were isolated from Singh's *A. albopictus* cells obtained from CMDNJ, Rutgers Medical School, New Jersey, U. S. A. The first cloning was done in the presence of anti-chikungunya (CHIK) serum, and each clone was tested for its sensitivity to DEN-1, 2, 3, 4, and CHIK viruses by assaying virus yields by FFUT. Clone No. 6 showed 10-100 fold higher yield for each virus than the original uncloned cells (SAAR). The clone No. 6 was subcultured in the presence of anti-dengue sera for 10 weeks and the second cloning was performed in the presence of anti-dengue sera. Forty two clones showed almost the same virus sensitivity as clone No. 6. One of these clones, C6/36 was studied further. Virus sensitivity of the clone was unchanged when it was incubated with the medium which had been used to grow the original SAAR cells. Neither the sensitivity of the SAAR was enhanced by the medium used for C6/36. However, the clone C6/36 became resistant to CHIK when it was incubated with the medium used for another Singh's *A. albopictus* cell line (SAAK), which had been obtained from Kobe university School of Medicine and was resistant to CHIK. On the other hand, the sensitivity to DEN or Sindbis viruses did not change by the same procedure. The transfer of specific resistance to CHIK is presumably attributable to some latent virus persistently infecting the SAAK cells.

3. Electron microscopic observation on the morphogenesis of dengue viruses in cultured *A. albopictus* cell clone C6/36

Formation of vesicles was the most striking change in cells infected with each one of the 4 types of dengue viruses. From early stage of infection (24 hr), electron-dense particles were observed on the membrane of desert-like structure derived from distended endoplasmic reticulum. In the middle stage of infection (48-96 hr), many mature particles were present inside the desert-like structure. Electron-dense cytoplasmic inclusions were also found to contain some mature particles. At later stage of infection (96-168 hr), electron-dense desmosome-like structure could be found, some of which was connected with mature particles. Crystalloid aggregates of viral particles and naked nucleoids were found in the cytoplasm of cells persistently infected with DEN-2 virus.

4. Amino acid requirement for the growth of Singh's *A. albopictus* cell clone C6/36 and for the growth of dengue and chikungunya viruses

Growth of clone C6/36 and the growth of DEN and CHIK viruses were tested in Eagle's media from which each one of the 20 amino acids was omitted. Growth of the cells and CHIK virus was greatly inhibited when cystine was omitted from the medium, while the growth of DEN viruses was more reduced in the medium without glycine. On the other hand, alanine, asparagine, aspartic acid, and glutamic acid were not required for the cell and virus growths. Other amino acids seemed to be essential. From these and other results in which some combination of amino acids was omitted, the minimal essential amino acid mixture was determined, which was devoid of the above 4 kinds of amino acids.

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REPORT FROM THE DEPARTMENT OF MICROBIOLOGY  
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Extraneural infection of DEN virus in mice and its application to therapeutic experiments

The Mochizuki strain of DEN-1 virus was isolated in 1943 by the mouse-intracerebral inoculation method. During the earlier mouse passages, the virus killed young mice not only by the ic route but also by the extraneural routes, such as ip, sc, and/or iv. After the Mochizuki virus had been "human-attenuated," its capacity of infecting mice through the extraneural routes decreased markedly and practically was lost. Recently, however, it was noted by chance that the Mochizuki virus, of the 211th mouse-passage, had become virulent to mice through the extraneural routes. Possibility of contamination by other mouse-pathogenic viruses was eliminated by biological examinations and by immunological tests using anti-DEN reference antibodies (kindly provided by Dr. R. E. Shope of YARU).

The ip or sc injection of the recent Mochizuki virus produced apparent infection of 1 to 10-day-old mice, with typical symptoms and causing eventual death, similarly as by the ic injection. Ranges of LD<sub>50</sub> for suckling (1 to 10-day-old) and young adult (3 to 4-week-old) mice were: ic > ip > sc, and ic > iv > ip, respectively. The LD<sub>50</sub> values became lower in accordance with the age of mice, and also were different between different strains of mice.

Using mice infected through the extraneural routes, therapeutic experiments were performed. Anti-DEN mouse immune ascitic fluid administered intramuscularly was effective to suppress the experimental mouse infection. The effect was obvious when the immune fluid was given prior to, or simultaneously with the virus infection, while the same material given after the virus infection was much less effective. Repeated ip injection of mouse serum interferon (MSIF) was effective on the ip infected mice and the treatment with MSIF by ic and iv routes also showed an effect on the intranasally infected mice. When the virus was given by the ic or iv route, the IF treatment through any routes was ineffective. PAA (phosphonoacetic acid) was completely ineffective, at least under the conditions studied.

The infection systems presented here may be useful for investigations of anti-DEN therapeutics. Further experiments are being undertaken.

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(Reported by S.Hotta)

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE, ENTEBBE  
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DISTRIBUTION OF CHF-CONGO VIRUS AND SOME OTHER TICK-BORNE VIRUSES  
IN EAST AFRICA (A SEROLOGICAL SURVEY)

The methods of preparation and some characteristics of tick-borne virus antigens have been described in "Arthropod-Borne Virus Information Exchange" No. 30, p. 68. This communication presents the second part of the work done in the period from October 1974 to April 1975 in the East African Virus Research Institute.

1. Distribution of CHF-Congo virus and other tick-borne viruses in Uganda.

Examination of the cattle sera in the AGDP test

In the period from November 1974 to March 1975, more than 1200 samples of cattle sera originating from different parts of Uganda were collected at Uganda Meat Packers (UMP), Kampala. The average rate of antibody to Congo virus was 23.3 (280 positive sera out of 1200 examined). According to these results, circulation of Congo virus was found to occur in Acholi (7.5%), Ankole (26.9%), Buganda (33%), Masaka (14.7%), Teso (30.0%), Lira (10.0%) and Toro (7.7%) regions (Table 1).

Precipitin antibody to Bhanja virus

Ranged from 8.9% in Acholi district to 75.3% in Buganda region (an average of 58% of cases). The low level of antibody to Dugbe virus was found again in Acholi district (3.0%) maximum levels in Buganda (63.1%), Teso (80%) and Karamoja (5 positive sera out of 5 tested).

Comparing the results of examinations of cattle sera from Acholi district and other places in Uganda covered by the survey, it can be summarized that circulation of Congo, Bhanja and Dugbe viruses in Acholi is limited (Table 2).

Among 689 sera tested in AGDP test against Kadam virus antigen only 4 sera (0.6%) contained antibody to this agent: 3 positive sera from Ankole cattle and 1 from Acholi.

Examination of cattle sera in HI test

The HA antigens of Kadam, Bhanja, Dhori, and Thogoto viruses were used for examination of Uganda cattle sera collected during the period from November 1974 to March 1975 at UMP. The average percentages of positive sera to those were 2.1, 44.2, 0.8, and 4.2, respectively (Table 3).

The five sera that were shown to react positively with Kadam antigen were not tested by the HI test against other group B arboviruses.

The results of serological survey using HI test showed, like in AGDP test, limited circulation of Bhanja virus in Acholi district as compared to other parts of Uganda covered by this study.

One-hundred-fifty-eight cattle sera were examined in parallel by AGDP and HI tests with Bhanja antigens. Out of these, 76 (48.1%) contained precipitin antibody and 72 (45.6%) HI antibody. Fifty-three sera were positive and 63 were negative in both tests. The correlation of these results in AgDP and HI tests put together is 73.4%.

## 2. Distribution of CHF-Congo virus and other tick-borne viruses in Tanzania

In January 1975, 422 cattle sera were collected by Dr. Minja from Northern and Central Tanzania (Longido, Monduli, Tengeru, and Mawapwa districts).

Four-hundred-seventeen samples of cattle sera from some regions of Tanzania surrounding Lake Victoria were collected from Uganda Meat Packers, Kampala; 209 cattle sera taken in December 1974 from Sukumaland district were kindly supplied by the Director of EAVRO, Muguga, Kenya.

### Examination of the cattle sera in AGDP test

All 166 sera from Central Tanzania were negative with Bhanja antigen, and only one was positive with Congo virus antigen (0.6%). In different parts of Northern Tanzania the occurrence of antibody to Congo virus ranged from 4.8 to 16.3% (Table 4).

Among the cattle originating from along the coast of Lake Victoria, the percentage of antibody to Bhanja and Dugbe viruses was 74.7 and 75.5, respectively, whereas only one out of 288 sera tested gave positive results with Kadam antigen (0.3%).

### Examination of cattle sera in HI test

Using Kadam, Bhanja, Dhori, and Thogoto antigens, 74, 19, 72, and 23 sera, respectively, were tested by the HI method. These samples were collected from UMP. Antibody to Bhanja and Dhori viruses were found in 67.9% and 5.5% of sera, respectively, but no antibody to Kadam and Thogoto viruses was found.

## 3. Distribution of CHF-Congo, Bhanja and Dugbe viruses in Kenya

### Examination of cattle sera in AGDP test

Three-hundred-forty-seven cattle serum samples, collected in December 1974, were kindly supplied by the Director of EAVRO, Muguga, Kenya. Precipitin antibody to Congo virus was found in the sera from West Pokot (3.0%), Kajiado (1.1%), Eldoret (7.3%), and Isiolo (8.3%) districts, giving an average seropositivity of 4.7% (Table 5).

The percentage of antibody to Bhanja virus ranged from 4.1 to 58.2 (an average of 27.4%). Antibody to Dugbe virus was absent in Eldoret district but was found in Kajiado (12.2%) and West Pokot (56.7%) districts.

#### 4. Examination of sera collected from wild animals

The EAVRI material collection included more than 600 wild game sera obtained in 1971 from Kenya. These sera were examined by AGDP method using Congo, Bhanja, and Dugbe antigens. Among 628 sera collected from 16 different species of wild animals, none had antibody to Congo virus. The average percent of positive findings to Bhanja and Dugbe viruses was 3.0 and 3.5, respectively (Table 6).

Among 98 sera collected from 10 different species of wild animals in December 1974 (EAVRO, Muguga, Kenya) no precipitin antibody to Congo virus was found either. Antibody to Bhanja and Dugbe viruses were detected in 11.3% (11 positives out of 97) and 9.4% (9 out of 96) cases respectively, in the same sera (Table 7).

In summary, the results of examinations of the Kenyan wild animal sera show that precipitin antibody to Bhanja virus occurs in 9 species of animals: buffalo, giraffe, kongoni, Th. gazelle, impala, ringed water buck, wild beast, and zebra. Using AGDP test, antibody to Dugbe virus was found in buffalo, giraffe, kongoni, and Th. gazelle sera.

#### 5. Serological examination of sera collected from the rural population in Uganda

Some sera collected in 1967, 1970, and 1972 from the rural population of Karamoja, Busoga, and Ankole districts were tested in AGDP test against Congo, Bhanja and Dugbe antigens (813, 408, and 325 specimens, respectively).

Antibody to Congo virus was found in two persons, both of whom were from Ankole district (Table 8).

Serum SG 28106 taken from a 45-year-old female contained precipitin antibody (1:4) as well as CF antibody (1:32). Serum SG 28181 from a 20-year-old female contained precipitin antibody (1:2) and demonstrated anticomplementarity in CF test.

By the AGDP test, antibody to Bhanja virus was found only in one serum (SG 22596) from Ankole district. This serum was also positive in HI (1:40) and CF (1:80) tests with Bhanja antigens.

Two-hundred-six serum samples from the rural population were tested in parallel by AGDP and HI tests using Bhanja antigen with negative results, with the exception of one sample (SG 22596). Among 325 sera tested by AGDP test with Dugbe antigen and 206 sera tested by HI with Dhorí antigen, none was found positive.

These results (in relation to Congo virus) tally to data of the human serological survey carried out earlier in the CHF foci in the USSR using AGDP, CF and neutralization tests. However, the results of examinations of the sera that have been stored for a long period in refrigerators of EAVRI (sera of human beings and wild animals) must be interpreted very carefully because the freezing facilities did not always provide and maintain constant temperature.

The results of examinations of the recently collected cattle sera showed that Congo, Bhanja, and Dugbe viruses were widely distributed in Uganda, Tanzania, and Kenya (the maximum of positive findings achieved 33%, 75% and 80%, respectively). It is interesting to note that antibody to these viruses occur very rarely or are absent in the arid zones such as Acholi district in Uganda and Mpwapwa region in Central Tanzania.

These data agree with the observations of Terstra concerning the absence of circulation of NSD virus in northern Uganda where the vector of this infection (Rh. appendiculatus) "is absent due to conditions of low rainfall". It is possible to suggest that there is a direct correlation in the territorial distribution of at least four tick-borne viruses: Congo, Bhanja, Dugbe, and NSD in this region.

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Table 1

AGDP test. Examination of sera collected from Uganda cattle in November 1974 to March 1975 at UMP, Kampala

Regions of Uganda	A n t i g e n s											
	Congo			Bhanja			Dugbe			Kadam		
	T <sup>x)</sup>	P	%	T	P	%	T	P	%	T	P	%
Acholi	187	15	7.5	190	17	8.9	198	6	3.0	145	1	0.7
Ankole	565	152	26.9	578	396	68.5	590	311	57.7	310	3	0.97
Buganda	273	90	33.0	272	205	75.3	271	171	63.1	177	0	0
Bunyoro	2	0	0	2	0	0	2	0	0	2	0	0
Entebbe	3	0	0	3	0	0	3	0	0			
Jinja, Njeru	3	0	0	3	0	0	3	0	0	3	0	0
Karamoja	5	0	0	5	5	100	5	5	100	5	0	0
Lira, Lango	10	1	10.0	10	4	40.0	10	6	60.0	10	0	0
Masaka	129	19	14.7	129	62	48.1	131	38	29.0	27	0	0
Teso	10	3	30.0	10	7	70.0	10	8	80.0	10	0	0
Toro	13	1	7.7	13	9	69.2	13	3	23.1			
Total	1200	280	23.3	1215	707	58.0	1236	548	44.3	689	4	0.6

x) T = number of sera tested, P = Number of positives; % = percentage of positive

Table 2

Distribution of precipitin antibody to Congo, Bhanja and Dugbe viruses among cattle population in Acholi and other regions of Uganda

Regions of Uganda	Congo			Bhanja			Dugbe		
	No. of sera tested	No. positive	% positive	No. of sera tested	No. positive	% positive	No. of sera tested	No. positive	% positive
Acholi	187	14	7.5	190	17	8.9	198	6	3.0
All others (sum)	1013	266	26.2	1025	688	67.1	1038	542	52.2
Total	1200	280	23.3	1215	705	58.0	1236	548	44.3

Table 3

HI test. Examination of sera collected from Uganda cattle in November 1974 to March 1975

Regions of Uganda	A n t i g e n s											
	Kadam			Bhanja			Dhøri			Thogoto		
	T	P	%	T	P	%	T	P	%	T	P	%
Acholi	33	2	6.0	12	2	16.7	33	0	0	6	0	0
Ankole	87	1	1.1	35	23	65.7	87	1	1.1	29	1	3.4
Buganda	40	1	2.5	23	11	47.9	42	0	0	12	1	8.3
Entebbe	3	0	0	3	0	0	3	0	0	3	0	0
Karamoja	5	0	0				5	0	0			
Masaka	68	1	1.5	65	25	38.7	69	1	1.4	69	3	4.3
Total	263	5 <sup>x</sup>	2.1	138	61	44.2	239	2	0.8	119	5	4.2

T = No. of sera tested, P - No. positive, % = per cent positive  
 x) titres of antibody were 1:10, 1:10, 1:10, 1:10, 1:20

Table 4

AGDP test. Examination of cattle sera from Tanzania, using Congo, Bhanja and Dugbe virus antigens

Place of Origin	Congo			Bhanja			Dugbe		
	No. of sera tested	No. positive	% positive	No. of sera tested	No. positive	% positive	No. of sera tested	No. positive	% positive
Central Tanzania (Mawapwa)	166	1	0.6	166	0	0			
Northern Tanzania (Longido, Monduli, Tengeru)	256	19	7.4	259	33	12.7			
Sukumaland	209	10	4.8	205	121	59.0	205	96	46.8
Regions around Lake Victoria coast	417	68	16.3	427	319	74.7	429	324	75.5
Total	1048	98	9.0	1057	473	44.7	634	420	66.2

Table 5

AGDP test. Examination of cattle sera from Kenya, using Congo, Bhanja, and Dugbe antigens

Districts	Congo			Bhanja			Digbe		
	T	P	%	T	P	%	T	P	%
Eldoret	55	4	7.3	55	7	12.7	54	0	0
Isiolo	96	8	8.3	98	57	58.2	97	36	37.1
Kajiado	92	1	1.1	98	4	4.1	98	12	12.2
West Pokot	100	3	3.0	96	27	28.1	93	53	56.7
Total	343	16	4.7	347	95	27.4	342	101	29.5

T = number of sera tested, P = Number positive, % = percent positive.

Table 6

AGDP test. Examination of wild animal sera collected in Kenya, 1971.

(The specimens were kept in refrigerators of EAVRI)

Animal species	A n t i g e n s								
	Congo		Bhanja			Dugbe			
	T	P	T	P	%	T	P	%	
Baboon	132	0	200	0	0				
Giraffe	44	0	43	7	16.3	42	7	16.7	
Gr.gazelle	61	0	16	2	12.5	16	0	0	
Th.gazelle	83	0	59	1	1.7	45	0	0	
Dik-dik	11	0	11	0	0	11	0	0	
Kongoni	58	0	55	3	5.4	55	5	9.1	
Impala	71	0	73	2	2.7	70	0	0	
Eland	6	0	6	0	0	6	0	0	
Oryx	2	0	2	0	0	2	0	0	
Reed buck	22	0	12	0	0	12	0	0	
Oribi	14	0	17	0	0	17	0	0	
Ringed water buck	25	0	24	1	4.2	26	0	0	
Buffalo	8	0	8	0	0	8	0	0	
Wild beast	35	0	30	1	3.3				
Rhinoceros	21	0	22	0	0				
Zebra	35	0	36	2	5.5	36	0	0	
Total	628	0	624	19	3.0	346	12	3.5	

T = number of sera tested, P = number positive, % = percentage positive

Table 7

AGDP test. Examination of wild animal sera obtained from EAVRO,  
Muguga, Kenya

Species	Time of collection	Place of collection	A n t i g e n s					
			Congo		Bhanja		Dugba	
			T	P	T	P	T	P
Buffalo	1974	Kabete	10	0	9	6	9	8
Giraffe	1974	Kabete	10	0	10	3	9	0
Th.gazelle	1974	Kabete	7	0	7	1	7	1
Kongoni	1972	Akira	1	0	1	0	1	0
	1974	Kabete	9	0	9	1	9	0
Impala	1974	Kabete	10	0	10	0	10	0
Gr.gazelle	1971	Kakira	2	0	2	0	2	0
	1971	Kabete	5	0	5	0	5	0
	1974	Kabete	5	0	5	0	5	0
Wild beast	1974	Kabete	10	0	10	0	10	0
Ringed water buck	1970	Kabete	10	0	10	0	10	0
Zebra	1971	Kabete	1	0	1	0	1	0
	1971	Kajado	3	0	3	0	3	0
	1971	Narosura	5	0	5	0	5	0
Eland	1971	Akira	4	0	4	0	4	0
	1971	Kabete	6	0	6	0	6	0
Total			98	0	97	11	96	9

Table 8

AGDP test. Examination of sera collected from the rural population  
in Uganda

Place of collection (district)	Year of collection	A n t i g e n s					
		Congo		Bhanja		Dugbe	
		No. of sera tested	No. posi- tive	No. of sera tested	No. posi- tive	No. of sera tested	No. posi- tive
Karamoja	1967	202	0				
Karamoja	1970	293	0	191	0	117	0
Ankole	1972	258	2	208	1	208	0
Others	1970	60	0	9	0		
<b>Total</b>		<b>813</b>	<b>2</b>	<b>408</b>	<b>1</b>	<b>325</b>	<b>0</b>

REPORT FROM WHO COLLABORATING CENTER  
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Serological survey with some arboviruses in human population in Austria (Steiermark and Burgenland)\*

A total of 284 sera were collected from randomly chosen agricultural workers in the Seewinkel (Burgenland) in the last years. During the same period 334 sera were collected from pregnant women as a control group in Steiermark (Styria) and Burgenland.

All sera were examined by HI and VN tests.

Results of HI test revealed that 1% (Steiermark) and 42% (Burgenland) of healthy agricultural workers had antibodies against Sindbis virus. Sera of these workers contained antibodies against TBE virus in 2.1 and 7.3%, respectively; and in the same sera antibodies against WN virus in 3.1% and 10.5%, respectively, were also found (Table 1). In a control group of pregnant women only a small proportion of sera reacted positively with antigens of TBE virus and WN virus. Titres of antibodies against Sindbis virus varied between 1:10 - 1:20, and those against TBE virus & WN virus, from 1:10 to 1:40.

The specificity of HI antibodies against TBE virus and WN virus in some sera tested was proven by VN test (Table 2). Only one serum reacted with Sindbis virus in VN test in a titre 1:4.

Titres of VN antibodies against TBE virus or WN virus varied from 1:4 to 1:32 or from 1:4 to 1:16, respectively.

Comparison of the levels of HI and VN antibodies against Sindbis virus showed that titres of 1:20 of HI antibodies could not be considered as specific, because the sera with such HI antibody levels were negative when examined by VN test (Table 3).

When the levels of HI and VN antibodies against TBE virus were compared, it was found that some sera reacting in HI test in a titre of 1:20 contained also VN antibodies and all sera with a titre of 1:40 of HI antibodies reacted positively in VN test (Table 4).

Similar results were obtained when comparing the levels of HI and VN antibodies against WN virus (Table 5).

Of interest are results of serological examination with Tahyna virus in VN test. Whereas sera collected from agricultural workers in the one year reacted in 88%, only a small proportion of sera collected in the other year were found to be positive. Levels of VN antibodies against Tahyna virus varied from 1:4 to 1:128.

\*Report received by editor, AIE, May, 1977.

It was impossible to analyse the human population under study according to profession because almost everybody, whether working in the forest or as hunter, fisher or workman, works usually on his own farm. As follows from the professional structure of the human population in Seewinkel and Burgenland, everybody can be considered to be a winegrower and therefore as an agricultural worker, i.e., the length of his exposure to infectious agents in nature may vary during year, depending on his second job. These facts should be taken into consideration in epidemiological analyses of all anthroozoonoses in the given biotope.

1) 1) 1) 2)  
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TABLE 1 Haemagglutination-inhibiting antibodies to some arboviruses in human healthy population

Origin of serum samples	No of tested sera	Percentage of positive reactions with antigen		
		Sindbis	Tick-borne encephalitis	West Nile
Farmer s	95	4.2	2.1	10.5
Farmer s	189	1.0	7.3	3.1
Pregnant women	334	0	1.2	3.0

TABLE 2

VN antibodies to some arboviruses in human  
healthy population in Austria

Origin of serum samples	No of tested sera	Percentage of positive reactions with antigen		
		Sindbis	Tick-borne encephalitis	West Nile
Farmer s	84	0	0	4.7
Farmer s	148	0.6	6.7	2.7
Pregnant women	308	0	1.9	2.9

TABLE 3

Comparison of HI and VN antibody titres to Sindbis virus in human population in Austria

Origin of serum samples	Protocol No	No of serum	HI titre with antigen	VN titre with antigen
			Sindbis	Sindbis
Farmer s	-	54	1 : 20	0
	-	60	1 : 20	-
	-	62	1 : 20	0
	-	80	1 : 20	-
	-			
Farmer s	26	3228	1 : 20	0
		4056	1 : 20	T
	25	1949	0	1 : 4
Pregnant women			0	0

T = toxic

0 = negative results

- = not examined

TABLE 4 Comparison of HI and VN antibody titres to tick-borne encephalitis virus in human population

Origin of serum samples	Proto-col No	No of serum	HI titre	VN titre
			Tick-borne encephalitis	Tick-borne encephalitis
Farmer s	-	32	1 : 20	O
	-	84	1 : 20	T
Farmer s	26	4141	1 : 20	T
		4118	1 : 40	1 : 16
		4050	1 : 20	O
		4140	1 : 40	T
	27	2104	1 : 20	1 : 16
		3189	1 : 40	T
		2013	1 : 20	-
		4023	1 : 20	-
		4036	1 : 20	1 : 16
		4015	1 : 20	1 : 16
		1789	1 : 20	-
	29	2959	1 : 20	1 : 8
		2728	1 : 20	1 : 8
		1805	1 : 20	1 : 8
	18	4387	1 : 20	1 : 4
		4386	1 : 20	1 : 8
21	4320	1 : 20	1 : 4	
Pregnant women	VI	4560	1 : 40	1 : 16
		3887	0	1 : 4
	1+2	1494	1 : 20	1 : 16
	14	1560	1 : 40	1 : 32
	15	1452	1 : 20	1 : 4
	9	2843	0	1 : 4

T = toxic

O = negative results

- = not examined

TABLE 5 Comparison of HI and VN antibody titres to West Nile virus  
in human population in Austria

Origin of serum samples	Protocol No	No of serum	HI titre with antigen	VN titre with antigen
			West Nile	With Nile
Farmer s	-	23	1 : 20	-
		24	1 : 20	-
		25	1 : 20	1 : 4
		28	1 : 20	-
		32	1 : 40	1 : 4
		44	1 : 40	1 : 16
		67	1 : 20	0
		71	1 : 20	0
		79	1 : 40	1 : 4
		84	1 : 40	T
Farmer s	26	4118	0	1 : 8
	29	2728	1 : 20	1 : 8
		1768	1 : 20	T
		1805	1 : 20	0
		1801	1 : 20	0
	18	4386	1 : 20	1 : 8
	21	4320	1 : 20	1 : 4
Pregnant women	9	2428	1 : 20	-
		2843	1 : 20	1 : 8
	11	1627	1 : 20	1 : 4
		2321	0	1 : 4
	12	1148	1 : 20	1 : 4
	15	1452	1 : 20	1 : 4
		2735	1 : 20	1 : 4
	10	2795	1 : 20	-
	VI	3884	1 : 20	-
		3887	0	1 : 4
		3883	1 : 20	1 : 8
	IV	1549	1 : 20	-
	3	3942	0	1 : 4

T = toxic

0 = negative results

- = not examined

REPORT FROM THE INSTITUTE OF PARASITOLOGY,  
CZECHOSLOVAK ACADEMY OF SCIENCES, PRAGUE , CZECHOSLOVAKIA

Bhanja virus antibodies in sheep from Bulgaria

In the No. 32 of Information Exchange (page 111) we have reported on the isolation of Bhanja virus from ticks *Haemaphysalis punctata* and *H. sulcata* collected on October 21, 1974 from sheep near Akhtopol, southeast Bulgaria. The same day blood sera were sampled from 21 and 37 sheep in the localities Akhtos and Akhtopol, respectively. The infestation of sheep with ticks (predominantly *Haemaphysalis* spp.) was very high, almost 100 %. The sera were placed into polyethylene ampoules, sealed and transported to Czechoslovakia in liquid nitrogen. They were examined in plaque-reduction neutralization test (PRNT) on PS cells with the virus Bhanja, strain Bg 326 MS4, using about 35 PFU per well and L-15 medium. All sera were inactivated 30 min. at 56°C, and PRNT was performed according to de Madrid & Porterfield (1969) with some minor modifications. The dilution of serum causing 50 % reduction of the number of plaques after 5-day incubation was taken as the titer of the serum. Specific immune anti-Bhanja virus rabbit serum (titer in PRNT 1:128) and normal rabbit serum were used as positive and negative controls. The results of PRNT are shown in the table.

Reciprocal titer	32	64	128	256	512	1024	2048	4096
Locality	Number of sera							
Akhtos	2	2	2	11	7	4	7	2
Akhtopol	0	1	3	1	7	6	3	0

All 58 sera from both localities examined contained neutralizing antibodies against Bhanja virus in titers of 1:32 to 1:4096, geometrical mean titer (GMT) being 1:494. GMT of the sera from Akhtos was higher (1:547) than GMT of those from Akhtopol (1:466); the difference, however, is statistically insignificant ( $P > 0.50$ ).

This serological survey, as well as the isolation of Bhanja virus, proved the presence of a natural focus of this virus in the coastal region of southeast Bulgaria.

<sup>x)</sup>  
(P. Pavlov, B. Rosický, Z. Hubálek, M. Daniel, V. Bárdoš, J. Minář, Z. Juřicová : Folia parasitol. /Prague/ in press, 1977).

<sup>x)</sup> Institute of Hygiene of the Bulgarian Academy of Agriculture, Sofia.

#### Bhanja virus antibodies in goats and sheep of Czechoslovakia

Since sheep and goats were found serologically positive in natural foci of Bhanja virus we decided to undertake a small informative serological study of goats and sheep of Slovakia (Czechoslovakia) grazing in areas where

Haemaphysalis ticks were found. Twenty-eight sera from sheep and 19 sera from goats were tested in PRNT as mentioned in the previous article. Two sheep sera and 12 goat sera were found positive - details see in the table.

Sera	No. of sera tested	of sera positive	%	Reciprocal titers
Sheep	28	2	7.1	2x16
Goats	19	12	63.2	1x4, 1x8, 2x16, 2x32, 1x128, 3x256, 1x1024, 1x2048

These results are indicating that Bhanja virus or another closely related virus may be present also in Czechoslovakia.

(V.Bárdoš, Z.Hubálek, T.Mittermayer<sup>x</sup>); Felia parasitol. /Prague/ in press, 1977).

<sup>x</sup>) Regional Hospital, Košice

Survey for antibodies against tick-borne encephalitis, Uukuniemi, Bhanja and Ťahyňa arboviruses among forest workers in southern Moravia

Two strains of tick-borne encephalitis (TBE) and 6 strains of Uukuniemi (UUK) viruses were isolated in June 1976 from Ixodes ricinus ticks in southern Moravia (Information Exchange, No. 32, page 113).

Sera from 29 forest workers working from 1 to 40 years in this natural focus were tested in a plaque reduction neutralization test using PS cells.

The test dose of the respective arboviruses was the following. TBE virus (strain 402 MS1) - 20 plaques/0.1 ml and UUK virus (strain 393 MS3) - 15-20 plaques/0,1 ml. Both strains were isolated in this natural focus. Bhanja (BHA) virus (strain Bg 326 MS4) - 30 PFU/0.1 ml and řahyňa (TAH) virus (strain T/16 MS3) - 15 PFU/0.1 ml. The test was done according to de Madrid and Porterfield (1969) using MEM medium with 2 % calf serum. The test was read with the TBE virus after 5, with UUK and BHA viruses after 4 and with TAH virus after 3 days of incubation (36°C) in a 5 % CO<sub>2</sub> atmosphere. Each serum was tested simultaneously in duplicate. The dilution of serum causing 50 % reduction of the number of plaques was taken as the titer. Seven sera (24.1 %) were positive with TBE virus and 14 (48.3 %) with TAH virus. None was positive with BHA or UUK viruses. Details are seen in the table.

Years at work	tested persons	Number of sera positive to		Reciprocal of serum titres	
		TBE	TAH	TBE	TAH
1 - 4	4	0	1	-	1x256
5 - 9	5	1	3	1x > 64	1x16 2x1024
10-14	8	4	3	1x512 3x256	1x4 2x1024
15-19	5	1	2	1x > 256	1x1024 1x2048
20-40	7	1	5	1x256	1x128 3x4096 1x8192

Total            29            <sup>§</sup>  
                               7            14  
                               (24.1%) (48.3%)

§ In 3 persons history of encephalitis.  
                               x)                                xx)  
 (V.Bárdoš, K.Svoboda, I.Havelka )

x) Regional Hyg.Station, Brno

xx) District Hyg.Station, Znojmo

Experimental infection of Asiatic Polecat (*Putorium*  
*eversmani* Lesson, 1827) and Beech Marten  
(*Martes foina* Erxleben, 1777) with Ťahyňa virus

Three polecats (weighing 725-950 g) and 2 martens (1060 and 1230 g) about 9 months old were subcutaneously infected with approximately 260 suckling mouse LD50 of the extraneurally passaged strain "236" of Ťahyňa virus. One adult rabbit (1050 g) was simultaneously inoculated with the same amount of virus. Viraemia was tested at 48-96 hours after the infection and was detected in all animals. The peak titer in the polecats was 1.32 dex i.c. LD50/0.02 ml, in the martens 1.28 dex LD50 and in the rabbit 3.91 dex LD50. 15 days after the infection, sero-conversion was detected in all infected animals (from <1:4 to 1:8192 in the polecats, to 1:4096 in the martens and to 1:4096 in the rabbit), using plaque - reduction neutralization test with Ťahyňa virus.

(P.Rödl, Z.Hubálek, V.Bárdoš : Folia parasitol. /Prague/  
in press)

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REPORT FROM THE DEPARTMENT OF VIROLOGY, NEUROLOGIC CLINIC,  
UNIVERSITY OF COLOGNE, FEDERAL REPUBLIC OF GERMANY

The risk of infection with LCM virus by pet hamsters. Serologic Survey.

Out of 291 persons of all age groups from Cologne, Frankfurt and their respective vicinity who had had contact with Syrian hamsters, 17 (5.84%) had neutralizing antibodies against lymphocytic choriomeningitis virus (LCM virus). Within a group of 251 people from the same area without such contact only 5 (1.99%) revealed LCM antibodies.

The difference in antibody rates resulted in a chi square value of 5.13 indicating at the 5% confidence level a difference between the two groups.

Contrary to the usual pattern of regional (geographical) distribution the urban population and the younger females were more inflicted by pet hamster related LCM virus infections. An increased risk due to LCM virus in contact with Syrian hamsters is without question. Because of the danger of intrauterine fetal damage in case of infection during pregnancy, special precautions should be taken and any contacts with this pet should be carefully avoided.

(R.Ackermann: Dtsch.med.Wschr. 102 (1977), in press)

### Bluetongue and Culicoides

Culicoides nubeculosus from the laboratory colony stock have been shown to be readily infected when inoculated with bluetongue virus (BTV), but to be refractory by the oral route. C.variipennis is readily infected by both routes. Attempts to infect C.nubeculosus orally by altering the pH of the blood meal and by feeding blood-virus-sugar mixtures (so that the infective feed passes to the gut diverticulum) have failed so far. Stocks of C.nubeculosus obtained from the wild are as refractory to BTV as the colony stocks.

### Sandfly fever and Pacui viruses

Female Lutzomyia longipalpis have been tested for their susceptibility to sandfly fever (Naples), sandfly fever (Sicilian) viruses, and Pacui virus, by the oral route. Pacui virus persisted for up to 10 days but the two sandfly fever viruses disappeared in 3 days. Neither of the sandfly viruses multiplied in Lutzomyia when inoculated into the insects, but Pacui virus increased in the insect from 1.9 to 5.4 log<sub>10</sub> mouse LD<sub>50</sub>. None of these three viruses, nor Arumowot virus, multiplied in C.variipennis or in C.nubeculosus following ingestion or inoculation of virus, although traces of virus were still detectable 15 days after inoculation.

### Nigeria

The taxonomy and biology of Culicoides species in relation to the transmission of bluetongue and African horse sickness viruses is being studied in the field, in Nigeria. Fifty five species of Culicoides have been recorded from light traps; the most probable species involved in virus transmission are the Culicoides imicola (=pallidipennis) group (about 6 species), the C.milnei group (6 species) and the C.schultzei group (2 species).

Neutralisation tests with bluetongue virus types 1 - 16 have been carried out on the calf sera collected at Vom (see Information Exchange 31, 127 - September, 1976). High titre antibodies to types 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13 and 15 were found. This indicates the possible existence in Nigeria of types in addition to those already described.

### Passage of bluetongue virus across the placenta in sheep

Dorset Horn and Suffolk-cross ewes were inoculated during pregnancy with a type 4 bluetongue virus isolated from a case of bluetongue in Cyprus in 1969. At term, 6 dead lambs or fetuses were produced, from 5 of which bluetongue virus was isolated. Bluetongue virus was also isolated from 7 of the 11 live lambs produced. In one lamb virus was present for 2 months after birth. These findings suggest that bluetongue virus may overwinter by passage of virus across the placenta in sheep.

Possible spread of African horse sickness and bluetongue  
by infected midges on the wind.

In collaboration with the Centre for Overseas Pest Research, London, an analysis has been made of the spread of African horse sickness and bluetongue in North and West Africa, the Middle East, Cyprus and India. It was found that movement of infected Culicoides on the wind was most likely responsible for the spread of these diseases for distances of 40 to 700 km to Spain, Portugal, Cyprus, Cape Verde Islands, Algeria and India and in the Middle East. Flight occurred at temperatures between 15° and 40°C and lasted for variable periods up to 20 hours.

(J. Boorman, P. Mellor, M. Jennings, K. Herniman, P. Gibbs, M. Lawman and R. Sellers).

REPORT OF THE SECTION VIROLOGY OF THE MEDICAL RESEARCH CENTRE  
OF THE NETHERLANDS AND THE NATIONAL PUBLIC HEALTH LABORATORY  
SERVICES OF KENYA AT NAIROBI

In December an epidemic of a dengue like illness started on the Seychelles and lasted for several months. It is estimated that more than 50 percent of the population suffered from the disease. From serum specimens flown to Nairobi a number of virus strains were isolated. During a visit to the main island of the group, mosquitoes and more serum specimens were collected.

Virus was isolated from so many serum specimens that further attempts were stopped. Several *Aedes albopictus* and *Culex fatigans* mosquito pools yielded virus also.

The Adaptation of the isolates to infant mice was difficult and the incubation time remained long. Strains were forwarded to the WHO Reference Centre at Dakar (Dr. Y. Robin) and the London School of Hygiene and Tropical Medicine (Dr. D.I.H. Simpson) for identification.

So far, identification was completed till the Flavivirus genus.

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