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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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Bette A. Hall, Secretary

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COMMENTS FROM THE EDITOR

Thanks again for the excellent reports. Your attention to the standard format has made our job much easier.

The deadline for reports intended for the next issue of the Information Exchange (No. 36) is March 1, 1979. The address, as usual, is:

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ERRATA

Your editor was considerably embarrassed to note that the date on the cover of Information Exchange Issue No. 34 was in error--by a whole year. It should have read "March, 1978", not "March, 1977". Please make this change on your copies.

Also, I have been asked by Dr. Jack D. Poland, Chief Medical Epidemiologist, Vector-Borne Diseases Division, Ft. Collins, Colorado, to point out an oversight of credits for the report on St. Louis encephalitis in Memphis, Tennessee (pp. 147-152, issue number 34). The able assistance of the Viral Diseases Division, Bureau of Epidemiology, CDC should have been acknowledged for help in the 1976 case study as well as in the planning and execution of the serosurvey in February, 1977.

REPORT FROM ARBOVIRUS RESEARCH UNIT, NATIONAL INSTITUTE FOR VIROLOGY, DEPARTMENT OF HEALTH, PRIVATE BAG X4, SANDRINGHAM 2131, SOUTH AFRICA.

RIFT VALLEY FEVER (RVF).

Severe outbreaks of RVF among sheep, cattle and humans occurred on the inland plateau in South Africa during the abnormally wet years of 1974/75. At varying periods after outbreaks on 9 farms mosquitoes, Culicoides spp., and Simuliidae were collected and tested for virus. Laboratory transmission tests were also carried out on mosquito species and 1 tick species in which information on vector capability seemed necessary.

Table 1 shows mosquito collection and virus isolation data from the 9 farms concerning 4 mosquito species which are under suspicion as vectors. On Goodhope a period of 3 months had elapsed since the last known case of RVF but these results are of interest in that some 2000 deaths in sheep had occurred there and the collections were made in midwinter. The specimens of Cx. theileri were old and the failure to isolate virus is in agreement with observations on some of the other farms where there were also no isolations from Cx. theileri. Although Cx. theileri yielded most isolations a relatively larger number of this species was tested and all but 1 of the 12 isolations came from one farm. On 7 farms no isolations from this species were made, and it is difficult to overlook the absence of isolations from Cx. theileri on Skatryk and Goedendacht in which the elapsed period was short and numbers tested fairly large. Only small numbers of the other 3 species were tested and

firm conclusions are not possible but the indications concerning these species also suggest low infection rates overall and irregular infection among each species on the various farms. The results do, nevertheless, implicate 3 species, all of which feed readily on sheep and cattle. Cx. theileri is the most widespread and abundant species in the epizootic areas. Together with Cx. univittatus it is a species of high adult longevity and blood-seeking females persist well into winter as the collections on Goodhope demonstrate. Cx. theileri is the most probable vector in the autumn and during dry spells in the summer when Aedes species are virtually absent. Cx. univittatus is primarily ornithophilic and on this account its role as vector among domestic animals is probably limited.

On Fairfield there was 1 isolation from An. cinereus. There were no isolations from 1490 specimens of other mosquito species, nor from 10668 Culicoides spp., nor from 1135 Simuliidae.

Table 2 shows the results of laboratory transmission tests with RVF virus and 3 Aedes spp., 2 Culex spp., and Ornithodoros savignyi. Apart from the tick which failed to become infected or to transmit virus, all species were readily infected. In the transmission attempts with mosquitoes the transmission rates shown in the table are based on known infected insects only and in all tests each animal was bitten by a single mosquito. 27 Ae. caballus failed to transmit and only 1 of 18 Ae. juppi transmitted. Both Ae. lineatopennis and Cx. univittatus transmitted but only 6 of each species fed making the assessment of field transmission rates from the tests difficult. Furthermore, in the test with Cx. univittatus infant mice were used which we now believe tend to misleadingly increase vector capability. This is evident in the

transmission rates obtained with Cx. theileri shown in the table in which infant mice (8 days old) gave a rate of 70% as against 26% with sheep and 13 or 16% with hamsters. In the test with Cx. theileri using sheep high infecting doses of virus were used, hence transmission rates would consequently be increased but high viraemia levels frequently occur in sheep and cattle. It seems therefore that the transmission rates obtained are probably a fair reflection of rates during epizootics in domestic animals.

Tests for the presence of virus in the progeny reared from eggs laid by infected mosquitoes have so far proved negative in the case of Ae. juppi, Ae. lineatopennis, Cx. theileri, Cx. univittatus, and Eretmapodites quinquevittatus. The latter species is absent on the plateau but may be a vector on the coastal plain where the virus was once isolated from it. Experimentally, it has also been shown to be a vector.

In 1978 over 1,000 sera were collected from humans resident on 80 farms known to be infected during the recent epizootics. Of the 467 sera tested for antibodies so far 72 (15.4%) have reacted in the HI tested with RVF antigen. Among the 17 members of a Regional Veterinary Laboratory in the affected area, 9 were positive reactors. There is little doubt that these 9 infections were acquired during autopsies on domestic animals and the subsequent laboratory procedures concerning the isolation and identification of virus isolates. In contrast to this high infection rate all 13 staff members of the adjoining Agricultural College, not so occupied, and presumably equally exposed to mosquito vectors, were negative for antibody. So far there has been no authenticated

mosquito-borne epidemic on the plateau but mosquito-borne human infection must occur as all suspected vectors concerned in sheep and cattle infections feed readily on man.

It seems probable that the situation existing on the inland plateau region is largely as follows:-

1. Epizootics occur irregularly, with an adequate rainfall and the presence of large numbers of susceptible sheep and cattle being essential causal factors.
2. Biological transmission by mosquitoes occurs during epizootics in sheep and cattle and in some human infection.
3. On grounds of abundance, feeding preferences, geographical distribution, adult female longevity, natural infection rates and vector capability, Cx. theileri is responsible for most transmission.
4. Other mosquito species, viz., Ae. lineatopennis, Ae. juppi, and perhaps Cx. univittatus, are also responsible for transmission, and in certain situations, especially when very prevalent, one or the other of the Aedes species, assumes the dominant vectorial role.
5. None of the above species are particularly efficient vectors and it is difficult to believe that they alone are responsible for the explosive epizootic in which hundreds of infections occur in a few weeks.
6. While there is no evidence to indicate that mechanical transmission by all biting flies occurs, it seems likely that some transmission among domestic animals is of this kind.

7. Most human infection is acquired by direct contact with infected domestic animals.

8. The virus persists on the temperate plateau, even in the arid parts, but the method is unknown.

(B.M. McINTOSH, P.G. JUPP & I. DOS SANTOS).

TABLE 1. Isolation of RVF virus from mosquito species collected on 9 farms where domestic ungulates were recently infected.

Farm	Last case[a]	Cx. theileri	Ae. lineatopennis	Ae. juppi	Cx. univittatus
Goodhope	3m	868			5
Potfontein	1m	411			11
Fairfield	1w	3647 (11)[b]	2		2
Prospect	1m	567	210	2257 (3)	48
Bally-ho	1m	218	135	41	81
Skatryk	2w	1012	330	179	116
Goedendacht	1d	1485	387 (2)	127	124
Bospan	1d	1826 (1)	185	162	
Vlakpan	1d	216	48	16	7
Totals		10250 (12)	1297 (2)	2782 (3)	394

a - period elapsing between last known case of infection and collections, in days (d), weeks (w) or months (m)

b - 11 isolations from 3647 mosquitoes.

TABLE 2. Results of transmission tests with RVF virus and 5 species of mosquito and a tick.

Species	Titre of infective feed in logs	No. days after infective feed mosqs. tested	Infection rate[a]	Transmission Attempt[c]	
				No. days after infective feed	Transmission rate[b]
Ae. caballus	7.8 5.0-6.3 4.8-5.1 3.5-3.6	10-22	18/30 (60%) 12/30 (40%) 33/118 (28%) 13/67 (19%)	12 + 13	0/27
Ae. juppi	>8.0 7.7-7.8 4.8-4.9 3.6	8-21	20/30 (67%) 25/52 (48%) 25/61 (41%) 5/23 (22%)	17	1/18 (6%)
Ae. lineatopennis	7.0 6.0-6.3 5.5	12-18	11/15 (73%) 10/33 (30%) 12/37 (32%)	10 + 17	1/6
Cx. theileri	7.9-8.0 6.2-6.5 5.3	12-22	77/78 (99%) 53/70 (76%) 30/44 (68%)	11 + 13 19 20 21	6/23 (26%) sheep 2/15 (13%) 7/10 (70%) infant mice 3/19 (16%)
Cx. univittatus	6.5	20-21	26/30 (87%)	19	2/6 (33%) infant mice
Ornithodoros savignyi	5.7-5.8	65-66	0/26	66 + 67	0/26

a - numerator = No. insects infected
denominator = No. insects tested

b - numerator = No. insects which transmitted virus
denominator = No. infected insects which fed

c - a single mosquito fed on a single animal which was a hamster unless otherwise stated. In the case of O. savignyi several ticks fed on each hamster

REPORT FROM THE VETERINARY RESEARCH LABORATORY, SALISBURY, RHODESIA

1. Rift Valley fever

RVF virus was first isolated in Rhodesia in 1957 when nine isolations were made in the course of routine screening of aborted cattle foetuses. Investigators were not aware of a major outbreak at the time, but from the fact that the virus was obtained from 10 per cent of the foetuses tested during that year, we can surmise that an epizootic situation existed. RVF was not diagnosed again until a major epizootic occurred in 1969. The disease flared up again during the rains in 1970, and from that time onwards routine screening of autopsy specimens and sera from livestock has shown that a degree of virus activity occurs in most years in what are considered to be enzootic areas.

A major epizootic has occurred again with the heavy rains which fell in 1978. The virus has been isolated 125 times since February from specimens from cattle and sheep and in addition the diagnosis has been established serologically on numerous farms. The outbreak has followed the pattern of the 1969 epizootic closely with the disease appearing in February, reaching maximal intensity during March, April and May, and declining from June onwards. No accurate estimate of losses is yet available, but abortions and deaths in cattle and sheep must run into thousands. Both the present and the 1969 epizootics seem to have extended outwards from the enzootic areas.

A disturbing feature of this outbreak has been the recognition of encephalitis and haemorrhagic infections in man. Five human deaths were suspected to be due to RVF, but laboratory evidence to support the diagnosis was obtained in three cases only, the evidence being circumstantial in the two remaining cases. In varying degree, the patients had fever, myalgia, arthralgia, nausea, vomiting and diarrhoea with haematemesis and melaena, rash, jaundice, haemoglobin levels as low as 4 g per cent, pneumonitis, and signs of myocarditis in one instance. An interesting feature in most cases was searing throat pain as described in Lassa fever. All of the deceased patients were males, aged from 25 to 63 years, and all but one had contact with diseased cattle. It is presumed that the one case arose from mosquito transmission. Fresh tissue specimens were obtained from one case only and although virus could not be isolated, RVF antigen was demonstrated in an immunodiffusion (ID) test, the histopathological lesions seen in liver were consistent with RVF and bunyavirus-type particles were seen electron-microscopically. Liver with heavy bacterial contamination was received 13 days after the death of a second patient and again it was only possible to demonstrate RVF antigen in an ID test and characteristic histopathological lesions in liver submitted in formalin. A third patient who died after 12 days of illness had a RVF HAI titre of 1:1280 in his serum at death, but no autopsy was performed.

Two cases from which no specimens were received involved slaughtermen. They came from separate abattoirs and RVF HAI responses were demonstrated in acute and convalescent phase sera from workmates of the deceased patients who also became ill. Clustering of cases of RVF in time and by exact place of work at the abattoirs suggests that an isolated few diseased cattle carcases were involved. At one abattoir five human cases occurred in the by-products section where condemned carcases are processed. There is no indication that the disease posed a threat to consumers, but detailed investigations are in progress. In the meantime, a fuller account of the deaths reported here will appear in the Central African Journal of Medicine.

2. Other arboviruses

A previous report in this bulletin outlined a study of flavivirus antibodies in cattle sera which showed that despite the failure to isolate Wesselsbron virus from numerous livestock specimens tested over the years, the serological evidence is that the virus is widely active in Rhodesia. The first isolation of Wesselsbron virus from a vertebrate in Rhodesia has now been made from the organs of a cow which died on a property where RVF was active. Nevertheless, it is still felt that Wesselsbron virus is of relatively low pathogenicity for cattle and only occasionally causes serious disease.

Isolation of Ndumu virus from cattle organs was previously reported and a second isolate has now been obtained from a cow which died. These two isolations appear to be the only instances on record in which the virus has been isolated from vertebrate specimens. An unidentified member of the Simbu group has been isolated from the brain of a horse which died of suspected rabies. There was histological evidence of viral encephalitis. Several isolates of the unidentified Mazoe virus (reported previously) have been obtained from cattle foetuses recently.

R. Swanepoel and N.K. Blackburn

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE,

ENTEBBE, UGANDA

A. Virus Isolation and Identification:

1. Human Blood Specimens: 242 human sera from febrile patients visiting the Institute clinic were inoculated into suckling Albino mice for virus isolation. Nine isolates were made. Two of them belong to Congo virus, 1 to Wesslesbron, 1 to Dugbe, and 5 have not yet been identified.

The Congo virus agents were obtained from patients displaying fever, headache joint pain and myalgia. They kill suckling mice by IC and IP routes in 5 to 8 days but they are not pathogenic for adult mice by these routes. They are ether sensitive and pass through Seitz filters. These agents were identified as Congo virus by CF test but confirmatory NT has not yet been done. The isolates of Wesslesbron and Dugbe viruses were identified by both CF and NT.

One of the unidentified isolates was obtained from blood of a man with complaints of fever ($102^{o}F$), headache, joint pain, myalgia, nausea and vomiting. This isolate kills NB mice by both IC and IP routes but is non-pathogenic for adult mice. It is ether sensitive and passes through Seitz filters. It has not yet been identified.

The remaining four isolates were obtained from the Institute workers soon after isolating two bat agents to which they are related by CF (Table 1), HI and NT and this suggested a laboratory infection. They kill suckling and and adult mice 2-4 days post-inoculation by IC and IP routes. They are also ether sensitive and pass through Seitz filters. Serological tests have failed to relate them to any of the arboviruses in our stock. Their identification studies are continuing.

2. Bat Blood: 66 blood specimens from various places in Uganda were inoculated into suckling mice for virus isolation. Two isolates were made from female bats (Rousettus aegyptiacus) caught in Kasokero cave in Masaka District. These two agents have similar physical, chemical and biological properties as the 4 humans isolates described above. They are interrelated and are also related by CF (Table 1) HI and NT to the 4 human isolates described above. They have not yet been identified.

It is of interest to note that 2 of the 4 Institute workers infected by these viruses were in contact with these agents during the isolation trials. However, none of them recalls pricking himself. It can therefore be assumed that they got infected by aerosal. The infection of the two non-laboratory workers might have been derived from the laboratory by aerosal because it occurred at about the same time as that of the laboratory workers. Isolation of the bat virus from four sick Institute workers suggests that this virus has a potential role in human health. A sero-epidemiological survey is planned to determine the role of this virus in human diseases.

3. Ticks: 6445 ticks were collected from cattle in various places in Uganda. After identification, the ticks were pooled according to species and then processed for inoculation into newborn mice. Eight isolates were obtained. Two of the isolates acquired from Rhipicephalus appendiculatus ticks belong to Congo Virus and five from Amblyomma variegatum collected from two distant places are related to Dugbe virus by CF test. The remaining isolate obtained from A. variegatum has not yet been identified. This isolate (AMP 15023) was made from a pool of 85 A. variegatum ticks collected from cattle at Bwerenga. It is pathogenic for NB mice by both IC and IP routes but does not infect mice by these routes. It is ether sensitive and passes through Seitz filters. Identification studies are continuing.

Obtaining two isolates of Congo virus from R. appendiculatus was of interest because this might be the first time this tick has yielded Congo virus.

4. Mosquitoes: 9813 mosquitoes of different species were obtained from various places in Uganda and were grouped in 231 pools for inoculation into new-born mice. No virus was isolated.

5. Rodent Blood: 76 rodents' blood was examined and one isolate was made from a rat (Oenomys hypoxanthus) from Bwayise near Kampala. This agent is pathogenic for suckling mice by both IC and IP routes and for adult mice by IC but not by IP route. It is ether sensitive and passes through Seitz filters. It has been identified as Witwatersrand by both CF and NT.

Obtaining an isolate of Witwatersrand from a rat was of great interest because this might be the second isolate of this virus that has been made so far. The prototype was obtained from Culex rubinotus mosquitoes captured in South Africa in 1958. Further, obtaining this virus from Uganda shows that Witwatersrand virus is not confined to S. Africa. Serological surveys are planned on human, rodents and other animals from Uganda and elsewhere.

B. Serological Studies

1. Human Sera: HI test was conducted on human sera, received from different hospitals in Uganda and the Institute clinic between 1975-1977, against some arboviruses known to infect man.

The results (Table 2) showed that 21.8% of the 2366 reactions conducted were positive. Some of the positive results, particularly those with a titre of 1:40 and greater might have been due to cross reactions. The results with Ntaya virus were remarkable and might show that this virus is wide spread in Uganda. These results call for an investigation on the role of arboviruses in human infections in Uganda.

2. Cattle Sera: Serological studies were conducted on cattle sera collected from the Uganda Meat Packers. Animals slaughtered in this abattoir come from all over Uganda. 169 sera were tested by CF against Dhori (Ib 11313), Dugbe (AMP 5689), Jos (IBAN 17854) and Thogoto (2A). Of these 65.7% were positive for Dhori and 9% for Jos, the rest were negative.

3. Bat Sera: 265 bat sera from Masaka were tested by agar gel diffusion against Congo and Kadam antigens and they were all negative.

(M. Kalunda, L.G. Mukwaya, S.D.K. Sempala, M. Lule, E. Sekyalo, Y. Senkubuge, C. Mawejje, A. Mukuye and E. Mujomba, East African Virus Research Institute, P. O. Box 49, Entebbe, Uganda)

Table 1: CF Test on Human and Bat Isolates.

Isolate	IMMUNE SERUM					
	Z 52963	Z 52969	SG 38485	SG 38495	SG 38545	SG 38594
Z 52963	16/32	32/8	32/64	32/32	16/32	32/16
Z 52969	16/8	16/8	128/16	32/32	32/16	32/8
SG 38485	16/8	16/8	64/16	16/8	NT	16/8
SG 38495	0	0	NT	0	NT	0
SG 38545	16/32	32/32	NT	0	32/32	0
SG 38594	16/8	16/8	32/8	NT	32/8	32/8

NT = Not tested Z = Bat isolate SG = Human isolate

Table 2: HI Results on Human Sera Tested Against Various Arboviruses Known To Infect Man

Antigen	Virus Group	HA Units used	Positive Sera at Titre				Total	
			1/10	1/20	1/40	1/40	Pos.	Tested
CHIK	Alfavirus (A)	8	3	4	2	23	32	182
SFV	"	8	7	2	3	7	19	"
Sindbis	"	4	15	7	2	15	39	"
Zika	Flavivirus (B)	8	10	7	2	17	36	"
YF	"	8	40	4	4	11	59	"
WN	"	8	20	8	3	10	41	"
Dengue	"	8	7	4	2	4	17	"
Ntaya	"	4	83	37	18	18	156	"
J.B.E.	"	16	11	2	3	8	24	"
Kadam	"	8	6	4	3	5	18	"
ENT.	"	4	16	4	2	10	32	"
Buk. Bat	"	8	10	5	0	8	32	"
Dakar Bat	"	16	8	2	2	3	15	"
Total			236	90	46	139	511	2366

REPORT FROM THE VIRUS LABORATORY, DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF GHANA MEDICAL SCHOOL, P.O. BOX 4236, ACCRA, GHANA.

1. SEROLOGICAL SURVEILLANCE OF YELLOW FEVER IN GHANA.

Of a total of 1396 blood samples collected from jaundiced patients from 8 of the 9 regions of Ghana and examined for Yellow Fever (YF) haemagglutination inhibiting (HI) and neutralizing (protective) (N) antibodies, it was found that whilst 35.7% of the cases contained YF HI antibodies, only 5.0% contained specific YF N antibodies.

It was found that the rates of both HI and N YF antibody incidence were highest in specimens from the Western Region. No HI nor N YF antibodies were found in specimens from the Upper Region of the country. No cases were examined from the Northern Region.

Peak incidence rates of both HI and N YF antibodies were observed between May and July, i.e., during the wet season. The difference in incidence rates between the wet and dry seasons was statistically significant ($X^2 = 4.49$, $p > 0.05$).

The incidence of YF antibodies was found to have a definite relationship to age; the rates increasing with increase in age reaching peak level among the age-group 21 - 30 years, but was found to be unrelated to sex ($X^2 = 0.225$, $p < 0.05$).

Although YF infections seem to be most rampant in the Western and Brong Ahafo Regions, a consideration of the entire results allows the suggestion that YF is endemic in Ghana and that the superendemic zone of this infection lies in the coastal swampy scrubland area which forms the southern sector of the country and comprises of the Greater Accra, Volta, Central and the Western Regions. YF in this sector is most likely to be the Urban type, whilst in the mountainous rain forest zone (Ashanti and Brong Ahafo and Eastern Regions) the prevailing epidemiological type of YF is most probably the "jungle" type.

Addy, P.A.K., Ahiabor, Theresa M., and Pappoe, M.A.

REPORT FROM THE ARBOVIRUS LABORATORY INSTITUT PASTEUR AND ORSTOM

INSTITUT PASTEUR - BP 220 DAKAR - SENEGAL

During the first half of 1978, virological and serological studies were carried out on samples from Senegal (Dakar-Eastern Senegal: Bandia, Kedougou).

I - Virological studies

1. Human blood samples

3 blood samples (a case from Dakar hospital, a mosquito-catcher from Kedougou and a febrile child at the clinic from Bandia), were inoculated into suckling mice for virus isolation without success.

2. Wild vertebrate samples

60 blood samples from monkeys killed in Kedougou area were inoculated into suckling mice without result.

11 blood samples from *Taterillus* caught in Bandia were inoculated with negative findings.

15 blood samples from *Mastomys* at Bandia might recover 2 new strains of Bandia virus.

3. Arthropods

Mosquitoes: 15863 mosquitoes alloted in 588 pools caught in Kedougou during the 1977 rainy season, were inoculated into suckling mice: 64 (sixty-four) strains of Yellow Fever Virus were isolated, i.e. on the whole 67 strains recovered from mosquitoes collected in 1977 rainy season:

- 56 strains from *Aedes gr. furcifer taylori* (273 pools of 8040 mosquitoes).
- 9 strains from 58 pools of 1586 *Aedes luteocephalus*
- 1 from 31 pools of 813 *Aedes vittatus*
- 1 from 6 pools of 97 *Aedes neoafricanus*

These isolates came after a first isolation of a strain of Yellow Fever Virus from a pool of *Aedes gr. furcifer taylori* collected in december 1976 near Kedougou.

In 3 pools of 85 males *Aedes gr. furcifer taylori*, we have found 3 strains of Yellow Fever Virus. Further investigations were taken on to confirm if it was transovarial or venereal transmission.

II - Serological studies

1. Human sera

210 serum samples collected from children under 10 years, in the Kedougou area, after 1977 rainy season, were studied in H.I., CF and SN tests for group A, group B and Bunyamweraviruses.

This investigation allowed to find again 93 from 240 children taken before 1977 rainy season. We have found serological conversions for yellow fever virus in four villages : Toubacouta, Malida, Sekoto et Bandafassi.

Epidemiological investigation have not showed increasing mortality during the 1977 rainy season.

2. Wild vertebrate sera

17 sera from monkeys caught in Kedougou were studied in HI, CF and SN tests for group A, group B and Bunyamweraviruses :

- 6 have CF isolated antibodies for yellow fever virus that confirms an epizootic disease during the 1977 rainy season.

(J. RENAUDET, Y. ROBIN and J.J SALAUN, Institut Pasteur,
M. CORNET, J.L CAMICAS et M. GERMAIN, ORSTOM, Dakar, Senegal).

REPORT FROM THE ARBOVIRUS RESEARCH UNIT
EGYPTIAN ORGANIZATION FOR BIOLOGICAL AND VACCINE PRODUCTION
AGOUZA, CAIRO, EGYPT

I. STUDIES ON ANTIBODIES AGAINST WEST NILE (WN) AND SINDBIS (SIN) VIRUSES

A. Prevalence of WN and SIN Viruses in Man

Three thousand five hundred and fourteen human sera from different governorates were tested by HI for antibodies against WN and SIN viruses. The overall prevalence rates were 51.8% and 14.2% for both WN and SIN, respectively (Table 1).

For WN virus, the incidence of hemagglutination-inhibiting (HI) antibodies is about 20% below 10 years of age and almost doubles between 10-15 years of age reflecting the high exposure at the beginning of the school age. The highest positivity rate for this prevalent endemic virus is reached at the age of 20 years and remains more or less levelled in a plateau up to the fifties. The progressive increase in WN incidence with age to reach considerably high levels at the young, adult and middle age is in accordance with the endemicity of the viral infection. If we try to analyse the 320 sera collected from children less than 10 years of age on yearly basis we can detect antibodies at all ages (although of low titer) indicating also the endemicity and prevalence of WN virus (Table 2).

Sindbis virus which is also transmitted by the same culicine mosquito, exhibits a lower prevalence, i.e. the overall incidence is 3.5 times less than that of WN. The age distribution of SIN positive sera is more or less similar to that of WN.

B. Prevalence of WN and SIN Viruses in Animal Sera

Table 3 shows that the overall percentage of positives for tested sera is higher for WN than for SIN, i.e. 25.8% vs 14.5%. For every animal species, WN virus showed a higher incidence than SIN. This finding in animal sera is along the same line with the higher prevalence of WN in man. The positivity of almost all the tested animal sera for WN and SIN antibodies is indicative of the prevalence of these 2 endemic viruses in the community. In fact previous studies in Egypt by Taylor et al. (1956) showed an overall mammal prevalence rate of 40%, whereas a more recent study on sera collected in 1969 exhibited a positivity rate of 21.2%.

Table 1

Age Distribution of Human Sera Containing HI Antibodies to West Nile and Sindbis Viruses in Egypt

Age in years	No. tested	Sindbis virus		West Nile virus	
		No.	Percent	No.	Percent
⩽ 1-5	228	42	18.4	44	19.3
5-10	92	12	13.0	22	23.9
10-15	2386	320	13.4	1184	49.6
15-20	196	20	10.2	104	53.1
20-25	92	14	15.2	84	91.3
25-30	156	18	11.5	112	71.8
30-35	128	30	23.4	86	67.2
35-40	60	14	23.3	50	83.3
40-45	84	12	14.2	66	78.5
45-50	62	10	16.1	50	80.6
50 and over	30	6	20.0	18	60.0
Total	3514	498	14.2	1820	51.8

Table 2

Age Distribution of Human Sera Containing HI Antibodies to West Nile and Sindbis Viruses in Egypt

Age in years	No. tested sera	Sindbis virus		West Nile virus	
		No.	Percent	No.	Percent
1	40	10	25.0	10	25.0
1-2	62	4	6.5	6	9.7
2-3	54	14	25.9	12	22.2
3-4	46	10	21.7	12	26.1
4-5	26	4	15.4	6	9.7
5-6	28	4	14.3	4	14.3
6-7	16	4	25.0	4	25.0
7-8	24	4	16.7	4	16.7
8-9	8	0	0	2	25.0
9-10	16	0	0	4	25.0
Total	320	54	16.9	64	20.0

Table 3

Hemagglutination-Inhibiting Antibodies Against West Nile and Sindbis Viruses in Domestic Animal Sera from Egypt

Animal species	No. tested sera	Sindbis virus		West Nile virus	
		No.	Percent	No.	Percent
Buffalo	262	76	29.8	100	38.2
Sheep	368	26	7.1	54	14.7
Cow	284	26	9.1	38	13.4
Camel	186	64	34.4	76	40.9
Goat	66	2	3.0	4	6.1
Pig	102	2	2.0	10	9.8
Horse	136	12	8.8	32	23.5
Donkey	190	36	18.9	118	62.1
Mule	7	0	0	2	28.6
Dog	98	2	2.4	4	4.1
Total	1699	248	14.5	438	25.8

II. PREVALENCE OF SANDFLY FEVER VIRUS

Sandfly Fever Virus represents the third endemic arbovirus in Egypt. Previous work had shown the predominance of the Sicilian virus in the community (Taylor 1959, Schmidt et al. 1971 & Darwish et al. 1971). It was decided to test certain number of the collected human sera for HI antibodies against the Sicilian strain of Sandfly Fever Virus (SFS).

Table 4 shows the distribution of HI antibodies among 1792 school children of age 10-18 years from Alexandria, Cairo and Asyut governorates. It is clear that the overall positivity rate is 18.9%. The coastal Alexandria governorate shows the lowest rate (10.7%), followed by Cairo (18.3%), and then Asyut governorate in Upper Egypt which exhibits the highest infection rate (23.1%). This north-to-south increase as we move to the more inland governorates has been noticed before with WN and SIN infections and was attributed to the decreased density of the vector in the coastal areas.

Table 5 which presents the age distribution of the positive sera, demonstrates that the overall rate was 18.0%. The prevalence rate which was 13.7% at the age group of 10-15, reached the peak (59.2) at the next age group (15-20 years) and decreased again to 26% at which it almost maintained its level till the age group of 40-45 years, before it dropped further.

The prevalence of SFS antibodies at all age groups is pertinent with the known endemicity of the virus in the country. The drop in the positivity rate at a certain age may also reflect the reported (Taylor 1959) transitory nature of Sandfly fever antibodies as compared with other arboviruses. Table 6 may also give support to the transient nature of SFS antibodies since about 60% of the positive HI reactors exhibited a titer of 1/10 or 1/20. Also, almost all the sera which exhibited HI titer of 1:80 and above were from school children between 10-18 years of age which may add evidence to the gradual waning of HI antibodies by time.

Table 4

Geographical Distribution of Human Sera Containing HI Antibodies to Sandfly Fever Virus in Egypt

Governorate	No. tested sera	Sandfly Fever Virus (Sicilian Strain, SFS) No.	Percent
Alexandria	354	38	10.7
Cairo	677	124	18.3
Asyut	761	176	23.1
Total	1792	338	18.9

Table 5

Age Distribution of Human Sera Containing HI Antibodies to Sandfly Fever (SFS) Virus in Egypt

Age in years	No. tested sera	Sandfly Fever Virus	
		No.	Percent
10-15	1473	202	13.7
15-20	98	58	59.2
20-25	46	12	26.1
25-30	78	22	28.2
30-35	64	19	29.7
35-40	30	7	23.3
40-45	42	10	23.8
45-50	31	6	19.3
50 and over	15	2	13.3
Total	1877	338	18.0

Table 6

Sandfly Fever (SFS) Virus, Hemagglutination-Inhibiting (HI) Titers in Human in Egypt

HI Titer	Sandfly Fever Virus Positive Sera	
	No.	Percent
10*	132	39.05
20	70	20.71
40	60	17.75
80	26	7.69
160	40	11.83
320	10	2.96
Total	338	99.99

*Reciprocal of serum end-point dilution.

III. EPIDEMIC OF RIFT VALLEY FEVER IN EGYPT

In October 1977 a Rift Valley Fever (RVF) epidemic occurred in Sharqiya Governorate, northeast of Cairo. The disease first presented as an acute febrile, dengue-like illness of man; however severe cases complicated by hemorrhage, jaundice, meningoencephalitis and retinitis were also encountered. The epidemic spread to more than 21 villages in Sharqiya Governorate to Qalyubiya and Giza Governorates in the Nile Delta. Later (about 11 December 1977) cases appeared in Upper Egypt (Minya and Asyut Governorates). By the end of December, the human epidemic was over. Data showed about 18,000 cases, with 598 deaths. At the beginning of the outbreak, RVF virus was isolated from the serum of 53 of 56 clinically suspected cases and also from 4 stool specimens and 2 throat washings. The virological diagnosis was later carried out serologically by using the HI test.

It is very unusual for RVF outbreaks to appear first in humans. It was therefore decided to explore the situation in the animal population. Search proved that death and abortions had occurred weeks or months before the human epidemic, but passed unnoticed or were misdiagnosed. Teams were sent to farms in different governorates upon notification of suspected cases and specimens were brought for virus isolation. RVF virus was isolated during the epidemic from 27 sheep, and from a cow, a camel, a goat, a horse and a rat (Rattus rattus). The isolation of virus from camel and horse represent the first records from these animals.

A retrospective serologic study was made on animal and human sera stored in our freezers from previous years. The survey included 5473 sera from 9 different animal species distributed over many Governorates representing most of the country. The results showed absence of RVF antibodies in 1974, 1975, and 1976 (about 3,000 sera). The earliest indication of viral activity (within the available sera) was shown on April 1977 (6 months before the human epidemic) for sheep, buffalo, cow, and goat sera collected from Matruh, Alexandria and Port Said Governorates. Sera collected in late September and early October 1977 (just before the Sharqiya human epidemic was brought to our attention) from domestic animals in Qena and Aswan Governorates also showed RVF virus antibodies. The overall positive rates for about 2,500 domestic animal sera collected during 1977 to April 1978 was 18%, ranging from 40% in sheep to 2.0% for goats with other animals.

The human serological data showed that 3,183 sera collected during 1972-76 were free of RVF HI antibodies. No human sera collected in 1977 prior to the epidemic could be found. After the start of the human outbreak in October 1977, all the human sera tested (1,705) had a certain degree of positivity, ranging from 1.2% to 66% with an average of 19.0%. The lowest incidence of positive reactions was in the coastal governorates, which together with the termination of the epidemic after insecticidal spray can be circumstantial evidences for RVF being mosquito or vector-borne. In this respect the only 2 isolates which we obtained from partially engorged Culex pipiens are being very cautiously interpreted in respect to incriminating a specific vector.

Certain epidemiological questions need to be answered: how was the virus introduced into Egypt (this outbreak represents the first record of RVF in a north African country). Was the virus introduced from the north or the south? (The fact that RVF epizootics, together with cases in man, had occurred in Sudan, south of Egypt in 1973 and 1976 may give a clue, and importation of infected animals is possible.) Wild birds have been suggested by Weiss to introduce RVF virus into a clean area. Concerning the reservoir(s) for RVF virus in Egypt - if overwintering occur - one has to consider domestic animals, rodents, and transovarial transmission in a proven efficient vector. The risk and potentiality of another human outbreak depends upon the herd immunity, reservoir and/or amplifier hosts, and efficiency of the transmitting vector. In June 1978, human cases of RVF started again at the same Sharqiya Governorate and the situation is being investigated.

Among the much needed aspects for investigation is a country-wide survey for the prevalence of antibodies among man and animals, to determine "virgin" areas. Mild and/or inapparent cases should not be belittled. Another important aspect for study is the nature of the Egyptian virus isolate as compared to the prototype and the Sudanese strain.

(Medhat A. Darwish and Imam Z. Imam)

REPORT FROM THE ARBOVIRUS AND MEDICAL ENTOMOLOGY UNITS OF THE PASTEUR INSTITUTE OF IRAN

This is the first contribution to the Information Exchange from the Arbovirus Unit and Medical Entomology Unit, both newly established in the Pasteur Institute of Iran at the beginning of 1978, and working in collaboration with the previously existing Epidemiology Unit.

A field investigation of tick-borne viruses has been conducted in April/June 1978 in the Khorassan Province of north-east Iran. From domestic and wild mammals the following species of ticks have been collected: Hyalomma asiaticum (from sheep and goats), H. anatolicum (from sheep and cattle), H. schulzei and H. dromaderii (from camels), Rhipicephalus turanicus (from sheep, goats and cattle and from Hemiechinus auritus), Alveonasus lahorensis (larvae from goats), Ornithodorus coniceps and Argas hermanni (in pigeon houses) and Argas persicus (in hen houses).

Virus isolation attempts, through inoculation into new-born white mice are in progress. At the present time only preliminary results may be given which indicate that isolates have been obtained from:

- a pool of 44 larvae of Alveonasus lahorensis collected on healthy goats at Ferdows (34°N-58°10'E) on April 25 1978 (N° Ar Th 193-3). The sucrose-aceton antigen prepared from the brain of baby mice of 2nd passage, harvested when paralyzed on 8th post-inoculation day, does not hemagglutinate goose erythrocytes; it gives a positive complement-fixation reaction with the Polyvalent Congo and Monovalent Congo Immune Ascitic Fluids of the N.I.H. (negative with all other IAF).
- two pools of 6 adult males of Hyalomma asiaticum collected on healthy goats at Ferdows on April 28,1978 (N° Ar Th 193-4 and Ar Th 193-5). The sucrose-aceton antigens prepared from the brain of baby mice of the 3rd passages, harvested when paralyzed on 11th day (ArTh193-4) and 8th day (ArTh193-5) do not agglutinate goose erythrocytes; both of them give a positive complement-fixation reaction with the Polyvalent 10 IAF of NIH (Dera Ghazi Khan, Dhori, Wanowrie).

The reactions are negative with all other available IAF tested (A-1, Anopheles A, B (Master), Bunyamwera, Bwamba, California, Congo, Kemerovo, Palyam, Phlebotomus fever, Quaranfil, Simbu, Poly 1, Poly 3, Poly 4, Poly 5, Poly 7, Poly 8, Poly 12 and Poly Rabies-LCM-NDV-Herpès-Vaccinia.

A serological survey conducted among sheep and goats of the same area indicates that 14,6% of sheep and 13,3% of goats have specific CHF-C antibodies in the agar-gel-diffusion-precipitation test; 22 of the 29 sheep and 9 of the 14 goats found to be positive in the AGDP test give a positive complement fixation test with the antigen prepared from the local isolate Ar Th 193-3. Among the same animals, CF antibodies have also been detected against the local isolate Ar Th 193-5.

(P.Sureau Arbovirus Unit, J.M.Klein Medical Entomology Unit, Y.Karimi Epidemiology Unit).

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

Third outbreak of Japanese encephalitis in six years in West Bengal, INDIA.

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The first epidemic of Japanese encephalitis(JE) in four districts of West Bengal broke out in 1973 in which 700 clinical cases with about 40% mortality was recorded. The epidemic recurred in one of the four districts(Burdwan) in 1976 when 277 clinical cases were investigated. In both the epidemics, JE virus was isolated from autopsy human brain tissues and mosquitoes. There was also serological evidence of JE virus infection.

In the month of May 1978, cases of encephalitis from Burdwan District were reported to this department for investigation. About 300 cases have been reported so far. The specimens received uptil now are:- acute blood - 67, C.S.F - 18, autopsy brain tissue - 2, convalescent blood-17, pig blood - 43, cattle blood - 42, goat blood - 17, Mosquitoes (of various species) - 931.

All the specimens are being studied, but virus could not be isolated uptil now. Serological tests(both HI & CF) show that 5 out of 17 paired sera show definite evidence and 3 more pairs are suggestive of JE virus infection. Results of 7 of the 23 single sera from clinical cases show strong evidence of JE infection.

Apart from these, 600 samples of blood from unaffected people of the area have been collected for antibody studies.

The work is in progress.

(M.S.Chakravarty, K.K.Mukherjee, S.K.Chakravarti, AC.Mitra, K.K.Mitra and J.K.Sarkar)

REPORT FROM THE JAKARTA DETACHMENT OF U.S. NAVAL MEDICAL RESEARCH UNIT
APO SAN FRANCISCO 96356

Neutralizing Antibodies to Arboviruses in Children of Indonesia

We have recently begun expanded studies on the epidemiology of arboviral diseases in Indonesia by seeking baseline information from sera collected during health surveys and investigations of disease outbreaks. In order to determine which arboviruses have infected these study populations in the relatively recent past, we have assayed for antibody in children less than 10 years of age.

Sera from 3 areas were tested: (1) from Ngawi, East Java, collected in 1977 during an investigation of a dengue hemorrhagic fever outbreak; (2) from the island of Lombok, collected in 1977 from children who had recovered from a febrile illness which was associated with morbilliform rash and was identified as measles on clinical and epidemiologic grounds; (3) from West Sumatra and Jambi Provinces of Sumatra from children being studied as part of a general health survey.

We screened sera from children under 10 years of age by hemagglutination inhibition (HI) using hemagglutinating antigens (HA) prepared with Japanese encephalitis (JE), dengue type 2 (DEN-2), chikungunya (CHIK) and Ross River (RR) viruses. Subsequently we tested [1] sera which had $\geq$ 1:80 HI antibody titers against JE or DEN-2 or which had $\geq$ 1:20 titers against CHIK or RR for neutralizing (Nt) antibodies to arboviruses active in nearby areas of Southeast Asia.

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[1] Sukhavachana, P., Yuill, T.M. and Russell, P.K. (1969). Assay of arbovirus neutralizing antibody by micro methods. Trans. Roy. Soc. Trop. Med. Hyg., 63: 446-455

We herein report results of Nt testing of those sera which were positive by HI test.

Data presented in table 1 show that 19/30 (63%) sera tested from Ngawi had $\geq$ 1:4 titers of Nt antibody to Zika virus (ZIKA). Thirteen of the 19 had antibody only to ZIKA, 5 others had titers higher against ZIKA than other viruses used in the test and one had equal titers to ZIKA and Langat virus (LAN). One serum (6375) had a titer of 1:4 to only JE and another (6330) had a 1:8 titer only to Murray Valley encephalitis virus (MVE). Antibody titers detected in other sera to Kunjin (KUN), LAN, Tembusu (TMU) and West Nile (WN) viruses were lower than the ZIKA antibody titer in the same serum. No antibody titers of $\geq$ 1:4 were detected using Sepik virus (SEP). No micro-neutralization tests with group A arboviruses were done on sera collected from Ngawi because HI titers using CHIK and RR HA were all $<$ 1:20.

Data presented in table 2 show that 2/15 (13%) sera tested from Lombok had Nt antibody to JE and 1/15 (7%) had antibody to MVE. The sera 5680 and 5681 had antibody to JE and TMU but JE was present in higher titer. Nt antibody to ZIKA was detected at low titer (1:4) in 2/15 (13%) sera tested. We did not test for KUN, LAN, WN or any of the group A arboviruses.

Data in table 3 show that 4/5 (80%) sera tested from Sumatra had highest neutralizing titers to ZIKA and that a single serum (1831) had equal titers of Nt antibody to JE and MVE. The JE and MVE titers in this serum were higher than those of other group B arboviruses used in the test. Antibody to TMU was detected in 3 sera but at lower titers. Low antibody titers (1:4)

to GET were detected in 7/17 (41%) of sera tested with group A arboviruses. Five of these 7 sera had no detectable titer against any of the other test viruses, but 2 (1938 and 2171) had equal titers of antibody to RR. No antibody was detected specific for CHIK or SIN.

We conclude from these preliminary studies that arboviruses other than those already isolated in Indonesia (JE and DEN) have recently infected the study populations. Viruses likely to be recovered from arthropods and possibly from humans with febrile illnesses include ZIKA, MVE and possibly JE in Central Java; JE, MVE and ZIKA in Lombok; and GET, ZIKA and MVE or JE and possibly RR in Sumatra. It is also possible that low titers of neutralizing antibodies indicate the presence of viruses closely related but distinct from those tested for.

Suharyono, I., Lubis, D.J. Gubler, T.G. Ksiazek, V.H. Lee and J.G. Olson

Table 1. Neutralizing antibodies to selected flaviviruses in sera* from Ngawi, Java.

Serum No.	Reciprocal of antibody titer							
	JE	KUN	LAN	MVE	SEP	TMU	WN	ZIKA
6321	-**	-***	-	-	-	8	8	-
6325	-	-	-	-	-	-	-	64
6326	-	-	-	-	-	-	-	128
6327	-	-	8	-	-	-	-	8
6330	-	-	-	8	-	-	-	-
6331	-	-	-	-	-	-	-	16
6332	4	8	-	-	-	8	8	16
6333	-	-	-	-	-	-	-	-
6334	-	-	-	-	-	-	-	32
6336	-	-	-	-	-	-	-	32
6340	-	-	-	-	-	-	-	-
6343	-	-	-	-	-	-	-	≥256
6344	-	-	-	-	-	-	-	16
6345	-	-	-	-	-	-	-	16
6346	-	-	-	-	-	-	-	16
6347	-	-	-	-	-	-	-	-
6349	-	-	-	-	-	-	-	-
6355	-	-	-	-	-	-	-	16
6357	-	-	-	-	-	-	-	-
6360	-	-	-	-	-	-	-	-

see cont'

Table 1. cont'

Serum No.	Reciprocal of antibody titer							
	JE	KUN	LAN	MVE	SEP	TMU	WN	ZIKA
6362	4	-	-	-	-	-	-	64
6363	-	-	-	-	-	-	-	64
6369	4	-	-	-	-	-	-	8
6370	-	-	-	-	-	-	8	16
6375	4	-	-	-	-	-	-	-
6383	-	-	-	-	-	-	-	-
6384	-	-	-	-	-	-	-	128
6386	-	-	-	-	-	-	-	-
6390	-	8	8	-	-	-	-	64
6391	-	-	-	-	-	-	-	32
Log10 TCID50	1.6	1.8	1.5	1.6	1.6	2.0	1.6	2.5

* Sera were screened by HI test using JE and DEN-2 and only those $\geq$ 1:80 were tested by micro-neutralization

** JE test were recorded as - if $\leq$ 1:4

*** All other tests recorded as - if $<$ 1:8

Table 2. Neutralizing antibodies to selected flaviviruses in human sera* from Lombok.

Serum No.	Reciprocal antibody of titer JE	MVE	TMU	ZIKA
5678	< 32	< 16	0	0
5679	0**	0	0	0
5680	16	< 16	8	0
5681	16	0	8	0
5692	< 16	< 16	< 16	< 16
5693	0	0	0	0
5713	-***	4	-	-
5715	-	-	-	-
5716	0	-	0	0
5717	0	-	-	-
5737	-	-	-	4
5742	-	0	0	0
5747	-	-	-	4
5749	0	0	0	0
5751	0	0	0	0
Log10 TCID50	1.8	1.8	1.5	2.5

* Sera screened by HI had $\geq$ 1:80 titer to JE
** Titers < 1:8 recorded as 0
*** Titers < 1:4 recorded as -

Table 3. Neutralizing antibodies to selected arboviruses in human sera* from West Sumatra and Jambi Provinces, Sumatra.

Serum No.	Reciprocal of antibody titer							
	JE	MVE	TMU	ZIKA	CHIK	GET	RR	SIN
1829					-	4	-	-
1831	32	32	4	4	-	4	-	-
1857					-	-	-	-
1863	-**	-	-	32	0***	0	0	0
1883	4	4	-	8	-	-	-	-
1892					-	-	-	-
1938					-	4	4	-
1978					0	0	0	-
1981					-	-	-	-
1991					0	0	0	0
2088					-	4	-	-
2112					-	4	-	-
2127					-	-	-	-
2138	4	-	4	⩾ 256	-	-	-	-
2156					-	-	-	-
2160	-	-	4	64	-	4	-	-
2171					-	4	4	-
Log10 TCID50	1.8	1.8	1.5	2.5	2.0	1.5	1.3	1.6

* Sera screened by HI and ⩾ 1:80 for JE and ⩾ 1:20 CHIK, GET or RR
** Titers < 1:4 recorded as -
*** Titers < 1:8 recorded as 0

REPORT FROM THE ARBOVIRUS RESEARCH UNIT

UNIVERSITY OF CALIFORNIA INTERNATIONAL CENTER

FOR MEDICAL RESEARCH

HOOPER FOUNDATION, SAN FRANCISCO

AND

THE UNIVERSITY OF MALAYA, KUALA LUMPUR

I. Telok Forest Virus.

An agent (P72-4 strain) isolated in suckling mice from the blood of a wild Macaca nemestrina monkey has been characterized as a newly-identified virus related to the ungrouped Tanjong Rabok virus. The source monkey was trapped in a fresh-water peat swamp forest in Peninsular Malaysia. Tanjong Rabok virus had been isolated previously from the blood of a sentinel monkey in an adjacent forest. The two viruses are indistinguishable by CF test, but show no cross-reaction by HAI and PRNt in Vero cells.

Serum	Antigen					
	P72-4			TR (P8-1542)		
	HAI	CF	PRNt	HAI	CF	PRNt
P72-4	80	128	470	<10	128	< 10
P8-1542	<10	64	< 10	320	64	400

In Vero cells, Telok Forest virus results in complete cell destruction within 5 days and produces 5-6 mm clear plaques under agar in 2 days. It results in death in suckling mice in 3 days and weanling mice in 7 days by the intracerebral route.

II. Dengue Virus Isolates in Aedes pseudoscutellaris Cells.

Ten low passage strains of untyped dengues (all probable type 3), detected in suckling mice by the dengue-challenge technique, failed to produce CPE or plaques in cell lines including Vero, PS, and Aedes albopictus. When inoculated in Aedes pseudoscutellaris cells, 9 of the 10 resulted in the production of readily observed syncitia in varying periods of time from 3 to 8 days. One of the ten strains produced dramatic syncitial formation within 3 days and it had been isolated from a DHF case in Malaysia believed to have been fatal (the final medical history has yet to be reviewed).

III. Toxorhynchites splendens Mosquitoes for the Detection and Assay of Dengue Viruses by Parenteral Inoculation.

With the successful establishment of a laboratory colony of Toxorhynchites splendens mosquitoes in our Malaysian unit, studies have been initiated to compare sensitivity for dengue viruses in these mosquitoes, in Aedes pseudoscutellaris cells, in LLC-MK_2 cells, and in mice employing the challenge technique. A medical student from YARU is participating in these studies.

IV. Jungle and Rural Dengue Studies.

One of our current principal objectives in the study of jungle dengue is to assess the importance of the Aedes (Finlaya) niveus subgroup of mosquitoes in different types of forest habitats in Malaysia. Our studies in the isolated hill dipterocarp forest in the Gunong Besout Reserve led us to the conclusion that jungle dengue is primarily a high canopy infection of monkeys transmitted by mosquitoes of the Ae. (Finlaya) niveus subgroup. This conclusion was based on analysis of mosquito collections in the high canopy where dengue transmission was detected versus the ground where no dengue transmission could be detected, mosquitoes attracted to and feeding on monkeys, and the isolation of a strain of dengue from A. niveus. This conclusion has yet to be tested by laboratory transmission experiments. This subgroup includes eleven known species and subspecies in Peninsular Malaysia. Unfortunately, specific identification of some of these (e.g., pseudoniveus and subniveus) cannot be made by examination of the adult females. Others can only be identified with difficulty in the adult female, especially if the specimens are damaged, as wild-caught specimens often are. All, however, can be identified in the larval stage and from study of the male terminalia. Our surveys for mosquitoes of this subgroup in different forest habitats, therefore, include: 1) collection of adults, both male and female, attracted to humans in the canopy and on the ground, 2) larval collections, and 3) obtaining eggs from gravid wild females, when possible, and rearing them through for positive identification. By this combination of methods, we hope to be able to make reasonably accurate assessments of the niveus subgroup populations in the various forest habitats.

Study sites include fresh-water peat swamp, mangrove swamps, disused rubber, and hill dipterocarp forests. Additional sites being considered for study include lowland dipterocarp forest and coconut and oil palm plantations.

Since dengue fever and dengue hemorrhagic fever are regularly recognized in urban and town areas of Malaysia but not in rural areas, where A. albopictus is common and A. aegypti usually absent, we have initiated a study to assess dengue virus infection in man in a rural area. This area is a valley surrounded on three sides by forest and leading into developed areas on the fourth. The study includes assessment of the vector populations, examination of pyrexias of undetermined origin for dengue, and detection of possible inapparent dengue infections. The study area has a population of almost 12,000 persons in over 2,000 houses. A detailed census has been completed and a pilot study involving 1500 individuals is now in its final phase.

(Albert Rudnick, John J.S. Burton, and William A. Neill)

REPORT FROM THE DEPARTMENT OF VIROLOGY, UNITED STATES COMPONENT, ARMED FORCES RESEARCH INSTITUTE OF THE MEDICAL SCIENCES, BANGKOK, THAILAND

Broadly cross reacting antibodies elicited by successive dengue virus infections make the serological diagnosis of the infecting serotype difficult or impossible. Isolation of the virus is often the only method of identifying the infecting serotype. Classically, dengue viruses have been obtained from serum or plasma; however, the recovery rates from these specimens have been dismally low. Recent studies invitro and in experimental animals have indicated that viruses may be associated with the formed elements of the blood. Therefore, we attempted to isolate dengue viruses from the leukocytes of naturally infected dengue patients and to compare the rate of isolation with that obtained from plasma.

Samples of heparinized blood were obtained from children admitted to the Bangkok Children's Hospital with clinical hemorrhagic fever. Plasma was separated by centrifugation and leukocytes prepared by standard methods. During 1976 and 1977 90 patients yielded dengue viruses. Of these, 86 were isolated from the leukocyte preparations while 28 were from plasma samples. Among 332 patients that had serological evidence of dengue infection, virus was isolated from 24% using leukocytes, but only 7% using plasma. Further, in samples obtained during the first four days of illness, leukocytes yielded isolates in 44% of cases while in plasma, virus was found in 15%.

Leukocytes proved to be a rich source of dengue virus allowing for the isolation of more than three times as many dengue strains as plasma.

Studies are continuing to determine exactly which leukocytes are infected by dengue viruses.

Submitted by, Robert McNair Scott, Ananda Nisalak, and Suchitra Nimmannitya.

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REPORT FROM THE VIROLOGY SECTION

NATIONAL HEALTH LABORATORY, 35, STEWART ROAD

RANGOON, BURMA

Japanese B encephalitis outbreak in horses in Burma

An outbreak of encephalitis among horses was reported at the Animal Breeding Centre and Fodder Farm of Ba Htoo Myo in November and December 1977. Ba Htoo Myo is a town situated in the northeastern part of Burma, i.e. in Shan State. The place is about 3000 feet above the sea level.

Approximately 3% of the total animals (19 horses and 1 ass) were infected, and 70% of the sick animals (13 horses and 1 ass) succumbed to the disease. It was also noted that out of the 14 deaths, 12 animals were under 3 years of age and the remaining two were 7 and 8 years of age. Just before the outbreak, animal attendents noted that two pigeons fell from their perch, trembled, turned around and around on one side and died.

By inoculating a suspension of brain of a horse that died of encephalitis into suckling mice, we isolated a virus. The isolated virus was identified as Japanese B encephalitis virus by Haemagglutination Inhibition and Complement Fixation Tests. The finding was confirmed by the Viral Research Centre, Poona.

In February of 1978, a field study group sent to the affected area collected sera from the sick animals that survived the outbreak. Of six animals which survived, four had detectable CF antibody and all six had detectable HI antibody against Japanese B encephalitis virus.

This is the first time a JBE virus has been isolated in this country. To quote some previous incidences of JBE in Burma:

(a). In a survey conducted in Rangoon city area in 1968, 16% of the human sera tested had detectable neutralizing antibody against JBE.

(b). In 1973 a survey conducted in pigs brought to Rangoon from various parts of the country for slaughter, showed a high prevalence of JBE HI antibody, but low prevalence was observed in cattle and buffalos, especially in the latter.

(c). In 1974, an outbreak of encephalitis in humans occurred in Tachileik, a border town close to Chainmai Valley, Thailand, where JBE was known to exist. Of the 5 cases 4 succumbed to the disease (80% case fatality rate). The sole survivor showed HI titre of 1 in 640; Plug Neutralizing titer of 1 in 80 against JBE.

(d). In 1975 there was reported incidence of encephalitis in Lashio, a town situated in northern Shan State. Of the 23 human cases the disease claimed 17 lives (73.9% case fatality rate); and in Kengtung township there were 15 deaths among 19 cases (78.9% case fatality rate).

The presently reported area where horses died or encephalitis, Ba Htoo Myo, is closely linked to the previously stricken area of Shan State socially and economically.

The isolation and identification of JBE virus in the horses confirmed the existance of the virus in Burma.

(Than Swe and Mi Khin Khin Soe)

REPORT FROM THE VIROLOGY AND ENTOMOLOGY DIVISIONS
U. S. NAVAL MEDICAL RESEARCH UNIT-2, TAIPEI, TAIWAN

1. Isolation of Japanese encephalitis virus from the Republic of the Philippines

There have been many reports of serological tests indicating the presence of JE in the Philippines. However, to our knowledge, there have not been any reports of isolation of the virus.

During studies of the role of diptera in transmission of disease in Southeast Asia we have been collecting mosquitoes at various locations in the Republic of the Philippines. These studies have been in collaboration with the Bureau of Research and Laboratories, Philippine Health Department.

During a field trip to Tagudin, Ilocos Sur Province, Luzon, R.P. in August 1977, 33,274 mosquitoes were collected. The species distribution of these mosquitoes is given in Table 1.

An isolate was obtained from both pools Ph Ar 281 and Ph Ar 384. The isolates were obtained by using BHK and Vero cells employing a direct and delayed plaquing technique. The isolations were confirmed by inoculation of an additional aliquot of the mosquito pool onto an Aedes albopictus cell line and observing for fluorescence using an FITC conjugated JE antisera.

Identification of the isolates was performed using a microneutralization test the results of which are shown in Table 2. The tests were performed in microtiter plates using the PS line of porcine kidney cells.

These isolations of JE virus positively demonstrate the presence of the virus in the Philippines, although this had been indicated by past serological surveys.

Furthermore, the isolates were obtained from vector species that have previously been demonstrated to take part in the transmission of JE elsewhere. These results indicate that the ecology of JE is probably the same in the Philippines as it is in other tropical areas of Southeast Asia. Our studies are continuing and field trips to other areas of the Philippines have been made. We shall report other isolates as they are made.

(J. H. Trosper, T. G. Ksiazek, V. Basaca-Sevilla & J. H. Cross)

2. Evaluation of two Asian rodent species as possible amplifiers of chikungunya virus

A report by B. M. McIntosh in Trans. Roy. Soc. Trop. Med. Hyg., 55: 63-68, which indicated that at least one species of South African rodent, Mystromys albicaudatus, might possibly serve as an amplyfying host for chikungunya (CHIK) virus. We evaluated two species of Southeast Asian rodent, Rattus mindanensis and Rattus exulans which had been colonized at NAMRU-2 for ability to produce viremia and antibody after subcutaneous inoculation with 3.3 $\log_{10}$ of CHIK, S-27 strain in it 177th SMB passage.

For viremia studies, six animals of each species were individually housed and bled from the retroorbital plexus on alternate days (3 each day for each species) for 6 days. Blood for serum antibody determination was obtained by the same route on days 7, 14, 28, and 56. Determination of viremia levels were attempted in both suckling mice and by plaquing on Vero cells. Antibody determinations were done by a microneutralization test employing Vero cells and serum dilutions challenged with 2.0 $\log_{10}$ of virus.

R. mindanensis responded with a trace of viremia (as measured by SMB-IC inoculation; there were no plaques on Vero cells) on days 1 and 2 (2 of 3 on each day). R. mindanensis did respond with neutralizing antibody as shown in the figure. R. exulans did not have any detectable viremia and only one of the six individuals responded with neutralizing antibody (not shown).

The study was performed with an African strain of CHIK virus which had undergone considerable laboratory passage so these results may be subject to considerable interpretation.

(T. G. Ksiazek, and S. J. Chang)

Table 1. Mosquitoes collected near Tagudin, Ilocos Sur Province, Luzon, R.P. August, 1977.

Species	No. mosquitoes	No. pools	No. positive pools
Culex tritaeniorhynchus*	19,677	210	1
Cx vishnui*	4,895	63	1
Cx fuscocephala	981	19	0
Cx bitaeniorhynchus	109	5	0
Cx gelidus	180	8	0
Cx whitmorei	15	1	0
Cx fuscanus	16	3	0
Cx fatigans	16	2	0
Cx pseudovishnui	14	1	0
An annularis	2,851	35	0
An vagus	1,210	17	0
An peditaeniatus	108	4	0
An indefinitus	431	5	0
An tessellatus	20	1	0
Mansonia uniformis	142	6	0
Aedeomyia catastica	1,244	21	0
Aedes vexans	1,134	24	0
Ae lineatopennis	215	8	0
Ae albopictus	9	1	0
Mimomyia luzonensis	7	1	0
Total	33,274	435	2

* Isolation: Ph Ar 281 Cx tritaeniorhynchus pool of 100

Ph Ar 384 Cx vishnui pool of 70

Table 2. Identification of isolates Ph Ar 281 and Ph Ar 384 by microneutralization tests using 1.5 - 2.5 Log_{10} virus dose against varying serum dilutions.

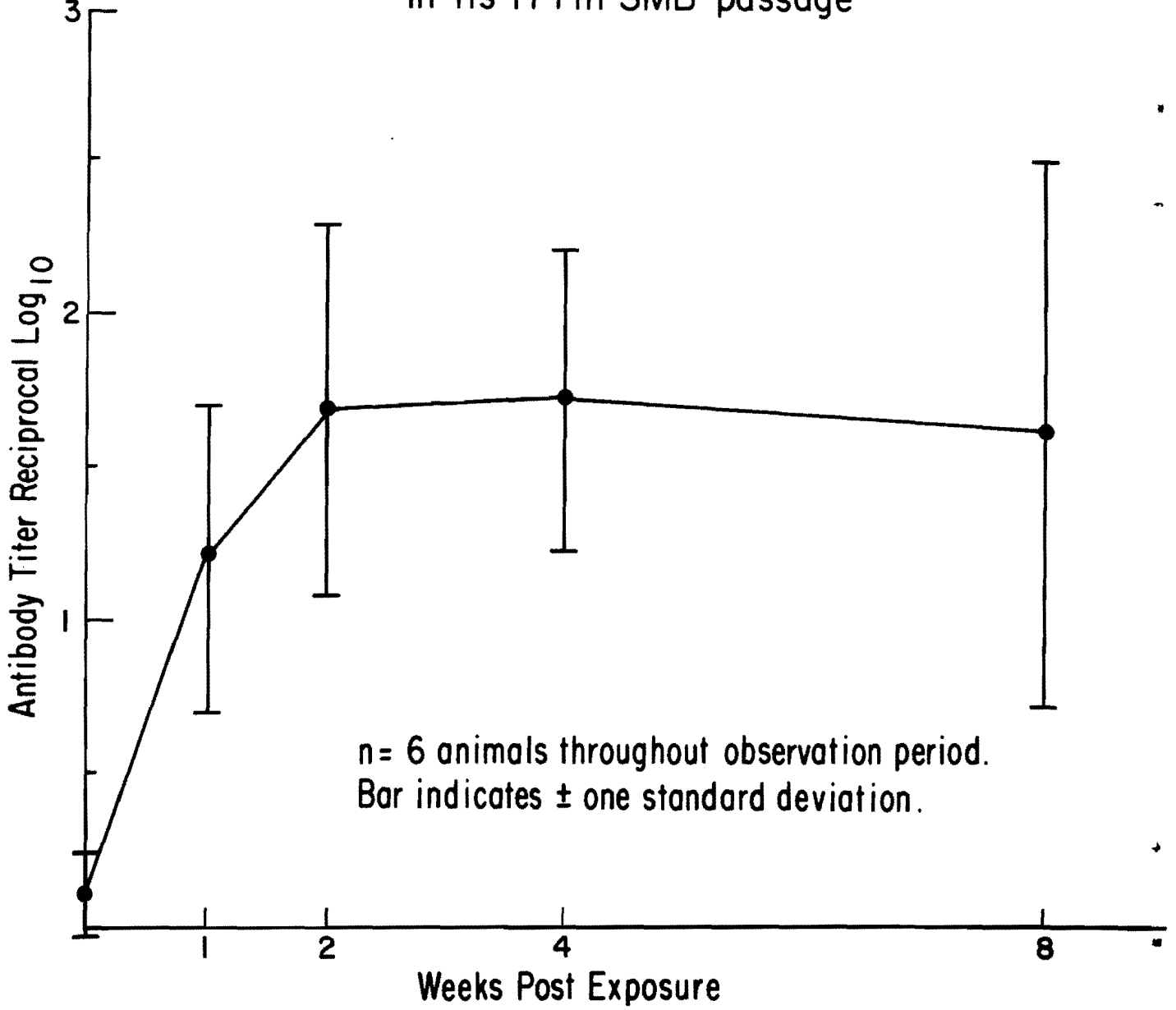
Sera	Virus					
	JE	281	384	WN	MVE	SLE
JE*	160	120	320	<10	<10	<10
281**	12	24	12	<4	ND	ND
384**	4	8	12	<4	ND	ND
WN*	<10	<10	<10	640	ND	ND
MVE*	<10	<10	<10	ND	160	ND
SLE*	<10	<10	<10	ND	ND	160

* Hyperimmune mouse ascitic fluid (HMAF).

** 2 dose mouse antisera.

In addition the following HMAF were tested against Ph Ar 281 and Ph Ar 384 viruses were found to react at <1:10: CHIK, GET, BEB, SIN, WHA, SAG, EEE, WEE, DEN-1, DEN-2, DEN-3, DEN-4, TEMB, LAN, ZIK, Y.F., SEP, KUN, ING, BUN, BAT, BAK, UMB, Normal MAF.

Geometric mean neutralizing antibody titer of Rattus mindanensis after subcutaneous inoculation with 3.3 $\log_{10}$ Vero cell plaque forming units of S-27 strain of Chikungunya virus in its 177th SMB passage



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

1. Viruses isolated from a Singh's Aedes albopictus cell line (SAAK) by plaquing on the clone C6/36 cells, a virus-sensitive clone isolated from another Singh's A. albopictus cell line (SAAR)

The SAAK cell line, obtained from Kobe University School of Medicine, is apparently resistant to chikungunya (CHIK) virus. By incubation with the medium used for the SAAK cells, the virus-sensitive C6/36 clone became resistant to CHIK virus. The SAAK medium produced two kinds of plaques on the C6/36 cells at 28°C under agar overlay. The large plaque (Lp) virus appears to be responsible for the CHIK-resistance of the SAAK cells and its transfer to the C6/36 cells. Purified Lp virus showed the same characteristics as the standard CHIK virus in terms of sedimentation rate, density, size of the genome RNA and core protein. However, mobility of the envelope protein of the Lp virus in SDS-polyacrylamide gels is faster than that of the standard CHIK virus grown in BHK21 cells: CHIK(BHK), but it is almost the same as that for the standard CHIK virus grown in C6/36 cells: CHIK(C6/36). HI titers of anti-CHIK(BHK) sera were 4 fold lower for SAAK-Lp and CHIK(C6/36) viruses than for the homologous CHIK(BHK) virus. On the other hand N-titers of the same sera were somewhat higher to the former than to the latter virus. The data indicate some degree of host-controlled modifications on the envelope glycoprotein. The Lp virus is remarkably more heat-labile than CHIK(BHK) or CHIK(C6/36) virus, and does not form plaques on BHK21 or LLC-MK_2 cells above 34°C nor is it pathogenic to suckling mice. The plating efficiency (eop) of the Lp virus is almost 100 fold higher on the C6/36 cells than on BHK21 cells at 28°C, while standard CHIK virus showed almost the same eop.

2. Curing of A. albopictus cells persistently infected with CHIK virus

Attempts were made to "cure" the A. albopictus, C6/36 cells persistently infected with CHIK virus. By 2 weeks subculturing of the cells with the medium containing 0.1 to 10 % of anti-CHIK serum (neutralizing titer: around 10000 by 50 % plaque reduction), cured cultures were obtained. Also by plating of the persistently infected cells under the medium containing 1 % of anti-CHIK serum, only such clones were obtained as "virus-negative". All the "cured cultures" and "virus-negative clones" were free of antigens cross-reacting with CHIK and were almost as sensitive to CHIK as the uninfected C6/36 cells. Similar results were obtained with the SAAK cells. Treatment with anti-HIK serum resulted in Lp(-) sp(+) clones or "cured" cultures. The results indicate remarkable easiness to remove contaminating CHIK or CHIK-related virus from A. albopictus cells by treatment with anti-CHIK serum.

3. Observations on A. albopictus cells persistently infected with dengue viruses

A. albopictus, C6/36, cells persistently infected with each of the 4 types of dengue viruses produce temperature sensitive (ts) viruses after a number of subcultures. The persistently infected cultures appear to be resistant not only to the homologous but also to the heterologous dengue viruses, however, they were sensitive to CHIK virus. The resistance were transferred to the virus-sensitive C6/36 cells by one weeks incubation with the cell-free medium from persistently infected cultures, but not by the medium preheated at 56°C for 30 min.

4. Serological survey on the dengue antibodies among the people in Okinawa islands

In collaboration with Dr. M. Ura of the Okinawa Prefecture Institute of Public Health, serological survey was undertaken by the HI tests on the prevalence of dengue antibodies among the people of north part of Okinawa main island (Kunigami district), Ishigaki island and Yonakuni island. Presence of dengue antibodies was observed in those persons older than 30 years of age in Ishigaki and Yonakuni islands and in those older than 50 years in Kunigami district. Antibody positive rate and also geometrical mean titers of antibodies were especially high against dengue type 4 antigen. The result may reflect the different chronological invasion of dengue viruses in these different parts of Okinawa islands.

(Konosuke Fukai)

REPORT FROM THE DEPARTMENT OF ECOLOGY OF VIRUSES

THE D.I. IVANOVSKY INSTITUTE OF VIROLOGY

AMS USSR, MOSCOW

In 1976-1977, as in recent years, main attention was paid to examination of ticks collected in basic places of physical-geographic regions of the country--in the Caucasus, in Middle Asia, in the Far East. More than 50,000 Ixodid ticks of 17 species and Argasid ticks of 5 species and 5,500 mosquitoes of 4 species were examined. 52 strains of arboviruses were isolated from them.

Three new viruses were identified. Identification was carried out by complement fixation test with antisera to various arboviruses representing all the known antigenic groups, and also with antisera to ungrouped arboviruses isolated from ticks. Determined in addition was the type of nucleic acid (according to susceptibility to 5b2 Deoxyuridine), the content of lipids in viral membrane (according to susceptibility to ether and DOCh Na), and the size of virion (according to filtration through Millipore filters of 220, 100 and 50 nm pore size).

Rasdan virus was isolated in the Armenian SSR from ticks Dermacentor marginatus (1 strain), collected from sheep. It is not antigenically related to the other arboviruses. The virus contains RNA and lipids in membrane, and the size is 90-110 nm (according to ultrafiltration and electronic microscope date). Electron microscope study of ultrathin sections of newborn mouse brains allow us to refer the virus to the Bunyamviridae family.

Burana virus was isolated from ticks, Haemaphysalis punctata, collected from sheep and cows, in the Kirgiz SSR. The virus is not antigenically related to the other arboviruses, it contains RNA and lipids in a membrane, and according to ultrafiltration its size is more than 50 nm and less 100 nm.

Sikhote Alin virus was isolated from ticks, Ixodes persulcatus, collected in the Primorye territory, in the Far East. The virus is antigenically related to CFT and NT to the encephalomyocarditis group, of the Picornaviridae family. The size of a virus is more than 20 nm and less 25 nm according to electronic microscope data.

Three strains of Tahyna virus were isolated from patients with a syndrome of influenza-like disease in July-August, 1976, in the Tajik SSR. The etiological association of the virus with these diseases was also confirmed by examinations of paired sera in the complement fixation, neutralization, and agar gel diffusion tests.

(D.K. Lvov, V.L. Gromashevsky)

REPORT FROM VIROLOGICAL DEPARTMENT OF THE ANDRIJA ŠTAMPAR SCHOOL OF PUBLIC HEALTH, MEDICAL FACULTY ZAGREB, YUGOSLAVIA AND CDC FORT COLLINS, USA

FIRST NATURAL CLINICAL HUMAN BHANJA INFECTION

Case history:

Patient: female (49).

The disease developed gradually,with pain in the back.On the second day fever developed (38.5° C),with photophobia. Vomited four times.On the third day became hypersensitive and then lost consciousness.

Epidemiology:

Resident of the city of Zagreb but 3 weeks before the onset of the disease stayed in the country.Hospitalized on the third day of the disease,in a serious condition,half-conscious, verbal contact impossible,at the slightest touch reacts violently and cries,otherwise lies quietly.The meningitis syndrome pronounced,stiffening the back.Kering positive. Tendon reflexes active,especially in lower extremities with a pronounced clonus.Abdominal reflexes cannot be induced, Babinski on both sides positive.Muscular tonus intensified in all extremities.The internal status normal except for pronounced tahycardia.

Course of disease:

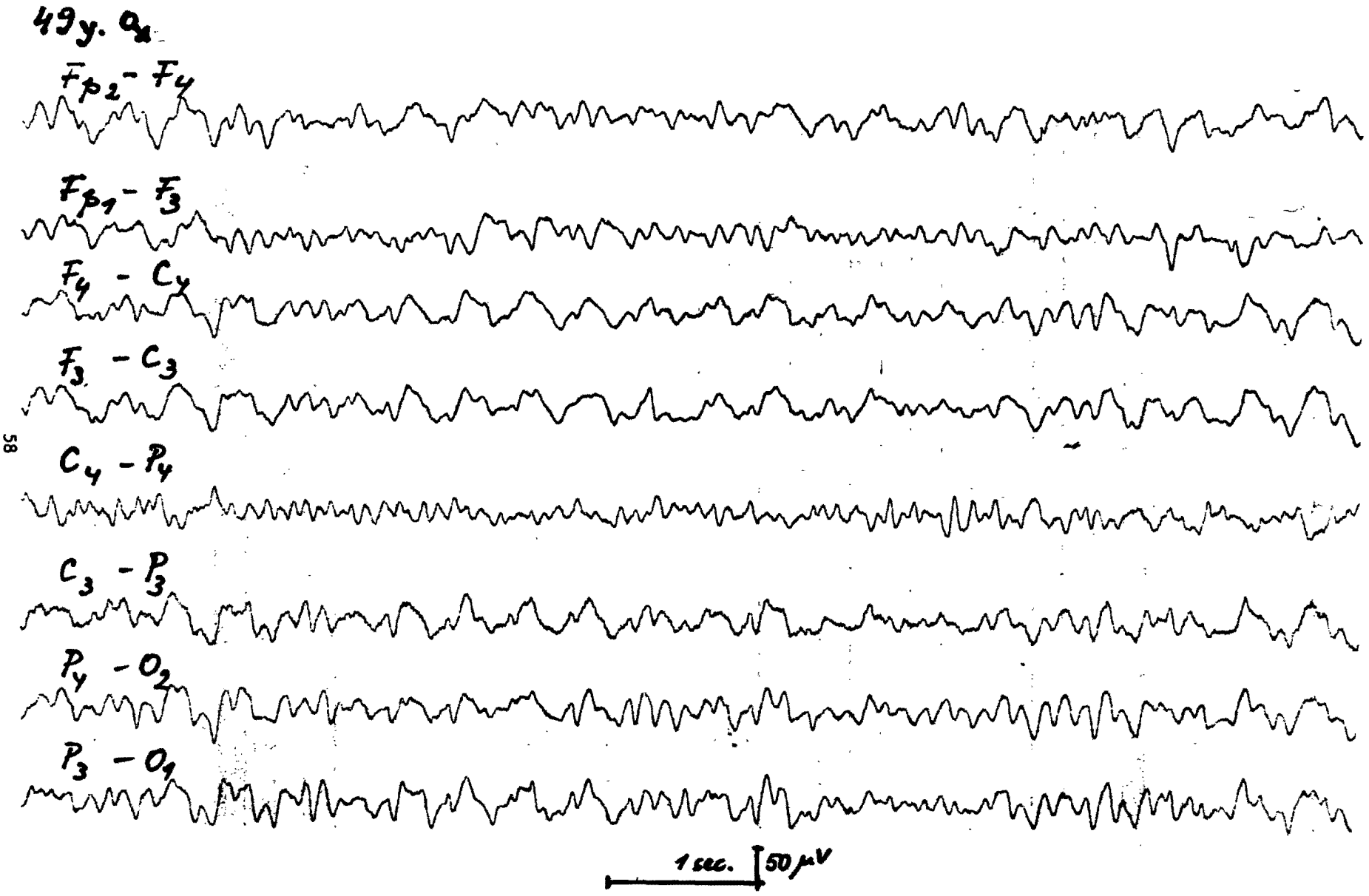
The patient was febrile for 11 days,the temperature ranging from 38°C in the morning to 39°C in the evening.It lythically decreased to normal values.The consciousness was disturbed for 16 days.Initially the patient appeared to be in a vigilant coma because she lay quietly,with the open eyes.From the seventh day short,sporadic talks with her possible but she still lacked orientation,both in time and space,and looked as being lost.Only on the 18th day did she become fully oriented and assume a critical attitude towards her disease. During all the time of treatment she had a hyperactivity of tendon reflexes,with the presence of Babinski and Rossolimo.

The following analyses were negative:blood and CSF culture, Dye and CF test for Toxoplasma.

On the 11th and 27th day the following virological examinations were made: TBE, LCM,Polio,ECHO,Coxsackie, Mumps- the results negative.Herpes simplex in both sera positive 1/8, NT for Bhanja virus showed seroconversion (0- 1/16o).

Discharged on the 25th day of the onset of the disease. The follow-up examination after one month: clinical and laboratory findings normal,except for a slight hypertonus of muscles and intense physiological reflexes.

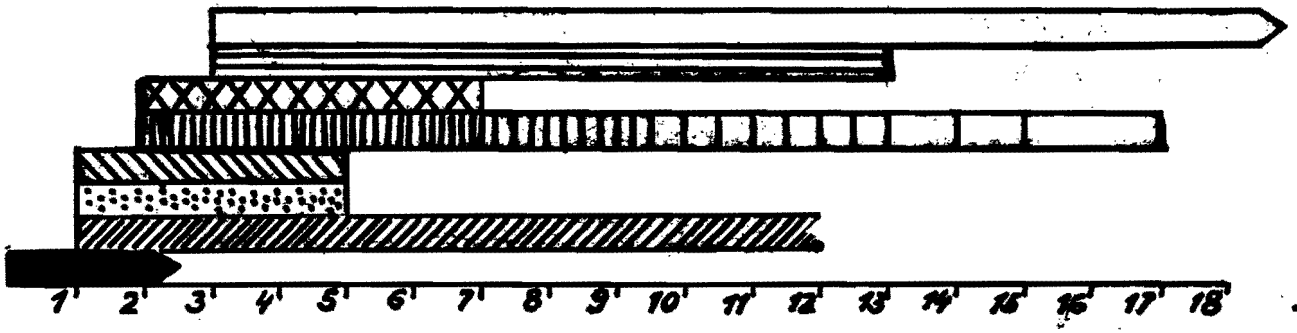
	DAYS OF ILLNESS		
	3	10	30
HEMATOLOGIC VALUES			
Erythrocyte sedimentation /Westergren/	21 in 1 h.	30 in 1 h.	18 in 1 h.
Erythrocytes	4.880.000		4.930.000
Hemoglobin	14.5 gm%		13.5 gm%
Leucocytes	7.350	15.400	10.600
Seg.neutrophils	67 %	63 %	53 %
Juv.neutrophils	7 %	16 %	3 %
Lymphocytes	17 %	14 %	41 %
Lymphocytes atypical	1 %	-	-
Monocytes	5 %	10 %	3 %
Plasma cells	1 %	-	-
SERUM PROTEINS			
Total proteins	7.2 gm%	6.6 gm%	6.9
Albumin	3.7 gm%	3.2 gm%	3.51
Globulin	3.5 gm%	3.4 gm%	3.39
Paper electrophoresis			
Albumin	51.5%	48.5%	51
Globulin			
$Alpha_1$	3,8%	4.3%	4.3
$Alpha_2$	10.2%	14.3%	13.2
Beta	13.2%	14.7%	12.7
Gamma	21.3%	18.2%	18.8
URINE			
Protein	-	-	0
Sugar	0	0	0
Urobilinogen	1:8	1:8	1:8
Acetone	0	0	0
Sediment	normal	normal	normal
ACID BASE BALANCE			
Sat. O_2	90,5%		
pH	7.36		
pCO_2	47.9		
BE	-1.0		
pO_2	62.5		
CEREBROSPINAL FLUID			
Cells	36/3	128/3	16/3
Polynuclear	1%	10%	43%
Mononuclear	99%	90%	57%
Protein	80.6 mg%	78.0 mg%	41,6 mg%
Glucose	61.0 mg% (blood 91 mg%)	61.0 mg% (blood 105 mg%)	61,0 mg% (blood 74mg%)
Chloride	694.5 mg%	713.0 mg%	731.0 mg%


49y. ♂
Fp2 - F4
Fp1 - F3
F4 - C4
F3 - C3
C4 - P4
C3 - P3
P4 - O2
P3 - O1
1 sec.
50 μV

ILLNESS

G.M. 49y. ♂

MENINGOENCEPHLITIS ACUTA
QUADRIPARESIS



BACK PAINS
FEVER
PHOTOPHOBIA
VOMITING
UNCONSCIOUSNESS
HYPERSENSIVITY
MENINGEAL SIGNS
HYPERREFLEXIA AND MUSCLES HYPERTONIA

LABORATORY INFECTIONS WITH BHANJA VIRUS

In the laboratory of the Virological Department of the A.Stampar School of Public Health,two laboratory infections caused by Bhanja virus developed:one clinical and one subclinical.

Clinical infection appeared in a laboratory worker 30 days after work with Bhanja virus.The disease started with headache in the frontal part of the head and photophobia. Fever developed on the fourth day (T 37.5 -39°C),along with nausea and retrobulbar pain.Fever lasted 7 and the whole infection 10 days.

In the other laboratory worker a subclinical infection caused by Bhanja virus was diagnosed.

The diagnosis of both infections was established on the basis of epidemiological data and specific viro-serological findings (neutralization and hemagglutination inhibition tests).

(Jelka Vesenjak-Hirjan, C.H. Calisher, Volga Punda and I. Beus)

BHANJA CLINICAL INFECTION

Serum taken in 1977	NT	HI
12 April	1o	ø
29July	1o	ø
12 August		80
15 September	160	16o
17 October	320	160

BHANJA SUBCLINICAL INFECTION

Serum taken	NT	HI
11 April 1976		40
20 December 1976		40
15 March 1977	8o	40
11 June 1977	80	40

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

Cross neutralization studies were conducted to assess possible antigenic variation among strains of Tahyna virus originating from various sources. The prototypestrain of La Crosse virus was also examined in these experiments.

Seed viruses for four Tahyna virus strains and for La Crosse virus strain were prepared by terminal dilution selection in green monkey kidney /GMK/ cell line. The passage history and the data of isolation of used virus strains are listed in Table 1.

For testing the cross reactivity of virus strains a serum dilution neutralization test in GMK cell cultures was used. Immune sera for the specific virus identification were produced in mice by one subcutaneous injection of one of the five listed purificated virus strains. The injected mice were exsanguinated by cardiac puncture 38 to 42 days later and the inactivated /56°C/30 min/ mono sera were tested for cross neutralization by serum dilution neutralization test with approximately 100 CPD_{50} per 0.1 ml of each seed virus against to twofold dilutions of each mono serum.

Table 2 presents the results of repeated tests. It clearly appears that the LAC virus strain is distinct from the four TAH strains. However, the one-way cross noted between LAC and TAH 669 suggest minor antigenic differences between these two virus strains, at least under the condition studied. It seems interesting to note that this TAH 669 virus strain was isolated from a clinically apparent, influenza-like Tahyna infection, while the other human Tahyna virus isolate - TAH 693, from a clinically inapparent Tahyna infection.

The comparison of four Tahyna virus strains by cross testing in cell culture serum dilution neutralization tests using virus strains purificated by terminal dilution selection and mouse mono antisera permits to detect antigenic similarities or dissimilarities also between the studied strains of Tahyna virus. Generally the homologous titers were significantly higher than the heterologous. Extensive cross reactivity in one direction was noted with the TAH 669 virus strain. The remaining three strains, i.e., the reference strain TAH 92, the strain TAH 6064 and the strain TAH 693 respectively, resemble each other in showing similar two-way differences of titer.

The problem of whether the antigenic chracters of Tahyna virus strains change during passages in various host systems has not been settled. Further investigations using additional fresh Tahyna virus isolates from human infections, from animal hosts and from mosquitoes would be of considerable value for obtaining more information about the antigenic variation, even also the ecology of this virus.

As a practical application of the presented study, investigations are under way to choose a more sensitive Tahyna antigen for immunological surveys as well as to select suitable virus strain for preparation of broad spectrum Tahyna diagnostic sera.

/ A. Šimková /

Presented at the Conference: "25 Years of Research Institute of Epidemiology and Microbiology" Bratislava, November 18,1977.

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Table 1 Characteristics of Virus Strains Used

Virus	Strain	Passage history+	Data of isolation			References
			Source	Lokality	Year	
Tahyna	TAH 92	M-5,SM-2,M-1,SM-1, GMK-6,SM-1	mosquito	Slovakia, CSSR	1958	Bárdoš and Danielová,1959
	TAH 6064	SM-9, GMK-6,SM-1	mosquito	Moravia, CSSR	1972	Málková et all.,1974
	TAH 669	SM-1, GMK-6,SM-1	human blood	Moravia, CSSR	1972	Šimková and Sluka,1973
	TAH 693	SM-1, GMK-6,SM-1	human blood	Moravia CSSR	1972	Šimková and Sluka,1977
La Crosse	LAC	M-5,SM-1, GMK-6,SM-1	human brain	Wisconsin, USA	1964	Thompson et all.,1965

+ Passage histories represent viruses before and after purification by terminal dilution selection in GMK cells. Abbreviations: M,mouse; SM,suckling mouse; GMK,green monkey kidney cells.

Table 2 Cross Reactivity of Tahyna Virus Strains and Virus La Crosse by Serum Dilution Neutralization Tests in GMK Cells

Virus[+]	Serum[++] dilution neutralization titer[+++]				
	TAH 92	TAH 6064	TAH 669	TAH 693	LAC
TAH 92	256	32	256	32	8
TAH 6064	32	128	256	32	8
TAH 669	16	16	128	16	8
TAH 693	32	32	256	256	4
LAC	4	4	64	4	64

\+ Purificated by terminal dilution selection in GMK cells.

++ Mono antisera prepared in mice.

+++ Titers given as reciprocals.

REPORT FROM WHO COLLABORATING CENTRE FOR ARBOVIRUS REFERENCE AND RESEARCH, INSTITUTE OF VIROLOGY, BRATISLAVA, CZECHOSLOVAKIA

Further isolations of Tettnang virus in Czechoslovakia

Further isolations of virus strains closely related to Tettnang virus from ticks, mosquitoes and small rodents are presented.

I. ricinus ticks were collected from vegetation in following localities: Posluchov, Lašťany and Červenka-Kráľová (north Moravia), in May and June, 1977. D. reticulatus ticks were collected in the locality Podunajské Biskupice near Bratislava, in October, 1976. Small rodents, namely 12 Clethrionomys glareolus and 7 Apodemus flavicollis were caught into the live-trap in the same locality, in September, 1967. Mosquitoes in the locality Koliňany were allowed to attack the collecting personnel and harvested with the use of battery aspirator.

Localities Posluchov, Lašťany and Červenka-Kráľová are situated on the foothills of Jeseníky mountains (north Moravia and belong to the area of mixed coniferous woods. The locality Podunajské Biskupice on the Danube alluvial plain is characterized by inundated forests. The locality Koliňany represents the community of the Prunion-union.

Suspensions were prepared from I. ricinus and D. reticulatus pools, each of 5 adults or 10 nymphs, in 1 ml of Eagle's basal salt solution supplemented with 10% heated calf serum. Two ml amounts of the same medium were used

for preparing the suspensions from mosquito pools consisting of 30 individuals each and from organs (the brain, liver, spleen, and kidney) of small rodents. Suspensions were clarified by low speed centrifugation at 3.000 r.p.m. for 10 minutes.

Isolation experiments were done by i.c. inoculation o 1-4 days old suckling white mice with 0.01 ml of suspension.

Three virus strains were isolated from a total of 985 I. ricinus ticks collected in Posluchov, Lašťany and Červenka-Kráľová (Table 1). One strain was isolated from nymphs in Lašťany (strain 787), one strain from female in Posluchov (strain 885) and one strain from males in Červenka-Kráľová (strain 766). One strain of the virus was isolated from females of a total of 180 D. reticulatus ticks collected in Podunajské Biskupice near Bratislava (strain 511). In parallel, two strains were isolated from the brain of Clethrionomys glareolus and Apodemus flavicollis (strains Clg 61 and Apf 64) trapped in the same locality. As shown in Table 1, one virus strain was isolated also from Aedes cantans mosquitoes collected in Koliňany (strain K 184). All virus strain isolated were pathogenic for suckling mice on i.c. inoculation. The incubation period in the 1st mouse passage was 7-12 days for strains isolated from ticks, 12 and 13 days for those isolated from small rodents and mosquitoes, respectively. This was shortened to 3-5 days in subsequent mouse passages. Attempts at virus re-isolation from original suspensions were successful in 4 cases (strain 885, Clg 61, Apf 64 and K 184 .

Results of the CF test using sucrose-acetone antigen of Tetnang virus strain 247 and mice antisera prepared to this strain as well as to newly isolated virus strains showed that these are closely related to Tettnang virus strain isolated previously in Czechoslovakia (Table 2).

Isolation of Tettnang virus from I. ricinus and D. reticulatus ticks and from Aedes cantans mosquito suggests that ticks and mosquitoes are possible vectors of this virus. The suggestion is supported by isolation of Tettnang virus from the brain of small rodents, when trophic relations are taken into consideration. Both ticks and mosquitoes occur in all localities, where we isolated the virus.

We assume that results obtained shoud be supplemented by studies on the relationship between Tettnang virus and birds as its possible hosts.

O.Kožuch, J.Nosek, M.Grešíková, M.Labuda, M.Sekeyová, J.Chmela (Acta virologica, in press/.

Table 1.

Isolation of Tettnang virus from tick, mosquitoes and small rodents

Locality and date of collection	Ticks					Mosquitoes			Small rodents	
	Ixodes ricinus			Dermacentor marginatus		Aedes cantans	Aedes anulipes	Aedes geniculatus	Clethrionomys glareolus	Apodemus flavicollis
	N	♀♀	♂♂	♀♀	♂♂					
Podunajské Biskupice 16.9.1976	-	-	-	-	-	-	-	-	1/12	1/7
Podunajské Biskupice 29.10.1976	-	-	-	1/114	66	-	-	-	-	-
Červenka-Kráľová 12.5.1977	106	1/10	20	-	-	-	-	-	-	-
Lašťany 13.5.1977	1/169	110	106	-	-	-	-	-	-	-
Posluchov 1.6.1977	364	49	1/51	-	-	-	-	-	-	-
Koliňany 1.7.1977	-	-	-	-	-	1/437	15	15	-	-
Number of strains isolated	1	1	1	1	-	1	-	-	1	1

Table 2

Identification of Tettnang virus strains by the complement-fixation/CF/ and virus--neutralization /VN/ tests

CF tests		VN tests with Tettnang /247/ strain
Antisera	CF titre with Tettnang /247/ antigen[x]	NI
885	40/40	2.5
776	80/20	2.5
787	40/20	2.5
K 184	80/20	2.5
Apf 64	32/128	2.5
Clg 61	16/128	2.5
511	16/128	2.0
Tettnang /prototype/	64/128	-

[x] Titre of serum/titre of antigen

- Not tested

REPORT FROM THE FEDERAL RESEARCH INSTITUTE FOR ANIMAL VIRUS DISEASES, TÜBINGEN, FEDERAL REPUBLIK OF GERMANY

RAPID DIAGNOSIS OF ALPHA- AND FLAVIVIRUSES BY RADIOIMMUNOASSAY

(P.-J.Enzmann)

A method for rapid determination of Tagovirus-antigen by solid phase radioimmunoassay was developed. The supernatants of cell cultures infected with Sindbis virus, Semliki Forest virus, and West Nile virus were serially diluted in 0.1 M phosphate buffer (pH 7.6) containing 0.1% bovine serum albumin. Twenty µl aliquots of each dilution of virus were added to the wells of a flexible microtitre plate. The plate was dried in a stream of warm air. The wells were washed first with distilled water and then with PBS and 20 µl of anti-virus IgG in a constant dilution (1:1,000) containing 0.1% bovine serum albumin were added. As a source of complement human serum in Tris-NaCl buffer containing EDTA was added in some instances, in these cases the same buffer was used for washing. The microtitre plates were covered and left at 37°C for one hour and afterwards washed 5 times with PBS containing 0.1% bovine serum albumin. Then 25 µl containing about 15,000 cpm of the second reactant labelled with ^{125}I (anti-rabbit IgG, anti-human-C1q, or protein A) were added to the wells, followed by a further incubation at 37°C for 30 min. Separation of bound and free radioactivity was accomplished by decanting the free counts while the bound remain absorbed. The plates were washed 5 times with PBS and dried. The wells were cut out and radioactivity was determined.

As an example Fig. 1 illustrates a typical titration of Sindbis virus. Cross reaction was observed between the alphaviruses Sindbis and Semliki Forest, whereas no cross reaction existed between the alphaviruses and a Flavivirus, West Nile virus. The indirect radioimmunoassay with the use of labelled anti-complement or labelled protein A as second reactant has the advantage that immune sera from different species can be used for the detection of viral antigens provided that the reference sera possess reactivity with complement or that specific IgG molecules are present to which protein A can bind. The immune sera for virus diagnosis have to be carefully tested for these prerequisites.

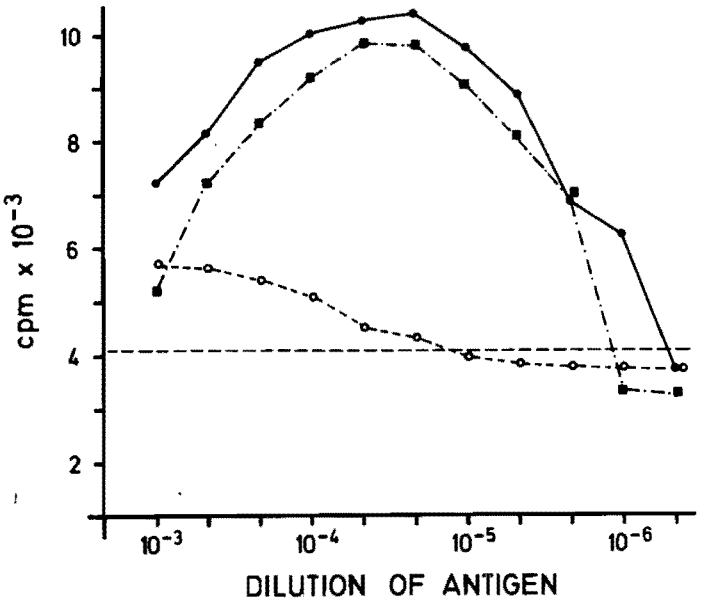

Fig.1 Rapid determination of Sindbis virus antigen by indirect solid phase radioimmunoassay.

Serial dilutions of Sindbis virus antigen: 10^{-3}, $5x10^{-4}$, $2.5x10^{-4}$, 10^{-4}, $5x10^{-5}$, $2.5x10^{-5}$, 10^{-5}, $5x10^{-6}$, $2.5x10^{-6}$, 10^{-6}, $5x10^{-7}$. The viral antigen absorbed to the wells of a microtitre plate was coated with IgG from rabbit hyperimmune serum. Squares: antigen-antibody-reaction in the presence of complement was measured by a second, labelled antibody against C1q-component of complement. Black circles: binding of IgG to immobilized antigen was determined by labelled protein A. Open circles: Semliki Forest virus used as a control.

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REPORT FROM ARBOVIRUS LABORATORY, VIRAL ECOLOGY UNIT
INSTITUT PASTEUR, PARIS, FRANCE.

1. DENGUE DIAGNOSTIC.

Among sera received between September 1977 and August 1978 for arbovirus diagnostic, 57 out of 83 from West Indies (Guadeloupe and Martinique) were positive for dengue viruses (by HI test). Likewise, from Reunion Island, 112 out of 177 sera had dengue antibodies suggestive of recent infections. Therefore, this diagnostic service appears to be a good surveillance system which allows to detect an epidemic phenomenon within 2 or 3months, to evaluate its importance, its geographical extension and its evolution with time.

Moreover, several dengue virus strains (3 from West Indies and 2 from Reunion Island) have been isolated from patients hospitalized in Paris or from blood samples received from infected territories. Typing of these strains is in progress.

2. GENETIC STUDIES ON LUMBO VIRUS.

Continuation of genetic studies on Lumbo virus has demonstrated the existence of a third group of complementation-recombination (ts mutants) which had been suggested from earlier studies (S. OZDEN and C. HANNOUN, Virology, 84, 210-212, 1978). Only one mutant of the third group has been obtained from a total of 92 ts selected clones. It seems that the viruses of the two first groups, corresponding to defects localized on the two larger segments of the viral genome, are capable of synthetizing RNA at non permissive temperature.

(C. HANNOUN, F. RODHAIN)

REPORT FROM THE VIRUS LABORATORY, FACULTY OF MEDICINE, BREST, FRANCE

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Serosurvey on arbovirus infections in selected occupationnal group of blood donnors in North Finistere, Brittany, France.

Between 1976 November to 1978 February, 454 sera from healthy blood donnors were collected in various areas of North Finistere, Brittany, and studied for antibody against ten arboviruses.

Antigens for Sindbis, West Nile, Dengue type 2, Tick-borne encephalitis (european type), Čalovo, Ťahyňa, Uukuniemi and Bhanja viruses were used in inhibition haemagglutination (IH) tests. On the other hand, Tribeč virus and a local isolate of Soldado-like virus (Hughes group) were used in complement fixation (FC) tests.

The blood donnors were selected on the basis of their occupationnal behaviour : agricultural workers, foresty employees, hunters, anglers.

By IH tests, 73 sera gave a positive result (16,07 %), with a titer equal or superior to 10, for one or more of the following viruses : Sindbis, West Nile, Dengue type 2 and Bhanja (*Table I*). The highest incidence of positive reactions occured in the central part of Finistere, i.e. into and around the "Parc Naturel Régional d'Armorique", an agricultural area not yet too modified with numerous forest areas (*Map*).

A number of positive reactions were easily explainable by a previous residence of some blood donnors abroad, in countries where arboviruses are endemic : Tropical Asia, Africa or America and mediterranean regions. But other positive reactions found in sera of blood donnors without such antecedent suggested the existence of a natural focus of arbovirus infections in Finistere, with the possible activity of a not yet identified flavivirus together with Bhanja virus.

[To be published in "Médecine et Maladies Infectieuses," Paris.]

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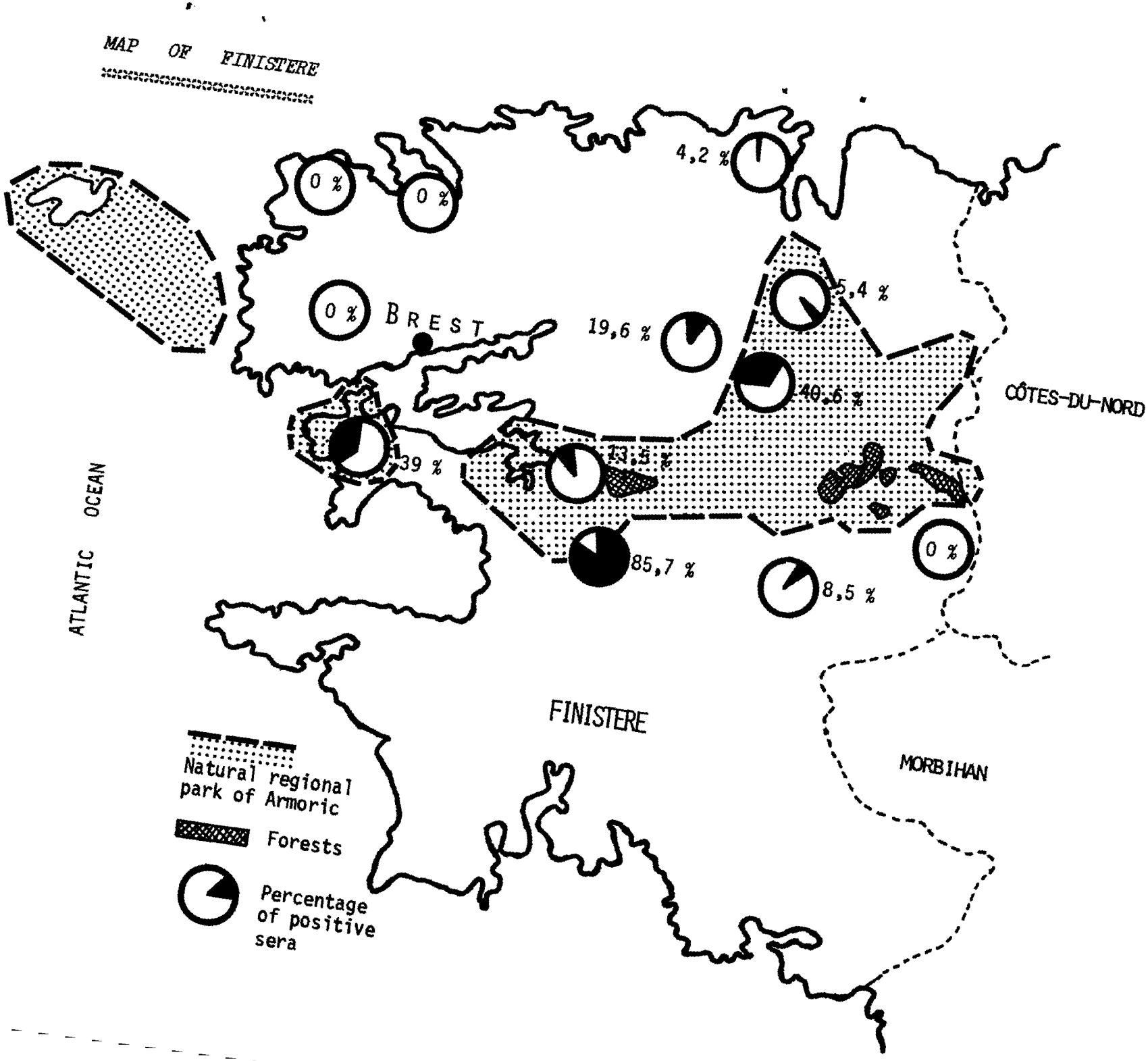
TABLE I - CHARACTERS, TITERS AND GEOGRAPHICAL DISTRIBUTION OF POSITIVE REACTIONS IN FINISTERE.

Biotopes	Donnors with previous residence abroad										Donnors without residence abroad						Total of studied sera
	Number of studied sera	SIN	WN			DEN 2				BHA	Number of studied sera	WN			DEN 2	BHA	
		20	10	20	40	10	20	40	160	10		10	20	40	10	10	
Littoral areas																	
St Pol de Léon	27		1				1			1	43	1				1	70
Lannilis	12										24						36
Ploudalmézeau	6										15						21
Le Faou	12		4								25	1					37
Camaret	10		4		1						13	4					23
Swampy areas																	
St Renan	8										26						34
Agricultural areas in the Central part of Finistere																	
La Martyre	11		2		1					1	55	9	1			1	66
Guiclan	17										20					2	37
Areas near the forests																	
Commana	6	1	3	1							26	8	1		1	1	32
Châteauneuf du Faou	9		2					1			38	2					47
Carhaix	8										15						23
Chateaulin	9		8		1				1	1	19	15					28
Total	135	1	24	1	3		1	1	1	3	319	40	2		1	5	454


MAP OF FINISTERE
0 %
0 %
4,2 %
0 %
BREST
19,6 %
5,4 %
40,6 %
CÔTES-DU-NORD
39 %
13,5 %
ATLANTIC OCEAN
85,7 %
8,5 %
0 %
FINISTERE
MORBIHAN
Natural regional park of Armoric
Forests
Percentage of positive sera

REPORT FROM THE ARBOVIRUS UNIT, DEPARTMENT OF MEDICAL MICROBIOLOGY,
LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

CROSS-NEUTRALIZATION ACTIVITY OF SEVEN CALIFORNIA GROUP VIRUSES IN HOMOIOTHERMOUS (PS) AND POIKILOTHERMOUS (XTC-2) VERTEBRATE CELLS

A study was carried out of cross-neutralization activity of California encephalitis (CE), La Crosse (LAC), Snowshoe hare (SSH), Lumbo (LUM), Tahyna (TAH), Jamestown Canyon (JC) and Trivittatus (TVT) viruses at 28°C in XTC-2 cells (Pudney, Varma and Leake, 1973, Experientia, 29: 466-467) and at 37°C in PS cells (Inoue and Ogura, 1962, Virol. 16: 205-207). The plaque reduction neutralization test was basically the constant virus-twofold serum dilution procedure of de Madrid and Porterfield (1969, Bull. WHO 40: 113-121) adapted to the microtechnique of Chanas, Johnson and Simpson (1976, J. Gen. Virol. 32: 295-300). Specific immune sera were prepared by a single intraperitoneal inoculation of 10% infected suckling mouse brain suspension into adult T.O. strain mice which were bled 11 days later. A control serum was prepared in the same manner against normal mouse brain. The sera were heat inactivated at 56°C for 30 minutes prior to testing.

Distinct plaques were produced in both cell lines by all viruses except TVT which did not form plaques in XTC-2 cells. In general, the plaques were clearer in XTC-2 than in PS cells. The results of cross-plaque reduction neutralization were similar in both cell lines (table I) and conformed to the findings of previous workers in that CE, LAC, SSH, TAH and LUM formed the major subgroup while JC and TVT viruses appeared to be antigenically different.

Table I

Virus	Cells	Immune sera (reciprocal 50% reduction titre values)						
		CE	LAC	SSH	LUM	TAH	JC	TVT
CE	XTC	512	128	128	128	64	8	8
	PS	256	64	32	64	16	8	8
LAC	XTC	32	128	32	32	8	8	2
	PS	32	128	32	16	4	2	4
SSH	XTC	64	128	128	128	32	16	2
	PS	32	64	128	32	8	4	4
LUM	XTC	128	32	128	512	128	8	8
	PS	128	16	32	512	64	8	8
TAH	XTC	256	128	128	512	512	32	4
	PS	64	64	64	256	128	32	4
JC	XTC	16	16	16	64	16	256	16
	PS	32	16	16	64	16	256	16
TVT	PS	32	32	32	32	16	8	64

ARGASID TICK - SOLDADO VIRUS ECOLOGY

An agent identified as Soldado (SOL) virus has been isolated from argasid ticks infesting sea bird nesting sites on Puffin Island, a small uninhabited island located off the coast of Anglesey, North Wales (Converse, et al., 1976, Acta Virol. 20: 243-246). We collected ticks from puffin Island on 3 occasions from under rocks and from within rock fissures from likely habitats of the tick Ornithodoros (Alectorobius) maritimus which have been described by Hobart and Whalley (1954, Nature 174: 936). The distribution of the ticks was highly focal with up to 100 individuals being found beneath a single rock, while adjacent, apparently identical rocks might contain none.

Ticks from 8 locations were pooled (up to 10 ticks per pool) according to location and developmental stage (27 pools-226 ticks) and inoculated I.C. into suckling mice. A virus similar or identical to SOL was recovered from 9 of the 27 pools (33.3%). This very high infection rate prompted us to test the remaining ticks, which had been collected from 5 sites (173 ticks), individually to determine the absolute infection rate; 34 of 173 individuals (19.6%) proved to be infected.

Table II compares the infection rates of the tick pools and the individually tested ticks which have been hypothetically "pooled" using the same criteria as used for the real pools. Infected ticks were randomly assigned to appropriate "pools". It can be seen that the hypothetical "pool" infection rate (69.6%) is significantly ($P = 0.02$) higher than the real pool infection rate (33.3%). The numbers of infected individuals from different locations ranged from 1 of 32 (3.1%) to 5 of 11 (45.4%). The calculated minimum field infection rate for the pools was 39.8/1000 and for the hypothetical "pools" 92.5/1000. The true infection rate for the latter was 196.5/1000.

TABLE II SOLDADO-LIKE VIRUS ISOLATION
FROM POOLED TICKS AND INDIVIDUAL TICKS

POOLS						INDIVIDUALS						
TICK GROUP	TICK POOL	ADULTS	LARGE NYMPHS	SMALL NYMPHS	VIRUS ISOLATION	TICK GROUP	HYPOTHETICAL "POOL"	ADULTS	LARGE NYMPHS	SMALL NYMPHS	VIRUS ISOLATION	ACTUAL NUMBER OF INFECTED TICKS IN EACH "POOL"
	11		9	*			38			10	-	
	12			9	+	9	39			10	-	
	13	4			+		40		7		-	
	14	8			+		41	3			-	
	15		10		-							
	16		9		+		42			10	+	(1/10)+
	17			10	-		43			10	-	
1	18			10	-	10	44			10	-	
	19			10	-		45			2	-	
	20			10	-							
	21			10	-		46			10	+	(2/10)
	22			10	-		47			10	+	(1/10)
	23			10	-		48			3	+	(2/3)
	36			10	-	11	49		10		+	(2/10)
							50		3		+	(3/3)
	32	7			-		51	4			+	(1/4)
	33		10		-							
2	34		10		-		52		10		+	(4/10)
	35		11		+	12	53		1		+	(1/1)
	37		10		+		54	2			+	(2/2)
	28	10			+		55			10	+	(5/10)
3	29	6			+		56			10	+	(4/10)
	30			2	-		57			10	+	(3/10)
						13	58			10	+	(1/10)
4	25			2	-		59			10	+	(1/10)
							60		8		+	(1/8)
5	24	3			-							
6	31	10			+							
7	26	1			-							
	27			9	-							
8	10			6	-							
TOTAL TICKS		49	69	108				9	39	125		
POOLS POSITIVE / POOLS TESTED		5/8	4/6	0/13				2/3	5/6	9/14		

* Tick pools were inadvertently mixed, the large nymph pool is considered positive on the table.

+ Actual number positive over number tested.

The major difference in the samples involved the infection rates among small nymphs. No isolations were made from pooled small nymphs (with the possible exception of pool 12 which was inadvertently mixed with another pool containing large nymphs), but 9 of 14 of the hypothetical "pools" were infected.

Considering the ecology of the ticks it is likely that the difference in infection rates is real. The distribution of the ticks in small foci and their opportunistic feeding behaviour would lead to many ticks from a given site feeding upon a single (and possibly infected) host. If subsequent infection and transovarial transmission followed, even more individuals from this location would be infected. The table shows that locations containing infected adults also contained infected immature ticks, if nymphs were present.

Another possible explanation for the difference in the infection rates of the samples is that pooled ticks may have an inhibitory effect upon the virus. A phenomenon of this type has been described by Yuill (1969, Am. J. Trop. Med. Hyg. 18: 609-613) using uninfected mosquitoes and several arboviruses in a tissue culture assay system. In our laboratory isolation of SOL virus from O. maritimus using suckling mice rarely resulted in sickness or death of all of the inoculated mice. Usually less than 25% of the mice exhibited signs of illness, and sometimes only 1 mouse of 16 inoculated showed signs of sickness. A low virus titre or lack of mouse adaptation might, if the tick suspensions contained virucidal material, affect the number of isolations from pools compared to single ticks ground up in equivalent volumes.

RIFT VALLEY FEVER IN EGYPT - 1978

In June, 1978, two members of the Unit were seconded to the Microbiology Department of the Faculty of Medicine at the Al-Azhar University Girls' College in Cairo, Egypt to assist Dr. K.S.E. Abdel-Wahab, the Department Head in determining whether Rift Valley fever was beginning to recur following the 1977 Egyptian epidemic. Reports of death and abortion in livestock in several governorates suggested that the disease may have recurred.

Dr. Esmat el Tayeb of the Central Microbiological Laboratory, Kobry el Kobbah, Cairo, collected 13 sera on 7 and 10 July, 1978, from febrile military recruits in the Inshas area of Sharqiya Governorate on the Nile Delta. Inoculation of the sera I.C. into suckling mice, I.P. into adult mice and into Vero cell monolayers yielded 10 strains of virus. Complement-fixation tests using mouse brain suspensions and neutralization tests in Vero cells using hyperimmune monkey serum against approximately 1000 $TCID_{50}$ of first passage Vero cell fluid derived virus indicated that the strains were RVF virus. Work is in progress on 14 additional sera or pharyngeal washings from the same area.

The possibility that RVF may have become endemic or enzootic poses a serious public health and veterinary problem in Egypt. It is essential that efforts must be made to control or eliminate this disease to prevent the spread of RVF within or outside of Egypt.

(B.K. Johnson, A.C. Chanas, Z. Hubalek* and D.I.H. Simpson)

*Czechoslovak Academy of Sciences, Institute of Parasitology, Prague.

Report from the MICROBIOLOGICAL RESEARCH ESTABLISHMENT, PORTON, SALISBURY, ENGLAND

T-lymphocyte factors in the regulation of brain infectivity following alphavirus infection of the mouse.

A report in the MARCH 1977 Information Exchange (No. 34, p. 117) summarized the general responses of nude-athymic mice to intraperitoneal infection by defined avirulent strains of Semliki Forest virus (A774) and Venezuelan equine encephalomyelitis virus (TC83). It was particularly noted that nude-athymic mice eliminate viraemia almost normally but fail to mount a regular and sustained antibody synthesis. The persistence of brain infectivity at up to 10^4 p.f.u. per brain was observed for up to five weeks.

In futher tests, nude-athymic mice and their normal litter mates were infected intraperitoneally at 40 to 70 days old by 10^3 to 10^4 p.f.u. of the avirulent A774 strain of SFV. Seven weeks later, and without any intermediate interference by bleeding, the mice were killed and their brains assayed for infectivity and bloods for serum neutralizing activity. The individual assays for 17 nude-athymic mice are summarized in the table below and show persistence of brain infectivity without detectable serum antibody activity in 9 cases. In contrast, high levels of serum antibody activity without detectable brain infectivity were observed in 6 of 17 nude mice.

Incidence in Nude mice	Brain Infectivity as log (p.f.u./brain)	Serum Neutralization Index or log (antibody activity)
9/17	5.4, 4.2, 4.1, 3.9, 3.8, 2.1, 1.1, 0.8, 0.6	all <1
2/17	both <0.3	both <1
6/17	all <0.3	all 4 to 5

The strong individual correlation of the persistence of brain infectivity with the absence of detectable serum neutralizing activity appears to divide the nude mice into two groups of distinct response. Alternatively, the nude mice showing positive or high brain infectivity at the time of sampling have only recently experienced a decline of antibody synthesis (local or circulating) and a concurrent proliferation of virus in brain. The latter possibility is consistent with similar observations at one month following infection and suggests a fluctuation of brain infectivity in nude-mice dependent upon subtle immunological balances in a host unable to sustain a regular synthesis of antibody.

When 14 'immunized' nude-mice similar to those above were challenged i.p. by the 'lethal' LIO.Cl. strain of SFV, eleven survivors at 14 days post-challenge all

showed persisting brain infectivities of 10^3 to $10^{4.3}$ p.f.u. per brain. This higher and probably more regular persistence of brain infectivity following virulent challenge was nevertheless not associated with detectable antibody synthesis or clinical signs.

These observations, and those already published (Doherty, 1973; Bradish et al. 1975), suggest that the normal regulation and clearance of brain infectivity, and thence the expression of virulence, may be mediated by a combination of macrophage and T-cell functions. Thus, in nude-athymic mice, the proliferation of SFV-strain A774 in brain is clearly limited by T-independent mechanisms to 'avirulent' levels of tolerated 'persistence' that are not finally cleared unless T-cell functions can be adopted or antibody synthesis stimulated through other routes. It is premature to draw close conclusions on the role of antibodies until the penetration or synthesis of specific immunoglobulins in brain has been assessed (Fleming, 1977).

This situation for SFV as a typical alphavirus is evidently distinct from that associated with infection by the viruses of ectromelia or LCM in which T-lymphocytes are an essential trigger for the inflammatory process (Cole et al., 1972).

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

Mosquito catches made at domiciliary environment were made at the Ribeira Valley, São Paulo State, Brazil. It follows a previous one and it is related to the study of the mechanisms of local encephalitis transmission by Culicidae. The same places were chosen, looking for its distribution between rural and urban areas. Significant presence of Aedes scapularis and Culex (Melanoconion) sp. at indoor catches suggested its participation on the domiciliary virus transmission. This pattern was more evident at the rural areas than the urban ones. It is possible to associate these findings to the occurrence of clinical cases of the disease in low age children.

(O.P.Forattini,A.C.Gomes, E.A.B.Galati, E.X.Rabello and L.B.Iversson)

REPORT FROM THE EVANDRO CHAGAS INSTITUTE, FSESP, BRAZILIAN
MINISTRY OF HEALTH, BELÉM, BRAZIL
AND THE
UNITED STATES ARMY MEDICAL RESEARCH UNIT-BELÉM
APO MIAMI 34030

SIMULTANEOUS OUTBREAKS OF MAYARO AND YELLOW FEVER VIRUSES IN BELTERRA, PARÁ, BRAZIL.

In February and March of 1978, several cases of an acute febrile disease were observed in Belterra, Pará, Brazil, and three fatalities were recorded. Belterra is an old rubber plantation located near the Tapajós River, about 40 km South of the city of Santarém (Figure 1). This plantation is currently managed by the Brazilian Ministry of Agriculture. The plantation occupies about 400 hectares and has a population of about 4.000 people as measured by a census in Dec., 1977.

Investigations were begun in March and two arboviruses were identified as responsible for this outbreak: Yellow Fever and Mayaro. All deaths were attributed to Yellow Fever virus. Preliminary results of clinical, ecological and epidemiological investigations of these outbreaks are included herein.

I. Mayaro Virus. Infection with Mayaro (MAY) virus was confirmed in 55 (76.4%) of 72 cases seen during the peak of the epidemic. Confirmation was accomplished by isolation of the virus from 43 cases, and serologically in an additional 12. Range of ages varied from 2 to 62 years, and both sexes were represented.

Clinical manifestation of disease due to MAY virus included fever, arthralgia and exanthema. Table 1 presents clinical signs and symptoms associated with MAY virus infection as seen in 43 cases from which virus was isolated. Arthralgia was most predominant in the fingers, hands, feet and ankles, and occasionally effecting the knee or elbow joints. Exanthema was either small maculopapular or micropapular, and was most commonly seen on the thorax, back, upper and lower extremities.

Clinical manifestations persisted for 3-5 days, except exanthema, which could be seen until the 8th or 9th day of illness. Arthralgia persisted in some patients for 2 months or more. No deaths were attributed to infection with MAY virus.

Serological surveys of the population were made between 10 and 14 April during the peak of the epidemic, and between 4 and 10 July after the epidemic had subsided.

In addition, results of a routine serological survey made during 1972 are available. Since no one questioned during the current epidemic recalled any illness clinically similar to the present MAY virus syndrome, results of the 1972 survey may serve as an estimate of the pre-epidemic MAY virus antibody prevalence rate. Antibody was measured in all surveys by hemagglutination inhibition (HI) tests, and results are currently being confirmed by neutralization tests. The MAY virus antibody prevalence rate among those over 10 years of age as estimated in 1972 was 10.3%. The rate among all age groups sampled in April 1978 was 22.3%, and in July was 29.7% (Table 2). All rates are age-adjusted to the 1977 census of Belterra. These results indicate that approximately 20% of the population was infected with MAY virus during this epidemic. Since only individuals above the age of 10 yrs were included in the 1972 survey, this estimate may actually be low.

A house-to-house survey was made during the last week of May in which 3941 people were questioned for illness compatable with MAY virus infection. Of these, 787 (19.9%) reported such illness. An epidemic curve depicting these cases is presented in Figure 2. Some cases occurred in Dec. 1977 and Jan. 1978, and the number of cases increased during Feb. and March and reached its peak during April. The last cases were seen during June.

II. Yellow Fever. Five fatal cases of Yellow Fever (YF) were identified in the Belterra outbreak between Jan. and April, 1978. Four of the fatalities resided in Belterra and one resided in a rural area adjacent to Belterra. Two cases were laboratory confirmed: one though the isolation of virus and the other by both virus isolation and characteristic liver histopathology. The remaining 3 cases were diagnosed based on clinical and epidemiological data. In addition, YF virus was diagnosed serologically from one non-fatal case. The first death from YF occurred on 29 Jan., the second in Feb., two in March and the last on 26 April. All deaths were among males, with ages ranging from 14 to 57 years.

A serological survey made in April, immediately before a vaccination program began, found 59 (18%) of 327 sera tested contained HI antibody to flaviviruses known to exist in Brazil. Eleven positive sera contained HI antibody to YF which titered $\geq$ 1:160, suggestive of recent infections or multiple vaccinations.

Ecological investigations were begun in Belterra in April and have continued unabated. Potential vectors have been collected, as well as

birds and mammals. Several species of mosquitoes and Culicoides have already been pooled and assayed for virus; 2 isolations of YF virus have been made and 8 isolations of MAY virus. All isolations were from Haemagogus capricornii. In addition, MAY virus has been isolated from the blood of a feral marmoset, Callithrix argentata. This marmoset is common in the area.

Between 11 and 20 April the Ministry of Health conducted a vaccination campaign against YF virus. After the death on 26 April, a second campaign was conducted. Following this second vaccination campaign, no new cases of YF virus were seen.

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James W. LeDuc, A. Lynn Hoch, N. E. Peterson. USAMRU-Belém.

PROBABLE LABORATORY ACQUIRED INFECTION WITH ARENAVIRUS

A probable laboratory infection with arenavirus occurred in a 21 y.o. female technician in February of 1978. The causative agent was a new member of the arenavirus group, isolated from Oryzomys rats captured in the Amazon region in 1975 (Pinheiro, F. P., Woodall, J. P., Travassos da Rosa, A. P. A., Travassos da Rosa, J. F. Studies on Arenaviruses in Brazil. Medicina 37: supl. 3, 175-181, 1977). Its prototype is designated BeAn 293022, and it has not yet been named or registered in the Arbovirus Catalogue.

Clinical manifestations began February 17, and consisted of: fever; chills; headache; generalized myalgia; back, eye and epigastric pain; nausea; vomiting; dizziness; and diarrhea. The fever sometimes reached 39°C. The headache was generalized and very severe. The diarrhea was watery and lasted for 1 week. The disease lasted for 4

weeks with flutuacting severity, but without distinctive changes in symptomatology.

Leukopenia (3400 WBC) was observed in the 4th day of illness. On the 12th day the WBC was 4300. The GOT, GPT, and bilirubin levels in the serum were within normal limits. The erythrocite sedimentation rate was 28 mm and 43 mm on the 4th and 14th days of disease, respectively. No abnormalities were observed in a urine sample taken on the 14th day of illness. Two blood cultures and 1 urine culture were negative for bacteria. Agglutination tests for salmonellosis and brucellosis also gave negative results. However, a virus strain (H340242), indistinguishable by CF test from BeAn 239022, was isolated from a blood sample obtained on the 4th day of illness.

Serum samples taken the patient at different times showed the following results by CF test againt the isolate (H340242):

Date	Days after onset	Titer
February, 20	4	< 8
February, 28	12	32+/16+
March, 3	15	32+/16+
April, 25	68	128+/16+

No neutralizing antibodies to the agent could be demonstrated in the two late serum samples by plaque reduction neutralization tests.

The mode of infection remains unknown. The patient denied contact with the infecting agent during 5 weeks prior to onset of disease and she reported no cuts or abrasions while working with it prior to that time. She had not been outside the city of Belēm for two months before onset.

This is the first documented human infection caused a Brazilian arenavirus.

Francisco P. Pinheiro, Amēlia P. A. Travassos da Rosa, Jorge F. Travassos da Rosa, Ronaldo B. de Freitas, Alexandre C. Linhares, Wyller A. Mello. Evandro Chagas Institute, FSESP, Brazilian Ministry of Health.

TABLE 1. Clinical signs and symptoms among 43 patients from which Mayaro virus was isolated during the epidemic in Belterra, Parā, Brazil, 1978.

Signs or symptoms	%
Fever	100
Arthralgia	100
Headache	86.0
Chills	81.3
Myalgia	74.4
Exanthema	66.6
Enlarged lymph nodes	52.6
Dizziness	41.8
Eye pain	37.8
Nausea	34.8
Articular edema	23.0
Vomiting	20.9
Photophobia	6.9
Diarrhea	4.6
Conjunctival congestion	2.3

TABLE 2. Summary of HI antibody prevalence rates to Mayaro virus among humans residing in Belterra, Parā, Brazil prior to, during and after the epidemic. All rates are age adjusted to the 1977 census of Belterra.

Date	Sample size	Mayaro Aby prevalence rate		
		Males	Females	Total
Nov-Dec 1972	164*	10.8%	3.2%	10.3%
April 1978	327+	32.0%	15.3%	22.3%
July 1978	365+	32.2%	26.1%	29.7%

* included only above age 10 yrs.; rates based on these denominators.
+ all ages included.

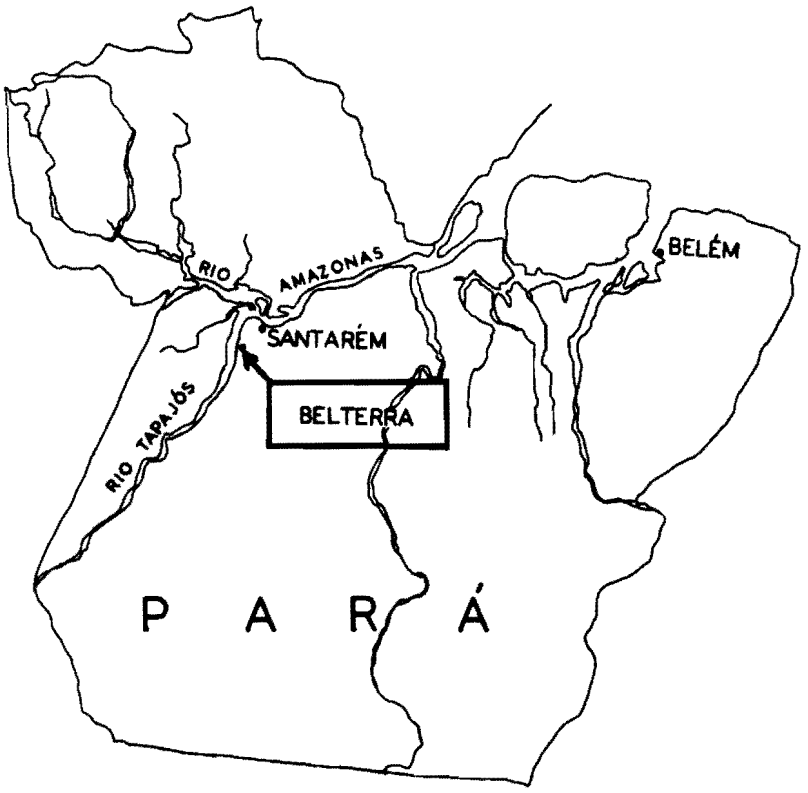


Figure 1. Map of the state of Parã, Brazil, showing location of Belterra.

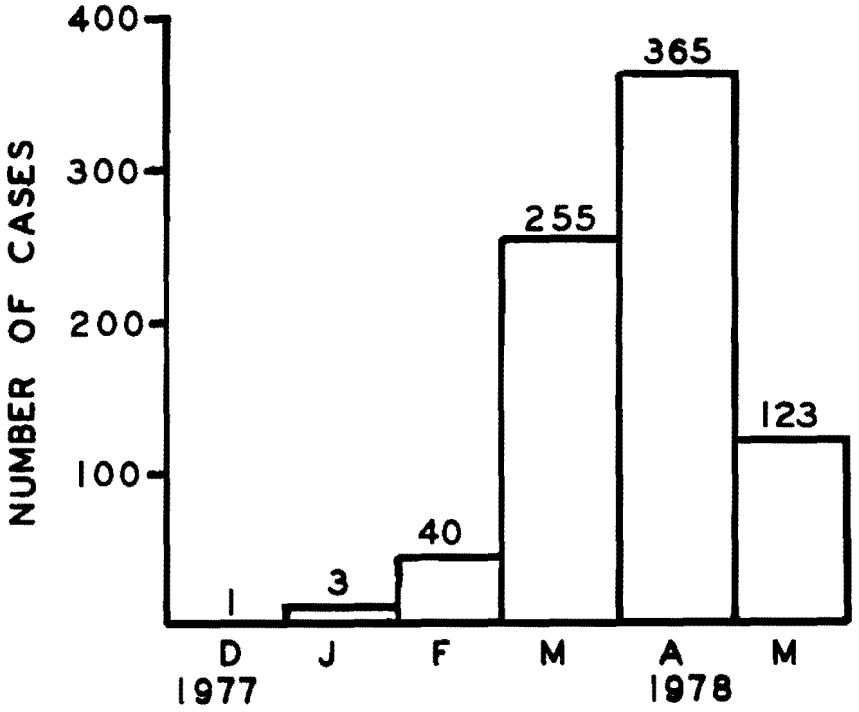


Figure 2. Month of onset of disease among 787 people with recent history of illness compatable with Mayaro virus infection based on a house-to-house survey made in May, 1978, Belterra, Parā, Brazil.

REPORT FROM THE ANIMAL VIRUS LABORATORY, INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS CARACAS, VENEZUELA

DENGUE ACTIVITY IN VENEZUELA DURING 1978

During the second half of May 1978, a febrile Dengue-like illness began to be clinically diagnosed in the northeastern and southeastern part of the country. By mid-June there were reports of some 170 cases in only one community in Ciudad Bolivar. Through the collaboration of the Epidemiology Division of Ministry of Public Health and the National Institute of Health, 29 acute serum specimens of suspected cases of Dengue fever were received at IVIC for virus isolation. In view of further informations from regional epidemiologists we agreed with a field team from National Institute of Health and also with Malariology Division to visit the region on June 7 to further investigate the apparent outbreak. During our house to house visits, 31 acute serum specimens were obtained from selected febrile individuals and contacts reporting illness within the 48 or 72 hours. In addition, A. aegypti (divided in 87 pools) and other mosquito species (divided into 128 pools) were collected from each house visited.

Virus isolations were done only in LSTM-AP-61 (Aedes pseudoscutellaris) cell system, from original materials and were detected by the cytopathogenic effect. Virus quantification, plaque morphology and identification by the reduction neutralisation test, following the method of Russell and McCown at Walter Reed Army Institute of Research, was achieved in LLC-MK_2 cell monolayers with the AP-61 grown virus as inocullum. We have succesfully isolated virus in the AP-61 cell system from 12/28 (43%) original serum specimens during the first generation. On the other hand we have also inoculated mosquitoes (A. aegeypti) and AP-61 cells with selected acute serum specimens (serum samples from team members returning from the field following Dengue fever; 4/4 cases), and virus was isolated in all instances.

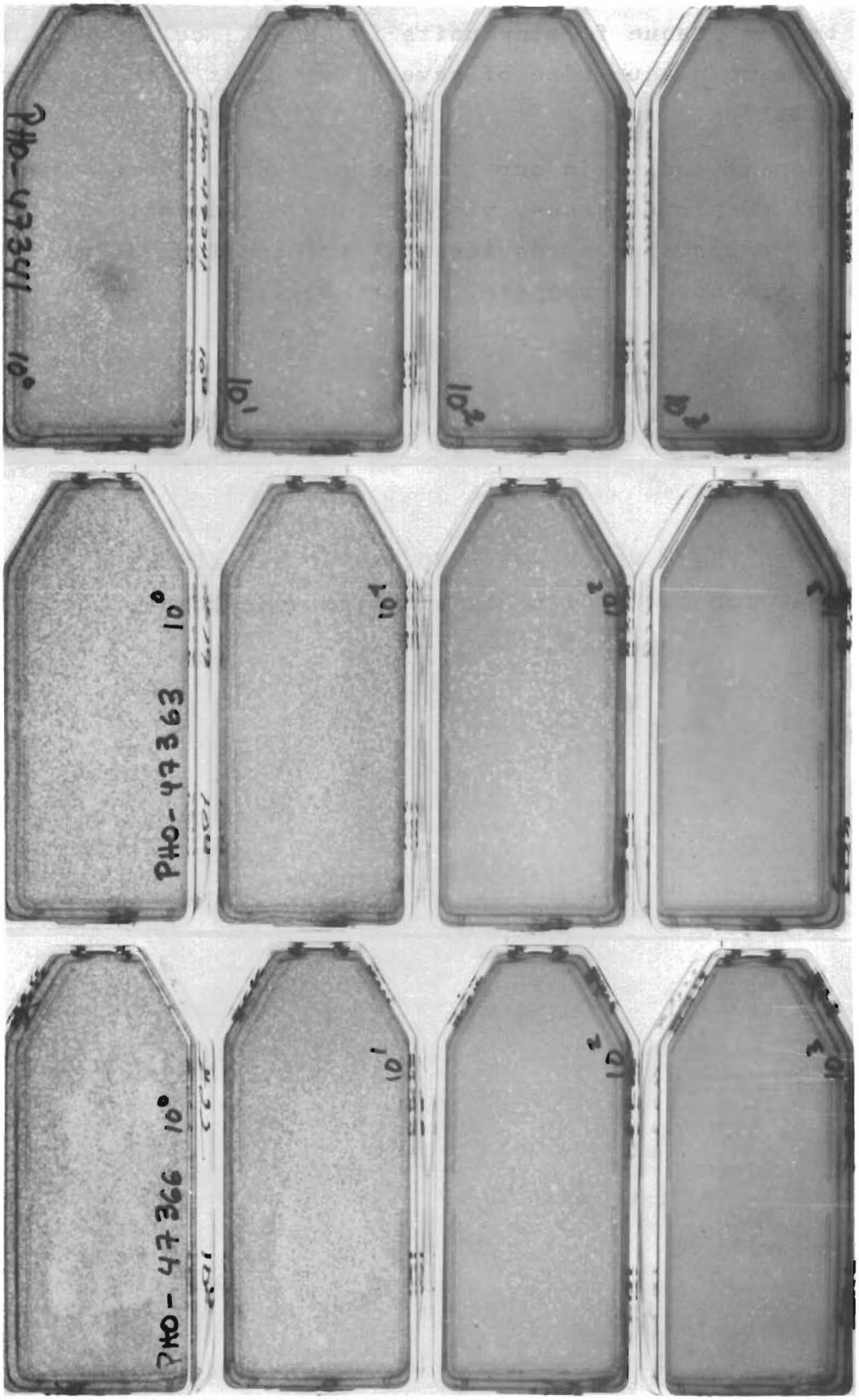
In our experience we achieved reasonably good titers with 1 to 3 AP-61 passaged material ($10^{3.3}$ to $10^{5.6}$ PFU/ml) as quantified by the plaque forming units in LLC-MK_2 cells (Fig. 1).With reference to plaque size of several isolates note heterogeneity of population.

Identification in our laboratory made to date with (4) isolates of suspected cases, yielded Dengue-1 serotype. Further isolations, reisolations and identifications of other virus isolates (10/28) are now in progress. Future additional confirmations will be done at WRAIR.

(Dr. Raúl Walder, O.M. Suarez, M. de Perez)

We wish to thank WRAIR and PAHO, Regional Adviser on Virus Diseases, for their valuable and opportune cooperation.

FIGURE 1


PHO-47341
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REPORT FROM THE SAN JUAN LABORATORIES,
CENTER FOR DISEASE CONTROL, SAN JUAN, PUERTO RICO

The 1978 Dengue Epidemic in Puerto Rico

The 1977 epidemic, reported in the March 1978 issue of the Infoexchange, ended in January 1978. Most virus isolations were of dengue types 2 and 3, but a few isolations of type 1 were made from patients with onset of illness in December 1977.

In April 1978, cases began to increase again, and type 1 virus was isolated from almost all cases from that point onward. From April 1 through August 23, 1978, a total of 9,071 suspected cases of dengue were reported from 76 of the 78 municipalities of Puerto Rico and its associated islands. These reports were accompanied by blood specimens. Paired specimens were received from 1,376 of these patients (15%), and serological tests confirmed that 1,107 (80%) had had a current infection with dengue. This compares with a serological confirmation rate of only 38% for the 1977 epidemic. However, the ratio of females to males was the same as in that epidemic (4:3) the most affected age was again the 20-30 year old group, and the geographic distribution was again proportional to the population, with 25% of the patients coming from the metropolitan area. The epidemic peaked in June and has now settled down to a plateau of between 300-400 cases reported weekly, representative of a much larger number actually infected. No deaths and relatively few hemorrhagic complications have been reported.

Over 100 isolations of dengue type 1 virus have been made by inoculating acute sera into Toxorhynchites amboinensis mosquitoes. Head squashes made after 6 days incubation are examined by direct FA staining with a conjugated human dengue convalescent serum, and positive mosquito pools are held alive until 14 days, then used as antigen in a CF test for identification.

Puerto Ricans traveling to the United States mainland became ill with serologically confirmed dengue in Illinois, New York and Pennsylvania, as also did tourists returning from Puerto Rico to Maine and Florida.

Larval indices for Aedes aegypti were higher during the early part of the epidemic than at similar periods during previous years. A dry period during July, together with 3 cycles of aerial ULV spraying with malathion in the metropolitan area in June, may have contributed to the decline in cases in July and August. Eight truck-mounted ULV spraying machines were

purchased in July to supplement the 9 units already available, and permit large-scale spraying of towns outside the metropolitan area. An island-wide source-reduction campaign is under way to assist in Aedes aegypti control.

Dengue in Other Caribbean Countries

a. Haiti

Type 1 virus was isolated from a 13 year old boy with onset of illness in November 1977. Suspected cases continued to be reported during each of the first 5 months of 1978, with a cumulative total as of April 1st of 54.

b. Dominican Republic

Type 1 virus was recovered from the sera of the following 4 patients:

Age	Sex	Onset date	Residence
9	M	September 1977	Santo Domingo
4	F	December 1977	San Pedro de Macoris
34	F	December 1977	San Pedro de Macoris
51	M	January 1978	Santo Domingo

There have been no reports of any epidemic dengue in the Dominican Republic, in spite of the fact that the Aedes aegypti control campaign in the capital city of Santo Domingo, begun in September 1977, was terminated at the end of January 1978, and larval indices in the city have returned to high levels.

c. U.S. Virgin Islands

A St. Thomas resident from whom type 2 virus was isolated in January 1977 became ill again in January 1978, and type 1 virus was isolated from him. Type 1 virus was also recovered from 6 patients from St. Thomas and St. John with onset of illness in July and August 1978.

d. British Virgin Islands

Type 1 virus was isolated from a case from Tortola with onset in May 1978.

e. Netherlands Antilles

Three strains of type 1 virus were obtained from patients from St. Maarten with onset of illness in November and December 1977.

f. Honduras

Six acute sera were received from Dr. David Harms of the Baptist Mission in Honduras from patients from an epidemic in San Pedro Sula and La Paz. Flaviviruses were isolated in Toxorhynchites from 5 of these and identified by CF as dengue type 1.

Dengue Imported into the United States

Serological confirmation of dengue infection has been obtained for travelers becoming ill in 1978 in Florida, Illinois, Maine, Maryland, North Carolina, New York, Ohio, Pennsylvania, and Texas after arriving from Curacao, Dominican Republic, U.S. Virgin Islands, St. Maarten, Puerto Rico and Tahiti. During 1977 there were 70 imported cases confirmed from 22 states and the District of Columbia.

(J. P. Woodall, R. H. López-Correa, C. G. Moore, G. E. Sather, G. Kuno, F. K. Banegura) San Juan Laboratories, CDC, GPO Box 4532, San Juan, Puerto Rico 00936.

REPORT FROM THE DEPARTAMENTO DE VIROLOGIA, HOSPITAL GENERAL DE MEXICO, S.S.A.
AND
INSTITUTO DE INVESTIGACIONES BIOMEDICAS, U.N.A.M.

HUMAN SERA FROM DIFFERENT PLACES IN MEXICO SURVEYED FOR VENEZUELAN ENCEPHALITIS ANTIBODIES

Origin of Samples	Date collected	Number of sera tested	Hemagglutination-inhibition titers (1)									Total of positives
			0	10	20	40	80	160	320	640	1280	
CHETUMAL	Jan.74	76	47	9	10	5	3	2	0	0	0	29
GUADALCAZAR	Jan.74	79	61	3	5	0	1	2	3	1	3	18
COZUMEL	Mar.74	50	43	4	1	1	1	0	0	0	0	7
MERIDA	Mar.74	40	30	4	2	1	1	1	1	0	0	10
CHIAPAS	Mar.74	13	6	3	2	0	2	0	0	0	0	7
CAMPECHE	Apr.74	13	10	3	0	0	0	0	0	0	0	3
	Total	271	197	26	20	7	8	5	4	1	3	74

(1) Reciprocal of highest dilution of serum that inhibited 4 HA units of VE antigen.

(Dr. Cesar Wong Chía)

REPORT - JANUARY-JUNE 1978
OFFICES OF LABORATORY SERVICES AND ENTOMOLOGY
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES, JACKSONVILLE, FLORIDA

Following the SLE activity experienced in 1977 in Florida, the level of arbovirus surveillance for 1978 has increased.

Human - Sera from a total of 885 patients with CNS symptoms were subjected to HI antibody tests against EEE, SLE and VEE antigens. Four patients were positive for EEE:

Patient	S_1	S_2	Remarks
64 year male	1:10	1:320	brain damage
8 year female	1:80	1:320	brain damage
7 year male	1:80	1:5120	death
41 year male	1:2560	1:10, 240	aseptic meningitis (recovered)

There were 26 patients with constant SLE and/or Dengue 2 titers probably due to the 1977 SLE outbreak in Florida or previous Group B Carribean-acquired infections. Also, there were two confirmed Dengue 2 cases and two in which laboratory serology could only determine that they were Group B infections. The Dengue 2 cases were documented as having been acquired in the Carribean area. There were no SLE cases detected during the period of this report.

Sentinel Chicks - A total of 831 young chickens (pretested for SLE and EEE) were placed in 105 sites in 32 counties located in peninsular Florida and in 2 panhandle counties. The chickens were bled monthly and tested against EEE and SLE antigens. A total of 2,228 sera yielded 5 chickens which converted to EEE in June. There were no SLE conversions.

Wild Birds - A total of 109 birds caught in the Lee County area yielded two which were HI positive for SLE. Placque neutralization tests yielded 8 with SLE antibody, probably maternal in origin since these were nestling and very young birds.

Mammals -	Rodents	11/115	SLE antibody representing past infection
	Raccoons	5/20	
	Opossums	14/57	
	Miscellaneous	3/83	

Mosquitoes - A total of 576 Culex species (nigripalpus, quinquefasciatus, and salinarius) were negative for SLE virus; one pool collected June 15 from Hillsborough County yielded EEE virus. A California trivittatus virus was isolated from one of 3 pools of Aedes infirmatus.

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

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

Arbovirus surveillance, July 1, 1977-December 31, 1977

For the period indicated abo e, 1834 pools totaling 28783 mosquitoes were inoculated into suckling mice. Listed below are the positives:

LOCALITY	COLLECTION DATE	SPECIES	# ISOLATIONS	VIRUS
Ft. Worth	6-13-77	C. quinque C. restuans C. quinque	1	Hart Park
Ft. Worth	6-13-77	C. tarsalis C. restuans	1	Hart Park
Ft. Worth	6-13-77	C. quinque C. restuans	1	Hart Park
Hidaldo Co.	6-14-77	C. quinque A. aegypti	1	Hart Park
Dallas Co.	6-15-77	C. quinque C. restuans C. quinque	1	Hart Park
Tarrant Co.	6-15-77	C. quinque C. restuans C. quinque	1	Hart Park
Ft. Worth	6-20-77	C. quinque C. restuans C. quinque	1	Hart Park
Ft. Worth	6-20-77	C. tarsalis C. restuans C. quinque	1	Hart Park
Hidalgo Co.	6-20-77	C. quinque A. aegypti	1	Hart Park
Hidalgo Co.	6-20-77	C. quinque A. aegypti C. quinque	1	Hart Park
Dallas	6-20/24-77	C. quinque C. restuans C. quinque	1	Hart Park
Dallas	6-20/24-77	C. restuans C. quinque C. restuans	1	Hart Park
Dallas	6-20/24-77	C. restuans C. quinque	1	Hart Park

San Antonio	6-21-77	C. quinque A. qegypti C. quinque	1	Hart Park
Amarillo	6-22-77	C. tarsalis	1	WEE
Amarillo	6-22-77	C. tarsalis	1	WEE
Ft. Worth	6-27-77	C. quinque C. tarsalis C. quinque	1	Hart Park
Dallas	6-30-77	C. quinque C. restuans C. quinque	1	Hart Park
Ft. Worth	7-5-77	C. quinque C. tarsalis C. quinque	1	Hart Park
Ft. Worth	7-5-77	C. restuans C. quinque	1	Hart Park
Randall Co.	7-7-77	C. quinque	1	WEE
Randall Co.	7-7-77	C. tarsalis	1	WEE
Ft. Worth	7-7-77	C. quinque C. tarsalis C. quinque C. restuans	1	WEE
Randall Co.	7-14-77	C. tarsalis	1	WEE
Ft. Worth	8-1-77	C. quinque	1	Hart Park
Randall Co.	8-2-77	C. quinque	1	WEE
Corpus Christi	8-3-77	C. quinque C. melanoconion C. quinque	1	Hart Park
Liberty Co.	8-4-77	A. atlanticus-tormentor	1	Keystone
Randall Co.	8-8-77	C. tarsalis	1	WEE
Ft. Worth	8-8-77	C. quinque	1	SLE
Randall Co.	8-8-77	C. tarsalis	1	WEE
Dallas Co.	8-9-77	C. quinque	1	WEE
Ft. Worth	8-15-77	C. quinque	1	SLE

Region I	8-16-77	C. quinque C. tarsalis A. vexans	1	Hart Park
Pt. Arthur	8-22-77	C. quinque A. quadrimaculatus A. taeniorhynchus	1	Hart Park
Cameron Co.	8-23-77	C. quinque A. pseudopunctupennis C. quinque	1	Hart Park
Dallas	8-26-77	C. quinque	1	Hart Park
Dallas	8-30/9-1/77	C. quinque	1	Hart Park
Bryan	9-6-77	C. quinque	1	SLE
Randall Co.	9-13-77	C. tarsalis	1	WEE
Randall Co.	9-20-77	C. tarsalis	1	WEE
Deaf Smith Co.	9-20-77	C. tarsalis	1	WEE
Dallas	10-19-77	C. quinque C. tarsalis C. restuans	1	Hart Park

WILD BIRD BLOODS FOR ISOLATION

A total of 217 wild bird bloods for isolation were submitted. Data is as follows:

LOCALITY	COLLECTION DATE	SPECIES	# ISOLATIONS	VIRUS
Lubbock	6-23-77	Sparrows	1	WEE
Lubbock	7-18-77	Sparrows	1	Hart Park
Lubbock	7-27-77	Sparrows	1	WEE
Lubbock	8-17-77	Sparrows	1	Hart Park

WILD BIRD BLOODS TESTED FOR ARBOVIRUS ANTIBODIES

LOCALITY	COLLECTION DATE	SPECIES	# POSITIVES	VIRUS
Dallas	7-7-77	1 pigeon 2 young chickens	0	--
Dallas	7-14-77	pigeons (13)	0	--
Dallas	7-15-77	pigeons (10)	0	--
Dallas	10-24-77	pigeons (17)	5	SLE $\geq$ 1:20
Dallas	10-27-77	sparrows (3)	0	--
Dallas	11-9-77	pigeons (11)	3	SLE $\geq$1:10

Dallas	12-2-77	sparrows (6) pigeons (8)	0	--
Dallas	12-14-77	sparrows (5) starling (1)	0	--
Dallas	[illegible]	Grackel (1)		
Dallas	12-20-77	Cowbird (2) Blackbird (2) Ducks (5) Sparrows (3)	7	SLE ≥1:10
San Antonio	7-20-77	? (9)	0	--

Temple-3 Non-sentinel chickens bloods were collected from area where SLE was isolated from mosquitoes, and where there was a case (human) SLE. These were collected on 9-30-77. Titers were 1;20, 1:160, and 1:640 against SLE antigen.

SENTINEL CHICKEN FLOCKS FOR ARBOVIRUS SEROLOGY

LOCALITY	COLLECTION DATE	# TESTED	# POSITIVE	ANTIBODY DETECTED
Dallas	7-5-77	100	1	SLE 1:10
Dallas	8-1-77	97	0	--
Dallas	9-13-77	94	4	SLE ≥1:10
Dallas	10-11-77	83	0	--
Dallas	11-7-77	85	2	SLE ≥1:10
Dallas Co.	7-13-77	96	0	--
Dallas Co.	8-15-77	95	0	--
Dallas Co.	9-20-77	80	0	--
Dallas Co.	10-3-77	71	2	WEE ≥1:80
Cameron Co.	6-28-77	87	1	WEE 1:10
Cameron Co.	7-14-77	85	0	--
Cameron Co.	8-11-77	81	0	--
Cameron Co.	9-8-77	74	0	--
Cameron Co.	10-6-77	69	0	--
Cameron Co.	11-3-77	66	0	--
Cameron Co.	12-1-77	62	1	WEE 1:20
Cameron Co.	12-29-77	62	0	--

Lubbock	7-19-77	56	4	WEE ≥1:80
Lubbock	8-17-77	58	18	WEE ≥1:80
Lubbock	9-12-77	46	4	WEE ≥1:80
Lubbock	10-18-77	44	1	WEE 1:320
Lubbock	11-8-77	37	1	SLE 1:20

Arbovirus surveillance, January 1, 1978-June 30, 1978

For the period indicated above, 429 pools totaling 3644 mosquitoes were inoculated in suckling mice. Listed below are the positives:

LOCALITY	COLLECTION DATE	SPECIES	# ISOLATIONS	VIRUS
Riviera	2-22-78	C. quinquefasciatus	1	VEE
Port O'Connor	5-9-78	A. crucians	1	WEE
Sea Drift	5-9-78	C. restuans	1	WEE
Calhoun Co.	5-9-78	C. quinquefasciatus	1	WEE
Calhoun Co.	5-9-78	C. salinarius	1	WEE
Calhoun Co.	5-9-78 " " "	C. (melanoconion sp) A. sollicitans A. taeniorhyncus P. columbiae	1	WEE
Port Arthur	5-9-78	C. quinquefasciatus	1	WEE
Port Arthur	5-9-78	C. salinarius	1	WEE
Port Arthur	5-9-78	C. salinarius	1	WEE
Hidalgo Co.	5-8-78	A. vexans	1	WEE
Willacy Co.	5-9-78	A. quiniquefasciatus	1	VEE
San Patricio Co.	5-10-78	C. salinarius	1	WEE
San Patricio Co.	5-10-78	C. quinquefasciatus	1	WEE
San Patricio Co.	5-10-78	C. quinquefasciatus	1	WEE
Galveston Co.	5-9-78	A. sollicitans	1	WEE
Galveston Co.	5-9-78	C. salinarius	1	WEE
Galveston Co.	5-9-78	A. taeniorhyncus	1	WEE
Galveston Co.	5-11-78 "	A. quadrimaculatus A. taeniorhyncus	1	WEE
Dallas	5-10/11-78 " "	C. restuans C. restuans C. tarsalis	1	WEE
Dallas	5-10/11-78	C. tarsalis	1	WEE

LOCALITY	COLLECTION DATE	SPECIES	# ISOLATION	VIRUS
Dallas	5-10/11-78	A. vexans	1	WEE
Dallas	"	A. vexans	1	WEE
Dallas	"	C. quinquefasciatus	1	WEE
Dallas	"	C. restuans	1	WEE
Dallas	"	C. quinquefasciatus	1	WEE
Dallas	"	C. quinquefasciatus	1	WEE
Dallas	"	C. tarsalis	1	WEE
Dallas	"	C. tarsalis	1	WEE
Dallas	"	C. restuans	1	WEE
Dallas	"	C. restuans	1	WEE
Dallas	"	C. quinquefasciatus	1	WEE
Dallas	"	C. quinquefasciatus	1	WEE
Victoria Co.	5-11-78	C. quinquefasciatus	1	WEE
Dallas	5-12/15-78	C. quinquefasciatus	1	WEE
Dallas	"	C. restuans A. punctipinnis A. vexans	1	WEE
Dallas	"	C. quinquefasciatus	1	WEE
Dallas	"	A. vexans	1	WEE
Dallas	"	C. quinquefasciatus	1	WEE
Dallas	"	C. tarsalis C. restuans C. (melanoconion sp)	1	WEE
Dallas	"	C. territans	1	WEE
Port Arthur	5-16-78	C. quinquefasciatus C. quniquefasciatus A. quadrimaculatus	1	WEE

LOCALITY	COLLECTION DATE	SPECIES	# ISOLATION	VIRUS
Cameron Co.	5-16-78	P. columbiae	1	WEE
Galveston Co.	5-16/17-78	A. sollicitans " " A. taeniorhyncus	1	WEE
Dallas	5-11/18-78	C. tarsalis	1	WEE
Dallas	"	C. restuans C. territans A. vexans	1	WEE
Liberty Co.	5-23-78	A. vexans	1	WEE
Kingsville	5-23-78	C. quinquefasciatus A. aegypti C. quinquefasciatus	1	WEE*
Port Arthur	5-23-78	A. quadrimaculatus	1	WEE

NESTLING BIRD BLOODS FOR ISOLATION

Testing of 48 wild bird bloods has yielded 2 Positives:

LOCALITY	COLLECTION DATE	SPECIES	# ISOLATIONS	VIRUS
Midland	9-1-77	Passer domesticus	1	VEE
Lamb Co.	9-6-77	Passer domesticus	1	VEE

WILD BIRD BLOODS FOR SEROLOGY

Dallas and San Antonio have submitted a total of 104 wild bird bloods for serology. None has been positive for the detection of antibody by HI for WEE, EEE, SLE, or VEE.

SENTINEL CHICKEN FLOCKS

Five hundred and forty eight chicken sera have been submitted by Dallas, Dallas Co., Lubbock, Uvalde, and Cameron Co. None has yielded a positive by HI for the presence of antibody to arbovirus antigens WEE, EEE, VEE, and SLE.

*Virus identified as WEE by C.F. by Texas Department of Health. Virus identified as mixture of VEE and WEE by CDC at Ft. Collins, Colorado.

(Charles E. Sweet and staff of the Medical Virology Branch)

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

CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

DATA THROUGH MID-AUGUST

Surveillance for Human Arbovirus Infection, United States, 1978

The 1978 arboviral season has been notably quiet in the continental United States. No cases of St. Louis Encephalitis (SLE) or Western Equine Encephalitis (WEE) have been identified. A total of 29 California Encephalitis (CE) cases including 2 deaths have been reported from 7 states and, although 4 Eastern Equine Encephalitis (EEE) cases have been diagnosed in Florida, they were scattered in place and time. In contrast, dengue activity in Puerto Rico has continued throughout the dry season, raising concern that major outbreaks may follow the rains now under way.

California Encephalitis

Arkansas - CE infection has been serologically confirmed in a fatal case involving an 8-year-old girl who had onset of acute encephalitis on July 13. A case from 1975 is the only previous case reported from Arkansas.

Illinois - The first report of the season (onset 6/30) involved a 3-year-old girl from Wabash County, Illinois who was hospitalized with acute encephalitis in Indiana. She was diagnosed by the Indiana State Laboratory and reported through the Illinois Department of Health. A second case of CE from Illinois involved a 6-year-old boy from Peoria.

Ohio - Two cases in children from Perry and Lorain Counties had onset in July.

Minnestoa and Wisconsin - On July 18, a 3-year-old girl hospitalized in LaCrosse, Wisconsin with acute encephalitis died (MMWR 27:31). Virus isolated from brain tissue was identified as LaCrosse or a closely related strain of CE on July 24 at the Department of Preventive Medicine, University of Wisconsin. The child's death and earlier flooding in the southeast counties of Minnesota and the southwest counties of Wisconsin led to concern that arboviral outbreaks might be developing. On request from the Minnesota and Wisconsin State Epidemiologists, teams from the Bureaus of Epidemiology, Laboratories, and Tropical Diseases assisted in investigating the situation and planning for appropriate controls. Surveillance for suspect cases was intensified and diagnostic tests by local medical centers, the State Laboratories, and CDC identified a total of 9 California cases (4 confirmed, 5 presumptive) from Minnesota and 7 from Wisconsin (4 confirmed, 3 presumptive). Most cases involved rural children in the hyperendemic area for LaCrosse virus. Close surveillance for further CE and other arboviral activity is continuing in both States.

New York - One confirmed and 8 presumptive cases have been reported, more than for any other year. CE was confirmed in an 11-year-old girl from Erie County in western New York. With the exception of 1 case from the finger lakes area, all of the presumptive cases were reported from northeast New York. One of the cases was unusual because it involved a 73-year-old man with very high titers. His illness is suspected to represent reinfection and further studies to confirm the diagnosis are underway.

Tennessee - Infection was confirmed in a 5-year-old boy from Memphis.

Eastern Equine Encephalitis

A total of 4 cases of EEE has been reported from widely scattered counties in Florida (Pasco, Collier, Alachua, Volusia). The case in Alachua County involved a 7-year-old boy with onset of acute encephalitis on July 2. Positive sera was collected on July 10 and he died on July 11. More than 100 laboratory diagnosed EEE cases in horses have been reported from 37 counties in Florida. Sentinel chickens maintained for SLE surveillance have shown evidence of EEE infection in 8 counties. EEE activity has also been noted in juvenile birds collected near the Mississippi coast.

St. Louis Encephalitis

No cases of SLE in humans have been reported. The Vector-Borne Diseases Division, CDC, at Fort Collins reported that little evidence of recent transmission has been found in birds or mosquitoes tested by surveillance systems across the United States.

Western Equine Encephalitis

No cases of WEE have been laboratory confirmed although a number of suspect cases are under investigation. Equine cases have been documented by the Arizona, California, and Colorado Laboratories.

Dengue

Caribbean - Over 9,000 cases of dengue-like illness have already been reported this year. Increased dengue transmission during the rainy season just begun is expected to result in another major outbreak of dengue (MMWR 27:33). If this occurs, it will be the third consecutive year of epidemic dengue in Puerto Rico. Although no recent outbreaks have been reported from other major Caribbean islands, dengue remains endemic in much of the area.

Central America - An abrupt outbreak of dengue with attack rates said to exceed 20% has been documented in the coastal areas of Honduras. Sera

collected from cases in the major port city of San Pedro Sula indicated recent dengue infection, but the type remains undetermined. Cases are suspected in Tegucigalpa, the inland capital, and extensive mosquito control has been undertaken.

Imported Cases - A total of 26 cases involving residents of 12 states were imported in the continental United States in 1977. Cases were imported from Tahiti, Puerto Rico and several locations in the Caribbean including Bequia, British Virgin Islands, Grenada, and St. Maarten. The simultaneous occurrence of dengue outbreaks abroad and maximum levels of Aedes aegypti populations in the areas of the U.S. where Aedes aegypti mosquitoes breed increases the possibility that an imported case may initiate indigenous transmission. Incoming flights from areas of dengue outbreaks are being disinsected. Increased surveillance in the southeastern states is continuing to detect any indigenous dengue transmission should it occur.

(Karl Kappus, Marjorie Pollack, Melinda Moore, Eugene Hurwitz, David Nelson, and Lawrence Schonberger)

REPORT FROM THE BUREAU OF TROPICAL DISEASES

CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

Development of Aedes aegypti Control Capabilities in Puerto Rico

Aedes aegypti surveillance and control methods used in Puerto Rico have undergone modification and diversification as a result of concern over the epidemic transmission of dengue in late 1975, in 1977, and again in 1978. The discovery of type 1 virus in December 1977 was a further stimulus to the improvement of methods.

Weekly sampling for Aedes aegypti has been established in each of the seven health regions of the island. Included in the sampling methods are larval inspections, oviposition traps, and adult traps in each area sampled. Information gained is used for planning emergency mosquito control measures related to dengue control and as a reference for comparison of progress in the long-term Aedes aegypti source reduction efforts.

Aedes aegypti control capabilities have also been improved. Prior to 1975, hand-compression sprayers were the main tool used in Aedes aegypti control in Puerto Rico. At present the department of environmental health has 17 heavy-duty ULV spray machines for use island-wide against adult mosquitoes. During periods of epidemic transmission of dengue, they have supplemented their truck-mounted adulticiding equipment by using contracted ULV-equipped aircraft for rapid coverage of large areas. In early 1978 a source reduction campaign against Aedes aegypti was begun in an attempt to reduce the potential for future epidemics of dengue. An integral part of both the emergency control and source reduction efforts has been use of public education and information efforts on

both the disease and the mosquito.

Currently, efforts are underway to speed up response time for mosquito adulticiding in areas from which cases are reported. Within 24 hours of the completion of weekly case summaries, the regions are notified of areas that should be sprayed and efforts are made to spray each of these areas two times during the week following notification.

Surveillance and combined control efforts appear to be having some effect. The number of cases reported declined during July and August after a peak of close to 4,000 cases in June. A second and perhaps greater peak in cases was anticipated when heavy rains began in August, but so far has not materialized.

Health departments in other dengue-receptive areas are urged to follow the example of the Department of Environmental Health in Puerto Rico in improving Aedes aegypti control capabilities through the use of a variety of both long-term source reduction and emergency adult control methods.

Donald A. Eliason

REPORT FROM THE
MEMPHIS & SHELBY COUNTY HEALTH DEPARTMENT
INSECT VECTOR CONTROL DIVISION
MEMPHIS, TENNESSEE

Since early April, 1978, the Insect Vector Control Division of the Memphis and Shelby County Health Department has maintained continuous daily surveillance of the SLE/WEE arboviral activity in the community. Surveillance consists of HI seropositive monitoring of wild-caught sparrows and seven (7) sentinel chicken flocks at strategic locations throughout the area. Mosquito vector control, consisting of larviciding and adulticiding, is integrated with surveillance.

Table 1, attached, summarizes our surveillance results through August 4, 1978.

Sparrow seropositive percentages generally have remained well below the danger threshold level of 4-5%, having risen above this level (to 7.3%) only once, in late June. This sparrow seropositive conversion rise coincided with a precipitous seropositive rise in sentinel chicken flocks, both of which were preceded by a sharp rise in mosquito population density. These seropositive increases peaked in the sentinel chickens in early July, then dropped again in mid-July. They continue to remain at a low level.

Immediately following the high seropositive conversion of sentinel chickens, larviciding and adulticiding activities were intensified in the specific problem loci. To date, no SLE virus has been isolated from mosquito pools taken from the high seropositive conversion areas.

(I. K. Moseley, James Hamm, John R. Oates and J. B. Mullenix)

TABLE 1. Tennessee Birds Tested for Hemagglutination-inhibition (HI) Antibodies to SLE Virus by Location, Date of Collection, and Age.

Location	Number (percent) with SLE HI Titer ≥20/No. Tested											
	Apr 4-7		Apr 10-14		Apr 17-21		Apr 24-28		May 1-5		May 8-12		May 15-19	
	Juv.	All ages	Juv.	All ages	Juv.	All ages	Juv.	All ages	Juv.	All ages	Juv.	All ages	Juv.	All ages
Memphis														
Wild birds		1/52 (2.0)	0/1	0/60	0/6	4/101 (4.0)	0/21	0/95	0/3	1/40 (2.5)	0/44	1/120 (0.8)	0/131	1/200 (0.5)
Sentinel chickens								0/12*						
	May 22-26		May 29-June 2		June 5-9		June 12-16		June 19-23		June 26-30		July 3-7	
Memphis														
Wild birds	0/84	2/122 (1.6)	0/55	0/119	0/79	0/141	0/84	0/150	0/54	0/111	0/64	4/55 (7.3)	0/36	0/116
Sentinel chickens	0/42		0/31		0/42		0/36		0/54		2/36(5.6)		6/42(14.3)**	
	July 10-14		July 17-21		July 24-28		July 31-Aug. 4							
Memphis														
Wild birds	0/28	0/114	0/47	1/95 (1.1)	0/53	0/59 (1.7%)	0/24	0/31						
Sentinel chickens	2/36(5.5)***		0/42		0/36		1/40 (2.5%)							

* Sentinel Chicken Seroconversions

** 9 additional chickens positive at 1:10

*** 1 chicken with WEE virus seroconversion

REPORT FROM INSTITUTE FOR CANCER RESEARCH
FOX CHASE CANCER CENTER
7701 BURHOLME AVENUE
PHILADELPHIA, PA 19111

Studies of the bedbug Cimex hemipterus (Fabr.) as a possible vector of hepatitis B virus

Bedbugs (Cimex hemipterus), colonized in the laboratory from a foundation stock collected in Senegal in 1977, were fed hepatitis B surface antigen-positive [HBsAg(+)] blood, donated by renal dialysis patients. This blood was also positive for the e-antigen (HBeAg), a marker of infectivity. Our first experiments (MS in prep.) indicated that HBsAg persists in the bedbug for up to six weeks after a single HBsAg(+) blood meal, but we obtained no evidence for multiplication of the virus in the insects' tissues.

We then tested the feces of bedbugs which were fed at weekly intervals on HBsAg(+) blood. Feces collected during the second week after the first HBsAg(+) blood meal were HBsAg(+), and the antigen continued to be detected until the second week after the last HBsAg(+) meal.

If the excreted HBsAg reflects the presence of infective virus, then the dust in the bug-infested bed of a chronic carrier of hepatitis B could be a source of infection for other household members.

(C. W. Ogston and W. T. London)

REPORT FROM THE STATE OF NEW JERSEY

DEPARTMENT OF HEALTH, TRENTON, NEW JERSEY

No isolations of arboviruses were made from mosquitoes, birds or equine animals in the first and second quarters, January 1 through June 30, 1978.

Two isolations of western encephalitis virus were isolated from Culiseta melanura mosquitoes collected in the Bass River area in July, 1978.

Isolations from Arthropods in August, 1978

Group	Virus and No. of Strains		Isolated from	Collected in	Month	Year
	EE	WE				
A		23	C. melanura	Bass River	August	1978
A		4	C. melanura	Burlington County	August	1978
A		1	C. restuans	Burlington County	August	1978
A		1	A. sollicitans	Burlington County	August	1978
A		5	C. melanura	Dennisville	August	1978
A		9	C. melanura	New Gretna	August	1978
A		4	C. melanura	Woodbine	August	1978
Totals		47				

(B. F. Taylor)

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

Arbovirus Surveillance 1978

During the month of June and July, 178 patients with signs of meningitis and/or encephalitis were tested for evidence of infection with Eastern encephalitis, Western encephalitis, St. Louis encephalitis, Powassan and California encephalitis (CAL) viruses. A current infection with CAL virus was indicated by hemagglutination-inhibition (HI) and neutralization tests in an 11-year-old girl with encephalitis. A presumptive diagnosis of recent infection with CAL virus was made in an additional 7 patients from whom only single sera were available: These sera reacted by HI and showed significant levels of neutralizing activity (neutralizing index ≥ 3.0).

Two CAL virus isolates were obtained, one each from Aedes canadensis and Aedes stimulans, collected in the same northeastern region of the state where 5 of the patients with CAL virus findings resided.

Field Investigation of CAL Virus in the Capital District, 1977-1978

In 1977, seroconversions to CAL virus were detected in 6 of 15 sentinel rabbits stationed between July 7 and September in 4 areas of the Capital District previously associated with human cases. From 498 pools of 3,048 mosquitoes collected in Albany and Schenectady counties from June 6 to September 9, two isolates of CAL virus were made, both from Aedes canadensis captured in July near our positive sentinel station. No agents were obtained from 160 pools of 914 laboratory-reared Aedes triseriatus mosquitoes examined for evidence of transovarial transmission of CAL virus.

Since May 1, 1978, 14 rabbits and 36 hamsters have been exposed in 21 sentinel sites of the Capital District area. Thus far, HI tests of serially-obtained blood specimens have not provided evidence of CAL virus infections in these sentinels.

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

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT
DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH
YALE UNIVERSITY, NEW HAVEN, CONNECTICUT 06510
IN COLLABORATION WITH THE PACIFIC RESEARCH SECTION, NIAID

In the previous Info-Exchange (No. 34) we reported transovarial passage of yellow fever (YF) virus in three geographic strains of Aedes aegypti mosquitoes. In brief review, mosquitoes were infected by intrathoracic inoculation of two strains of YF virus. Strain #1, designated Alouatta, was isolated from a howler monkey and passaged once in a night monkey. Strain #2, Haemagogus, was obtained from an unpassaged pool of Haemagogus mosquitoes. Following infection, experimental parent mosquitoes were incubated for 5 days at 27°C. They were then permitted to engorge noninfectious blood-meals at approximately weekly intervals. A portion of the egg progeny from these females was surface sterilized. After larval and pupal development, emerged adults were sexed, pooled and triturated. Suspensions of these progeny mosquitoes were inoculated intrathoracically into recipient uninfected mosquitoes which were incubated for 10-12 days at 27°C, then killed by freezing and headsquashed. Headsmears were stained with anti-YF conjugated antibody and examined for the presence of viral antigen with a fluorescence microscope. As reported, YF virus antigen was detected in 1st ovarian cycle progeny.

Additional ovarian cycles have now been tested and virus antigen is demonstrable in the 2nd and 3rd ovarian cycle progeny (Table 1). Altogether 115,668 individuals have been tested and 92 pools yielded YF virus antigen. Of the positive pools, 32 refer to the 48,137 progeny individuals associated with the Alouatta virus and 60 pools to 67,531 progeny associated with the Haemagogus virus. Approximately 1/2 of the 1st and 2nd ovarian cycle eggs were surface sterilized in order to delineate the mechanism of progeny infection. Forty-four YF positive pools (Table 2) were obtained from 33,379 progeny resulting from surface-sterilized eggs, as compared to 38 positive pools from 29,774 progeny resulting from nonsurface-sterilized eggs. Therefore, transovarial transmission, rather than transovum, would seem to be the mechanism of progeny infection.

By B.J. Beaty, T.H.G. Aitken, R. Tesh, and L. Rosen

Table 1

Filial Infection Rates of Aedes aegypti Infected with the Alouatta and Haemagogus Yellow Fever Virus Strains

Ovarian cycle	Alouatta virus				Haemagogus virus			
	Pools	Pools Positive	Progeny	MFIR*	Pools	Positive	Progeny	MFIR
1st OV	157	26	18,385	1:707	225	54	29,309	1:543
2nd OV	94	5	15,719	1:3144	156	3	22,709	1:7570
3rd OV	66	1	14,033	1:14033	77	3	15,513	1:5171
TOTAL	317	32	48,137	1:1504	458	60	67,531	1:1126

*Minimum Filial Infection Rate

Table 2

Effect of Surface Sterilization of Eggs on Filial Infection Rates

Ovarian cycle	Eggs Surface Sterilized				Eggs Nonsurface Sterilized			
	Pools	Pools Positive	Progeny	MFIR*	Pools	Positive	Progeny	MFIR
1st OV	216	41	26,104	1:637	163	37	20,292	1:548
2nd OV	62	3	7,275	1:2425	85	1	9,482	1:9482
TOTAL	278	44	33,379	1:758	248	38	29,774	1:784

*Minimum Filial Infection Rate

REPORT FROM THE UNIVERSITY OF NOTRE DAME LABORATORY FOR ARBOVIRUS RESEARCH AND SURVEILLANCE (UNDLARS), NOTRE DAME, INDIANA 46556

Again this summer UNDLARS is participating in an encephalitis "early warning" program sponsored by the Indiana State Board of Health (ISBH). Four 2-man teams have been continuously mist-netting primarily English (house) sparrows and will continue to do so through September, 1978. Collections will continue on an irregular basis through fall and winter, however.

To date (August 25) 4,520 birds have been captured, bled (0.5ml), and released. Whole blood samples are sent to UNDLARS (by air under special arrangement with a local commuter airline) within 48 hours of bleeding for serological screening. Samples are screened for SLE, EEE, and WEE by hemagglutination-inhibition (HI). All HI positive samples are confirmed via serum dilution neutralization tests (NT) in BHK_{21} cells in microtiter.

SLE SEROPOSITIVE AVIAN RESULTS:

Based on a HI titer of 1:20 or greater at 8 units, we have recorded 30 SLE seropositive birds to date; all are English sparrows. These birds have been recorded from 9 counties compared to 14 counties with seropositive birds in 1977. A total of 9 species of birds have been trapped; however, the vast majority have been English sparrows. Of the 30 HI/NT positive samples, only 4 have been from juvenile sparrows. Two additional HI positive samples were NT negative and are considered to be false positives.

Three sparrows have been recaptured once so far. HI and NT titers showed only a 2-fold rise over 13-day, 19-day, and 35-day periods between initial capture and banding and subsequent recapture/bleeding for each of the 3 birds.

An additional 19 avian samples did have a titer of $10 > HI > 20$, however, only 11 were NT confirmed with a titer of 1:8 or greater. Three of these 11 were juvenile sparrows. Included in this group of 11 were two recaptured birds that subsequently on recapture and serum testing were considered HI positive ($HI \geq 20$). Both were NT positive on the initial capture/bleeding, however. This indicates to us that NT screening, as we noted last year, does permit earlier detection of SLE seropositive birds than does HI screening.

WEE SEROPOSITIVE AVIAN RESULTS:

No avian samples HI or NT positive for WEE were detected in Indiana in 1977. However, to date in 1978 we have recorded 3 WEE HI positive birds from 3 northwestern counties. One male English sparrow with an HI of 1:40 was captured on

July 12th in Lake County (Gary-Hammond). A second male was captured in Tippecanoe County (Purdue University experimental farm) on July 20th; it also had an HI titer of 1:40. The third sparrow, a juvenile, was captured in La Porte County on August 9th; the HI titer was ≥ 10. In 1977 there was one horse positive for WEE recorded from La Porte County; also that year, one horse positive for WEE was detected in Warren County just west of Tippecanoe County.

MOSQUITO VIRUS ISOLATIONS:

Numerous pools of Culex restuans, Culex pipiens, and some Culex salinarius have been screened in primary duck embryo cells with no virus isolations made to date. In addition, Aedes triseriatus larvae and adults have been screened in VERO cells; no California group isolations have been made as yet.

HUMAN SEROLOGICAL SURVEY:

UNDLARS has just been awarded a contract to screen human sera for antibodies to EEE, SLE, WEE, and LAC. Plans call for the ISBH to collect 300 random samples in each of 30 counties over the next year. Samples will come through board pathologists with informed consent given for all samples. Included with each sample will be a brief questionaire including residence history, WW II and Korean duty, etc. Each sample will be screened in microtiter in tissue culture for the 4 viruses by NT (a total of 36,000 individual tests). The specific purpose of this project, from the standpoint of the ISBH, is to better evaluate county needs with respect to encephalitis monitoring and mosquito control. It is also hoped that information valuable to encephalitis sequellae studies will result. We additionally hope to locate previously undetected CE group foci.

LA CROSSE VIRUS MONITORING:

Allen County (Ft. Wayne) has had 6 recorded cases of La Crosse encephalitis in children since spring 1975. This summer we initiated a study to detect foci in that county by ovitrapping for A. triseriatus eggs. Three of the 6 children live on or very near property with substantial A. triseriatus populations. These children also had no travel history prior to onset of La Crosse encephalitis that would suggest their infection elsewhere.

A slight modification of the black painted beer can with black percale cloth liner trap, used routinely by Vector Biology Laboratory personnel, for A. triseriatus egg collection was made. Strips of 1/8" thick balsa wood, slightly less than can width and depth, were substituted for the black cloth. Field trials conducted in two separate locations this summer by VBL personnel indicated A. triseriatus would lay more eggs consistently on the balsa wood strips than on the black cloths. We are in the process of hatching all the eggs collected and

are employing a special regimen developed by Drs. Novak and Shroyer of VBL to obtain virtually a 100% hatch on the first flood. All resulting adults will be screened in VERO cells and/or suckling mice for La Crosse virus.

This method of egg trapping has been highly successful. It has allowed us to go into woodlots with no evidence of treeholes on initial inspection or adult females seeking bloodmeals, and readily detect the presence of A. triseriatus virtually overnight. Coupled with the new egg conditioning and hatching techniques we now practice, detection and evaluation of potential La Crosse virus foci has been greatly simplified.

A complete report of this and other ongoing research and surveillance studies will be presented in the next report.

POSSIBLE EVIDENCE FOR OVERWINTERING OF SLE IN INDIANA:

This spring, Culex reproduction was delayed considerably due to unfavorable weather. In the central part of the state monitoring of Culex began on 1 April by Marion County (Indianapolis) mosquito abatement personnel. Overwintered females were detected in the first week of April leaving the hibernacula. The first records of 4th instar larvae and pupae were made after the middle of May.

Nine adult sparrows were collected prior to the end of May that had SLE HI titers of 1:80 or less. We presume that these are last year's infections. However, 4 birds, including one juvenile, had antibody levels that are indicative of recent infection.

One adult male was collected on 1 May in Marion County. A HI titer of 1:640 with a confirming NT titer of 1:512 was noted. Allowing at least 21 days for SLE antibody titers to reach peak levels after initial infection (J. Hardy, T.M. Yuill, pers. comm.), we would have to conclude that this bird was infected during the first week of April-the exact time during which the first female Culex were detected leaving overwintering sites and seeking bloodmeals.

Two additional adults were collected in Bartholomew Co. during the last week of May. Both had HI titers greater than 1:160 and confirming NT titers of 1:128 and 1:256 respectively. Allowing again at least 21 days for antibody titer to peak sets the probable time of infection during early May. This again is prior to the observation of 4th instar Culex in that area. Presumably only overwintered adult Culex were present at this time. It was also noted that the emergence of early spring Aedes and other spring mosquitoes was drastically delayed or did not occur due to the unfavorable weather conditions and lack of moisture. The fourth bird was a juvenile,

collected in Tippecanoe Co. (Purdue University farms) the 25th of May. The HI titer was greater than 1:20, the NT titer was 1:8, possibly indicating a very recent (less than 21 days prior to capture) infection. Since this is also prior to the emergence of the first generation of Culex in that area, the involvement of overwintered Culex is suspected.

We acknowledge that explanations other than overwintering of SLE virus in adult female Culex are possible. But in the light of the findings by the WRAIR teams of virus isolations from overwintered Culex on the East Coast, and our detection of early season SLE infection in three widely separated locations in Indiana, the hypothesis that SLE virus overwinters in Indiana in hibernating female Culex remains tenable. We do intend to explore this more fully in the coming seasons.

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STATE OF ILLINOIS

DEPARTMENT OF PUBLIC HEALTH

DIVISION OF LABORATORIES

2121 WEST TAYLOR STREET

CHICAGO, ILLINOIS 60612

Results from arbovirus studies in Illinois through August, 1978, characterize this transmission season as one of low level endemicity for SLE virus. This is the third consecutive year at or near this level following the epidemic of 1975. Emphasis this year was on serologic study of resident avians and results are presented in Table 1. The percentage of juvenile or younger birds with antibodies to SLE virus is the same as for the corresponding period of 1977.

No viral isolations have been made on specimens collected in areas where isolations were made in previous years and where antibodies were detected this year. Isolation attempts have been made from blood of 850 nestling or fledgling birds, primarily house sparrows. Culex mosquito populations appear to be lower this year and only 4249 mosquitoes have been tested. To date, no human SLE infections have been confirmed in Illinois.

Two human LaCrosse encephalitis infections have been confirmed in Illinois. The first was a 3 year-old female (onset 6/20) from extreme southeastern Illinois and the second was a 6 year-old male (onset 7/9) from an area of recognized LaCrosse virus activity in north-central Illinois.

No WEE virus activity has been observed this year.

(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 ≥ 1:20/Number Tested								TOTAL	
	1 May - 28 May		29 May - 2 Jul		3 Jul - 30 Jul		31 Jul - 15 Aug			
	Juv.	All ages	Juv.	All ages	Juv.	All ages	Juv.	All ages	Juv.	All ages
NORTHERN ILLINOIS			0/91	1/140 (0.7)	5/354 (1.4)	5/476 (1.0)	4/327 (1.2)	4/371 (1.1)	9/772 (1.2)	10/987 (1.0)
CENTRAL ILLINOIS	0/10	1/139 (0.7)	3/184 (1.6)	11/381 (2.9)	0/113	0/198	0/114	0/116	3/421 (0.7)	12/834 (1.4)
SOUTHERN ILLINOIS	0/83	14/324* (4.3)	4/313 (1.3)	7/527 (1.3)	1/479 (0.2)	3/776 (0.4)	0/250	0/258	5/1125 (0.4)	24/1885 (1.3)
TOTAL	0/93	15/463 (3.2)	7/588 (1.2)	19/1048 (1.8)	6/946 (0.6)	8/1450 (0.6)	4/691 (0.6)	4/745 (0.5)	17/2318 (0.7)	46/3706 (1.2)

* 1 Adult House Sparrow with Antibodies to EEE Virus.

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

Isolation of La Crosse or closely related arbovirus from brain tissues of a fatal case of California encephalitis

La Crosse or a closely related arbovirus has been isolated from brain tissues from a fatal case of California encephalitis which occurred in a 3-year-old girl hospitalized on July 14, 1978 in La Crosse.

La Crosse virus was suspected as the etiologic agent because of the medical and epidemiologic history, clinical symptoms, and a positive reaction with the acute-phase serum in the counter-immuno electrophoresis test. (J. Clin. Microbiology, 7 (6): 603, 1978) conducted by Dr. Cameron Gundersen at the La Crosse hospital before death on July 18th (WMMR-CDC-8-4-78).

We obtained sections of brain tissue at autopsy and promptly examined them for LAC virus by direct fluorescent antibody technique. Smears of tissues from five portions of brain were stained with LAC antibody conjugated with FITC. Scattered foci of weak fluorescence were observed in smears of basal ganglia and cerebellum. Suspensions of these portions of brain were inoculated IC and IP into several litters of new-borne suckling mice. Numerous foci of brilliant fluorescence were observed in the brain tissues of a mouse which died with encephalitis four days after inoculation with basal ganglia tissues. LAC virus studies are continuing with these and the other sections of brain. Dr. Kalfayan, pathologist at Lutheran Hospital in La Crosse, is conducting an extensive histopathologic examination of the various tissues, which has perivascular cuffing and other lesions of encephalitis, in scattered foci. Additional fluorescent antibody studies are also underway to determine the distribution of LAC antigen in various sections of the brain.

This is the second known isolate from a fatal case of California encephalitis. This was similar in clinical symptoms and many other ways to the first one which also occurred near La Crosse, during 1960 (Amer. J. of Epidemiology 1965, 81: 230-253). Some non-fatal cases of California encephalitis, with antibodies to LAC virus in convalescent serums, have since been diagnosed in Wisconsin. Occasional suspected fatal cases have also occurred from which isolates were not obtained. Properly collected and preserved tissue specimens are not often available for laboratory studies.

The patient lived on a small farm on a forested hill near Desoto, near the Mississippi River in southwestern Wisconsin. Epidemiologic studies included collection of larvae of Aedes triseriatus and other mosquitoes from basal tree-holes, old automobile tires, an old boat, and other water holding containers near her home. Virus isolation and transmission studies are in process with Aedes triseriatus reared from these larvae.

A transmission and isolate of LAC virus had been previously obtained from adults reared from Aedes triseriatus larvae collected from basal tree-holes near La Crosse during the last week of June, 1978. LAC virus has been reappearing each season, overwintering by transovarial transmission in eggs deposited by infected females. Both male and female progeny of infected females are infected. Venereal transfer from infected male to female Aedes triseriatus has been demonstrated in laboratory studies. Horizontal transfer of LAC also occurs through blood meals on viremic mammals. Wisconsin and other portions of the upper-mid-west have had record amounts of rainfall during the spring and early summer of 1978.

A dozen other suspected cases of California encephalitis during July of 1978 were reported by physicians in the La Crosse area. Following the fatal case, health authorities and personnel from CDC collected histories and sera from suspected cases, some of which were diagnosed by the CEP test at La Crosse and the HI test at CDC Fort Collins. Further tests are underway in state puplic health laboratories of Wisconsin, Minnesota and Iowa. During previous years 80% of the cases in Wisconsin have had dates of onset after the end of July; during August, September and October.

(Wayne Thompson)

REPORT FROM THE DEPARTMENT OF VETERINARY SCIENCE
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Bluetongue Virus Typing by Mouse Passive Immunity Cross-Protection Test

Conflicting results and cross-reactivity have confused the antigenic classification of North American Bluetongue virus (BTV) isolates. Also the experimental animals, sheep and calves, have been expensive and difficult to house securely. We have developed a mouse passive immunity cross-protection test to distinguish between the four BTV serotypes currently recognized in the United States.

Groups of female white mice were immunized by i.p. inoculation of one of four mouse adapted BTV strains, each representing a different serotype. Immune mice were mated. Their offspring, and the offspring of unimmunized controls, were challenged by i.c. inoculation of either a homologous or a heterologous BTV isolate. Protective indices were calculated by subtracting the titer in the progeny from immunized dams from the titer in control offspring.

Table 1 summarizes the cross-protective indices, which clearly differentiate the four isolates. Table 2 summarizes plaque-reduction neutralization tests (PRNT) using hyperimmune mouse ascitic fluids and L cells. The PRNT also clearly differentiates the four isolates; results by this technique parallel those of the mouse passive immunity cross-protection test.

Modoc Virus Variant Isolates

Two virus strains were isolated and reisolated from blood clots of Peromyscus maniculatus collected in 1976 from northeastern Alberta. The two strains are identical by mouse neutralization test. Preliminary characterization of prototype strain PM00160 appeared in the most recent Arbovirus Information Exchange and showed it to be a member of arbovirus antigenic group B. Complement-fixation and plaque-reduction neutralization tests done at CDC-Fort Collins under the direction of C. H. Calisher have shown that strain PM00160 is very closely related to Modoc virus (Tables 3 and 4). However, PM00160 and Modoc antigens differed in the extent of their CF and neutralization reactions with other flavivirus antisera. The two P. maniculatus agents are thus classified as variants of Modoc virus.

Table 1 -- Log_{10} Protection Index* of Suckling Mice Immunized Against One of Four Bluetongue Strains

Immunizing Viruses	Challenge Viruses			
	BT8-166	Station-053	63-66B	71-1455WR
BT8-166	5,5†	0,1	0,8	0,5
Station-053	1	4,7	1,1	1
63-66B	0,8	0,1	5	0,8
71-1455WR	0,5	0	0	6,2

* Log_{10} Protection Index = Difference between log_{10} virus titer of the immunized group and log_{10} virus titer of unimmunized control group.

† Homologous Protection Index underlined.

Table 2 -- Result of Plaque Reduction Neutralization Test with Mouse Immune Ascitic Fluids to Bluetongue Virus Strains

Virus Strains	Mouse Immune Ascitic Fluids To Strains			
	BT8	Station	63-66B	71-145
BT8	500*	<10	<10	<10
Station	<10	340	<10	<10
63-66B	<10	<10	360	<10
71-1455WR	<10	<10	<10	860

* Homologous Titers Underlined.

Table 3 -- Complement-fixation Test Reactions of PM00160 and Modoc Viruses with Homologous and Heterologous Antisera

Antigen	Antibody	
	PM00160	Modoc
PM00160	256*	32
Modoc	256	64

*Inverse of serum dilution which fixed complement in standard test.

Table 4 -- Plaque-reduction Neutralization Test Reactions of PM00160 and Modoc Viruses with Homologous and Heterologous Antisera

Antigen	Antibody	
	PM00160	Modoc
PM00160	>640*	40
Modoc	>640	40

*Reciprocal of serum dilution which neutralized $\geq$ 90% of plaque-forming units in virus test dose.

F. D. Adu, R. L. Zarnke, T. M. Yuill

REPORT FROM THE VECTOR-BORNE DISEASES DIVISION

CENTER FOR DISEASE CONTROL

FORT COLLINS, COLORADO

Surveillance of St. Louis Encephalitis (SLE) by Serologic Testing of Wild Birds and Sentinel Flocks, U.S.A., 1976-1978

In 1975 the eastern U.S. suffered the largest outbreak of SLE on record, with 1,815 documented human cases. The following year a number of state and local health departments initiated new programs to monitor viral transmission, and several agencies expanded surveillance programs already in place. The emphasis was placed upon measurement of HI antibody prevalence in wild bird populations, principally house sparrows, as a means of detecting early viral activity. In most areas, the avian serology program was linked to an increased readiness for vector control, so that steps for intervention could be taken when the data suggested risk of viral "spillover" to man from the silent cycle. State and local health officials have cooperated and continue to cooperate with CDC by providing current data on avian infections, which are published in an informal, biweekly Encephalitis Surveillance report. Thus, the data from one state or region are made available to contiguous and remote regions; since SLE has historically affected more southerly areas in the Mississippi River Basin in advance of more northern localities, information shared through the surveillance bulletin has been a useful adjunct to local programs.

1976 was a year of high SLE viral activity; Mississippi and Texas sustained large outbreaks, and a number of other states reported moderate numbers of cases (table 1). The nationwide total reported cases in 1976 was 376. In 1977, fewer human cases were recognized in the east-central states, but SLE viral activity was nevertheless widely distributed. Florida sustained an epidemic (110 laboratory-documented cases), the first since 1962. In 1978, up to the time of this writing (August 22), no SLE cases have been diagnosed in the eastern U.S.

Avian serologic data are summarized in tables 1-3. The analyses, necessarily abbreviated for purposes of this report, underestimate the value of the data because they generally reflect infections over large areas, whereas viral activity has often been localized. For example, the data for Illinois are lumped, but human cases and avian immunity appears localized to certain southeastern counties.

In general, viral activity in 1976 and 1977 is reflected by presence of antibody in immature wild birds and by seroconversions in sentinel flocks. Antibody prevalences in juvenile wild birds captured in June are generally between 1 and 6% in areas subsequently reporting human cases. In areas which sustained epidemics in 1976 (Mississippi, Texas), a progression or accumulation of immunity was found in the wild bird

population, reaching high rates (7-8%) in August. Avian surveillance in Memphis, TN also indicated an impending human outbreak, which, however, never materialized (see Arbovirus Information Exchange No. 34, March 1977, pp. 147-152). In general, the monthly prevalence of immunity in avians in 1977, a year of reduced human infection, was lower than in 1976 (table 2). In Florida, however, where a large human outbreak occurred in October-November, sentinel flock seroconversions during the month preceding the epidemic reflected a high rate of viral transmission.

Data for the early and midsummer months of this year appear to indicate little risk of human epidemics (table 3). Seroconversions in sentinel chickens in Memphis during July stimulated intensive vector control efforts in the city. The situation in Florida is being watched closely; late August and September may represent the critical period for SLE viral amplification in that state.

Acknowledgments. The following individuals have kindly provided data for inclusion in Encephalitis Surveillance (edited by the Vector-Borne Diseases Division, Fort Collins) and in this summary: On human case incidence, K. Kappus, Viral Diseases Division, BE, CDC, Atlanta. On avian serology: Tennessee, I. K. Moseley, J. Hamm, J. Oates, J. Mullenix, and T. D. Gilbert; Indiana, M. J. Sinsko and P. R. Grimstad; Louisiana, G. Roy Hayes and H. B. Bradford; Texas, M. Jenevein, R. Bartnett, and L. Leffingwell; Mississippi, G. Crosby, D. Sykes, D. Blakey, E. Jumper, and J. McMillan; Kentucky, T. S. Wallace, Jr.; Florida, J. A. Tomas, F. M. Wellings, and N. Schneider; Ohio, T. Halpin, M. A. Parsons, and R. L. Berry; Michigan, A. A. Therrien; Illinois, G. Clark and H. Pretula; and Alabama, M. Maetz and P. Pate.

(T. P. Monath)

TABLE 1. Prevalence of SLE HI Antibodies in Juvenile Birds, 1976, by State and Month, and Correlation With Number of Confirmed and Presumptive Human Cases.

Locality	No. Birds with SLE HI Titer ≥20/No. Tested (%)								No. Human Cases (entire state)
	April	May	June	July	August	September	Oct	Total	
Mississippi	-	1/168(0.6)	5/508(1.0)	22/753(2.9)	47/615(7.6)	4/217(1.8)	-	79/2261(3.5)	79
Louisiana	-	0/87	6/365(1.6)	9/431(2.1)	8/503(1.6)	0/133(0)	-	23/1519(1.5)	11
Tennessee (Memphis) Wild birds	-	0/137	5/402(1.2)	15/428(3.5)	32/302(10.6)	9/102(8.8)	-	61/1371(4.5)	8
Sent. chickens	-	0/83	2/152(1.3)	4/190(2.1)	12/129(9.3)	19/132(14.4)	-	37/686(5.4)	
Illinois	-	0/30	3/84(3.5)	3/699(0.4)	10/617(1.6)	-	-	16/1430(1.1)	14
Ohio	-	-	0/108	1/428(0.2)	5/1113(0.5)	-	-	6/1649(0.4)	17
Michigan	-	-	-	0/387	1/1565(0.06)	1/279(0.4)	-	2/2231(.09)	0
Texas (Houston)	0/202	20/168(11.9)	6/259(2.3)	21/420(5.0)	20/280(7.1)	3/109(2.8)	-	70/1438(4.9)	96
TOTAL	0/202	21/673(3.1)	27/1878(1.4)	75/3736(2.0)	135/5124(2.6)	36/972(3.7)	-	294/12,585(2.3)	225

TABLE 2. Prevalence of SLE HI Antibodies in Juvenile Birds, 1977, by State and Month, and Correlation With Number of Confirmed and Presumptive Human Cases.

Locality	No. Birds with SLE HI Titer ≥20/No. Tested (%)								No. Human Cases (entire* state)
	April	May	June	July	August	September	October	Total	
Mississippi	-	5/162(3.0)	21/329(6.3)	11/400(2.8)	5/238(2.1)	-	-	42/1129(3.7)	5
Louisiana	0/25	0/55	0/343	0/427	1/318(0.3)	-	-	1/1168(0.1)	4
Tennessee (Memphis) Wild birds	0/40	0/211	15/312(4.8)	0/146	1/169(0.6)	-	-	16/878(1.8)	2
Sent. chickens		0/139	28/176(15.9)	0/88	0/77	-	-	28/480(5.8)	
Illinois	-	4/222(1.8)	7/713(1.0)	2/910(0.2)	-	-	-	13/1845(0.7)	12
Ohio	0/3	0/192	4/689(0.6)	2/1173(0.2)	0/21	-	-	6/2078(0.3)	4
Texas- Wild birds	-	0/396	0/486	0/17	-	-	-	0/899(0)	10
Sent. chickens	-	2/199(1.0)	3/371(0.8)	0/338	-	-	-	5/908(0.6)	
Indiana	-	-	1/59(1.7)	1/498(0.2)	4/161(2.5)	-	-	6/718(0.8)	8
Arkansas	-	-	0/58	0/161	0/3	-	-	0/222(0)	2
Alabama	-	-	1/544(0.2)	1/226(0.4)	-	-	-	2/770(0.3)	1
Florida Sent. chickens	-	-	-	-	0/60	10/62(16.1)	83/185(44.9)	93/247(37.7)	110
TOTAL	0/68	11/1576(0.7)	80/4080(2.0)	17/4384(0.4)	11/1047(1.1)	10/62(16.1)	83/185(44.9)	212/11,342(1.9)	158

* Preliminary data

TABLE #. Prevalence of SLE HI Antibodies in ~~Juvenile~~ wild Birds + sentinel chickens, 1978, by State and Month, and Correlation With Number of Confirmed and Presumptive Human Cases.

Locality	No. Birds with SLE HI Titer ≥20/No. Tested (%) by month							Total	No. Human Cases (entire* state)
	April	May	June	July	August	September	October		
Mississippi	-	0/180 ~~0/93~~	5/60 (8.3) ~~0/49~~	2/295 (0.7) ~~1/206(0.5)~~	3/168 (1.8)	0/50		10/753 (1.3) ~~1/348(0.3)~~	0
Louisiana wild birds	0/220 ~~0/53~~	~~[illegible]~~	5/641(0.8) ~~1/381(0.3)~~	1/655(0.2) ~~1/420(0.2)~~	10/832(1.2)	4/242(1.7)	29/3115 (0.9)	~~[illegible]~~ ~~2/1201(0.2)~~	0
Sent chickens	0/21	3/108( )	0/85	0/174	0/118			3/406(0.7)	
Tennessee (state)	~~5/308(1.6)~~	~~5/601( )~~	6/820( )	5/735(0.7)	719 1/[illegible](0.1)	8/473(1.7)	5/197 (2.5)	35/3853(0.9)	
Wild birds	~~0/28~~	~~0/317~~	~~0/201~~	~~0/111~~				~~0/737(0)~~	
Sent. chickens	0/12	0/73	2/168(1.2)	156 (5.1) ~~8/120(6.7)~~	10/152(6.6)	3/145(2.1)	1/34 (2.9)	24/740(3.2) ~~10/373(2.7)~~	2
Illinois	-	15/456(3.3) ~~0/92~~	18/991( ) ~~7/548(1.3)~~	8/1450(5.5) ~~6/870(0.7)~~	4/745(0.5)			45/3442(1.2) ~~13/1510(0.9)~~	0
Iowa			0/182	1/242 (0.5)	1/132 (0.8)	3/81 (3.7)		5/637 (0.8)	
Ohio	-	-	0/~~204~~ 431	~~1/600(0.1)~~ 1319	5/[illegible] (0.2) 2282	5/1354( )	0/118	~~1/1004(0.1)~~ 10/4185 (0.2)	4
Michigan	-	-	0/1042 ~~0/890~~	1/[illegible](0.1)				~~1/1902(0.05)~~ 1/2361 (0.04)	0
Texas (Houston)	-	11/252( ) ~~1/193(0.5)~~	6/749( ) ~~3/583(0.5)~~	2/519(0.4) ~~1/398(0.3)~~	1/482(0.2)	1/210 (0.5)		~~5/1174(0.09)~~ 21/2212(0.9)	0
Indiana	2/125( ) ~~0/7~~	11/678( ) ~~1/50(2)~~	~~1/707(0.1)~~ 6/1279( )	8/[illegible] ~~2/[illegible]~~	8/873(0.9)			~~1/1301(0.1)~~ 27/3055 (0.9)	1
Florida		0/592	0/1855	0/915	0/1148			0/5571 (0)	
Sent. chickens	0/415	~~0/701~~	~~0/621~~	~~0/1024~~	~~0/233~~	0/965	0/481	~~0/2479(0)~~	0
~~Kentucky~~									
TOTAL	~~0/538~~ ~~0/93~~ 7/1201(0.6)	~~5/1946(0.1)~~ ~~2/1946(0.1)~~ 54/3465(0.2)	~~25/5723(0.4)~~ ~~14/4372(0.3)~~ 48/7072(0.7)	~~36/7237(0.5)~~ ~~21/5468(0.4)~~ ~~36/9515~~ 41/9515(0.4)	35/5850(0.6) ~~0/233~~	19/2284(0.8)	6/712(0.8)	210/30,530 ~~37/12,109(0.3)~~ (0.7)	7

~~* Through August 26, 1978~~

*Preliminary data (courtesy Dr. K. Kappus, BE, CDC, Atlanta)

DELAYED INFLAMMATORY RESPONSES IN MICE IMMUNIZED WITH ST. LOUIS ENCEPHALITIS VIRUS.

The development of central nervous systems lesions after St. Louis encephalitis virus (SLE) infection has long been associated with cellular infiltrates and inflammatory responses.

It seems likely, therefore, that in vivo studies of the delayed inflammatory response and the associated cellular infiltrates might be useful in evaluating the immune response to flavivirus infection. As a portion of our studies of the development of immunity to SLE infection, we have investigated the use of labeled monocyte infiltration techniques for measurement of the peripheral delayed inflammatory response in mice immunized with living SLE virus.

In order to do this, immunized and control mice were inoculated IP with 20 μCi of ^{3}H-thymidine. Twenty-four hours later each mouse received an inoculation of 0.05 ml of undiluted 10% SLE-infected mouse brain suspension into the left footpad. Each mouse also received an exactly equivalent dosage of a NSMB suspension into the right footpad. One day after the footpad challenge, the feet were removed at the first joint, placed in 1 ml of NCS Tissue solubilizer and incubated for 8-16 hours at 50°C. The next day 10 ml of scintillant was added and the samples were held in the dark an additional 48 hours for equilibration. After scintillation counting, data uncorrected for quenching were used for calculating footpad cellular infiltration ratios (CIR), the CIR being equal to the ratio of CPM measured in the left and right footpads.

Mice were immunized by intraperitoneal inoculation with 6.5×10^{5} PFU of live SLE virus strain BeH 203235, (suckling mouse brain) in 0.02 ml of diluent. Control mice received equivalent inoculations of normal suckling mouse brain suspensions in diluent. The level of the delayed 24-hour inflammatory response was measured at intervals for 10 days after immunization.

Peripheral DTH responses in mice can be potentiated by live BCG administration, by splenectomy or by cyclophosphamide administered 1-2 days before immunization. Selective suppression of T-cell responses can be induced by administering phytohemagglutinin (PHA) followed by low doses of cyclophosphamide. Therefore, the experiment was also designed to evaluate the effects of various immunoregulatory treatments on the development of 24-hour cellular infiltration responses.

The results are shown in Figure 1, which presents the development of the footpad cellular infiltration response from day 3 to day 10 after immunization.

Figure 1B shows the development of the delayed cellular infiltration response in normal and sham splenectomized mice. The response begins by day 4 after immunization, reaches maximum values by day 5, and declines rapidly to negligible levels by day 10.

The effects of the various immunoregulatory agents are shown in Figure 1A. Splenectomy caused a marked enhancement of the response on day 5-6 after immunization. Cyclophosphamide given 2 days before immunization, BCG pretreatment, and splenectomy prolonged the response. In all three cases, greater than normal 24 hr cellular infiltrates were detected 10 days after immunization. The combined PHA-Cyclophosphamide treatment apparently eliminated the normal response.

The data presented in Figure 1 suggest that the 24-hour cellular infiltration may be T-cell mediated. Thus the treatments known to potentiate DTH also potentiate or prolong the 24-hour cellular infiltration responses, and the treatment known to suppress T-cell responses causes virtual elimination of 24-hour cellular infiltration.

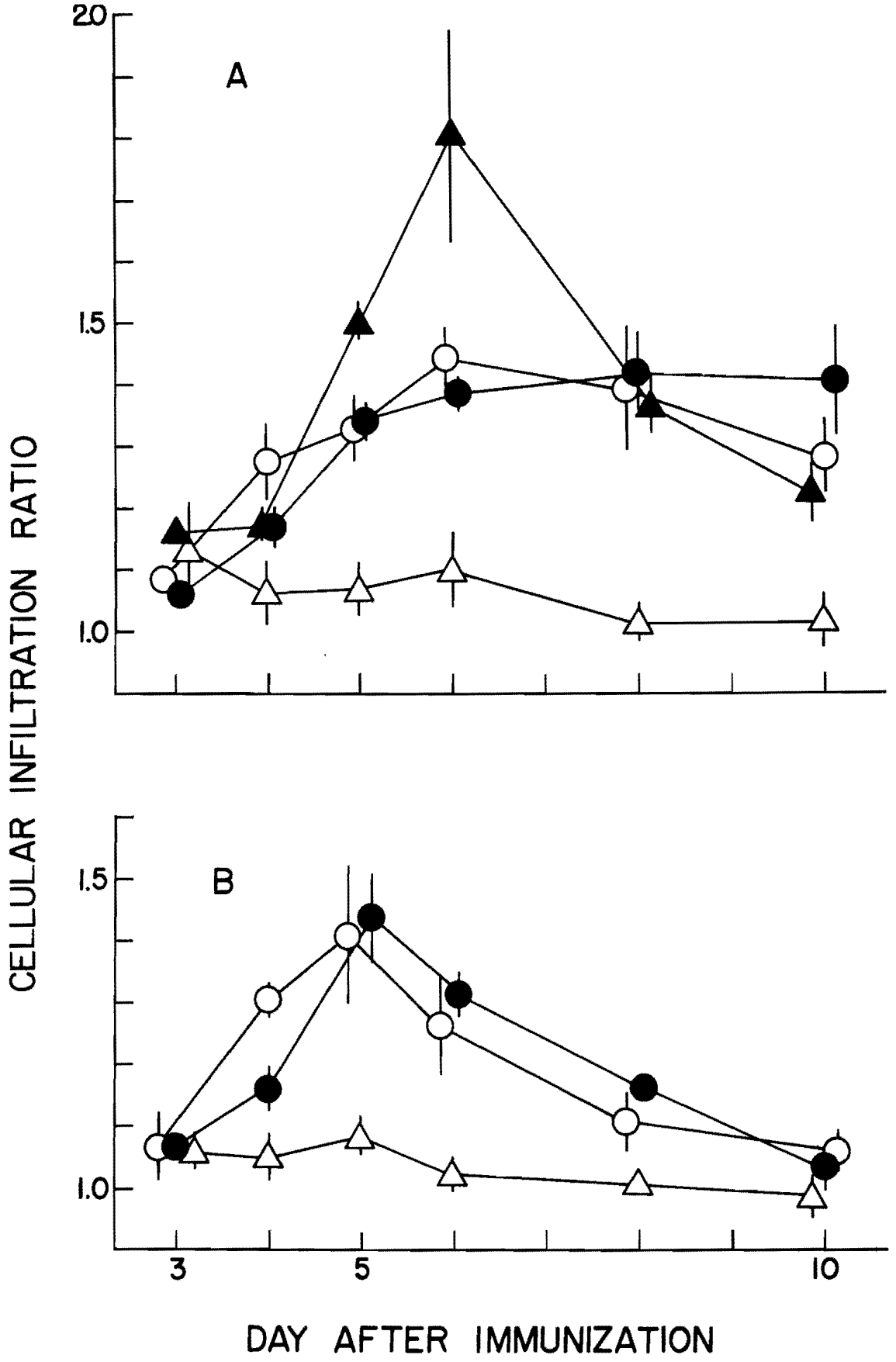
The T-cell mediated response, assessed by cytotoxicity assays or by splenocyte migration indices, has been shown to play both protective and immunopathologic roles in mice infected with a number of related flaviviruses. Differentiation of the relative contributions of cell mediated and humoral factors to immunity has been difficult, however, since the assessment of protection has usually depended on intracerebral inoculation of the virus into immunized animals which exhibit both cellular and humoral immunity. Since peripheral delayed inflammatory responses can be easily manipulated by established immunoregulatory techniques, it should be possible to assess the differential role of humoral and cell-mediated immunity in the development of resistance to SLE infection.

(B.W. Hudson)

Figure 1. The development of 24-hour footpad responses during the 10 days following intravenous immunization of mice with 6.5 X 10^5 plaque forming units of St. Louis encephalitis virus. The effect of immunoregulatory treatments on 24-hour labeled cell infiltration ratios. Mean values ± 1 Standard Error.

A. Treated mice immunized with St. Louis encephalitis virus. Solid triangles, splenectomized mice. Solid circles, cyclophosphamide (200 mg/kg) 2 days before immunization. Open circles, 10^7 live BCG 18 days before immunization. Open triangles, phytohemagglutinin (Ca 15 mg/kg) 10 days and cyclophosphamide (40 mg/kg) 5 days before immunization.

B. Controls. Closed circles, normal mice immunized with St. Louis encephalitis virus. Open circles, sham splenectomized mice immunized with St. Louis encephalitis virus. Open triangles, pooled data from mice immunized with normal suckling mouse brain.


2.0
1.5
1.0
A
B
1.5
1.0
3
5
10
CELLULAR INFILTRATION RATIO
DAY AFTER IMMUNIZATION

Antigenic Relationships Among Simbu Group Viruses

As a portion of our continuing study of the antigenic relationships of the Bunyaviridae we have recently begun a comparison of Simbu serogroup viruses. The 16 simbuviruses registered in the International Catalogue of Arboviruses as well as three unregistered viruses were tested by cross box complement-fixation (CF) and serum dilution plaque reduction neutralization (N) tests in serially propagated Vero cells. The results are summarized in Tables 1 and 2. Although the N test results are preliminary because not all viruses have been tested, a number of observations may be made. By CF six African (Simbu, Sabo, Sango, Shuni, (YABA-7) and Shamonda), one from India and Africa (Sathuperi), one from India (Kaikalur), and two from Australia and Asia (Akabane and Aino) viruses form a complex; however, subsequent N testing showed that individual members of the complex generally are separable with little difficulty. Shuni, Kaikalur and Aino appear to be closely related to each other with Kaikalur and Aino distinguishable one-way only; the latter two may be varieties of Aino virus.

The second complex identified by CF test includes one each from Asia and Africa (Ingwavuma), South America (Manzanilla) and North America (Mermet). From N test results it may be seen that, although distinguishable, these viruses are more closely related to each other than to any other viruses of the serogroup.

Oropouche virus, which has been isolated from mosquitoes, man and a sloth in Brazil, is related to the as yet unregistered Utinga and Bradypus viruses which were isolated from sloths in Brazil and Panama, respectively. N tests with these viruses have not yet been completed.

Finally, Buttonwillow, Thimiri and Nola appear by both CF and N tests to be distantly related to other members of the group with, perhaps, Buttonwillow somewhat more reactive with other New World viruses of the Simbu group.

These results and knowledge of the natural history of the Simbu viruses, including vector-host relationships, vertebrate susceptibility, and geographic distribution imply that the serogroup may occur in avian-mosquito or mammalian (ungulates, primates, lagomorphs) - Culicoides cycles in nature. The wide distribution of these viruses may indicate intercontinental movement by infected birds.

R. M. Kinney and C. H. Calisher

TABLE 1. CROSS-REACTIVITIES OF SIMBU GROUP VIRUSES BY COMPLEMENT-FIXATION TESTS

ANTIGEN	Reciprocal Antibody Titer to: SIM	SABO	SAN	SHU	SAT	YABA-7	KAI	SHA	AINO	AKA	ING	MAN	MER	ORO	(UTIN)	(BRAD)	BUT	THI	NOLA
SIMBU	≥1024	≥1024	64	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	8	-	8	16	32	16	16	8	-
SABO	128	≥1024	128	512	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	8	-	8	8	32	32	-	8	-
SANGO	128	≥1024	≥1024	≥1024	≥1024	512	≥1024	256	≥1024	≥1024	8	-	8	8	16	-	-	8	-
SHUNI	128	512	≥1024	≥1024	≥1024	256	≥1024	256	≥1024	≥1024	8	-	8	8	8	-	-	8	-
SATHUPERI	128	≥1024	64	512	≥1024	256	512	≥1024	128	≥1024	-	-	8	8	16	16	8	8	8
YABA-7	256	≥1024	64	512	≥1024	≥1024	≥1024	256	≥1024	≥1024	-	8	16	16	16	16	-	8	8
KAIYALUR	128	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	128	≥1024	≥1024	8	-	8	8	8	-	-	8	-
SHAMONDA	256	≥1024	64	≥1024	≥1024	≥1024	512	≥1024	≥1024	≥1024	-	-	8	8	16	16	-	-	-
AINO	128	512	≥1024	≥1024	≥1024	256	≥1024	≥1024	≥1024	≥1024	-	-	8	8	8	-	-	8	-
AKABANE	512	≥1024	256	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	-	8	8	8	8	8	-	8	-
INGWAVUMA	-	8	-	8	8	8	8	-	8	8	≥1024	512	≥1024	16	8	8	8	8	-
MANZANILLA	-	8	-	8	8	8	8	-	8	16	≥1024	≥1024	512	16	8	8	8	16	-
MERMET	-	16	8	16	8	16	8	8	-	32	≥1024	≥1024	≥1024	32	16	16	8	16	8
OROPOUCHE	32	32	8	32	≥1024	32	32	≥1024	32	256	32	16	64	≥1024	512	256	16	8	-
(UTINGA)	32	64	8	16	32	32	64	8	32	8	16	8	32	128	≥1024	≥1024	32	8	-
(BRADYPUS)	16	32	8	8	16	32	32	8	16	8	8	8	16	64	≥1024	≥1024	32	8	-
BUTTONWILLOW	8	8	-	8	16	8	32	8	8	8	16	16	16	16	16	32	≥1024	8	-
THIMIRI	8	16	-	8	16	8	32	8	8	16	8	-	8	16	8	-	8	≥1024	-
NOLA	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	≥1024

- = <8

TABLE 2. CROSS REACTIVITIES OF SIMBU GROUP VIRUSES BY NEUTRALIZATION IN VERO CELLS

VIRUS	Reciprocal Antibody Titer to: SIM	SABO	SAN	SHU	SAT	(YABA)	KAI	SHA	AINO	AKA	ING	MAN	MER	ORO	BUT	THI	NOLA
SIMBU	1280	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SABO	-	2560	-	-	-	-	-	10	-	10	-	-	-	-	-	-	-
SANGO	-	-	2560	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SHUNI	-	-	-	20480	160	-	1280	-	1280	-	-	-	-	-	-	-	-
SATHUPERI	-	-	-	10	5120	-	-	-	-	-	-	-	-	-	-	-	-
(YABA-7)	-	-	-	-	-	5120	-	10	-	40	-	-	-	-	-	-	-
KAIKALUR	-	-	-	160	-	-	2560	-	1280	-	-	-	-	-	-	-	-
SHAMONDA	-	-	-	-	-	-	-	640	-	20	-	-	-	-	-	-	-
AINO	-	-	-	320	-	-	2560	-	5120	-	-	-	-	-	-	-	-
INGWAVUMA	-	-	-	-	-	-	-	-	-	-	320	40	40	-	80	-	-
MANZANILLA	-	-	-	-	-	-	-	-	-	-	-	640	40	-	80	-	-
MERMET	-	-	-	-	-	-	-	-	-	-	40	160	320	-	80	-	-
OROPOUCHE	-	-	-	-	-	-	-	-	-	-	-	-	-	2560	-	-	-
(UTINGA)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(BRADYPUS)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BUTTONWILLOW	-	-	-	-	-	-	-	-	-	-	-	40	10	-	2560	-	-
THIMIRI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	320	-
NOLA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	640

- = <10

Colonization of the Swallow Bug, *Oeciacus vicarius*

It was shown in 1976 that a newly described alpha togavirus, Fort Morgan, can be maintained and transmitted by the Swallow Bug. To facilitate studies of vector characteristics, attempts were initiated at that time to colonize this species, and within about a year, a self-perpetuating colony had been established. This was accomplished by using suckling mice as sources of blood, and allowing bugs continuous access to hosts.

It was recognized that the methodology had played an important role, but it was also believed that genetic selection had contributed to the success of the enterprise. It was felt that if behavioral or reproductive differences could be identified between the wild and colony stock, that this would indicate that selection had in fact occurred. Accordingly, a number of comparisons were made.

Adult bugs were collected in the field and held in the laboratory under colony conditions. Progeny of these specimens were reared, and as new adults appeared they were matched, specimen for specimen, with colony stock. This matching is indicated in the upper portion of Table 1, where it may be seen, for example, that 27 male and 19 female newly emerged adults were isolated from each of the 2 stocks on April 10. The 2 experimental groups, each ultimately consisting of 85 males and 75 females, were placed in separate 500 ml wide-mouth Ehrlenmeyer flasks, and records were kept of oviposition and mortality. Mortality records are shown in Table 1. It is quickly apparent that mortality occurred earlier, particularly among females, in the colony than in the wild stock. The last female of the colony died during the period of June 26-July 3; the corresponding period for wild stock was July 31-August 7.

A second comparison was of egg production. The very different patterns of the 2 groups appear in Table 2. The colony group oviposited earlier, with greater total and *per capita* production, and, as indicated above, died sooner.

It was serendipitously possible to make a third comparison. Individually distinctive patterns of excrement were deposited in the flasks in which the 2 matched experimental groups were held. In the flask of wild stock, fecal deposits were concentrated in a small roughly-circular area at the center of the floor, while with the colony they were more generally distributed. The difference was very marked, as may be seen in Fig. 1.

The above findings indicate that genetic selection occurred during the colonization process and/or during subsequent rearing. That colonization can be achieved through suitable methodology alone is shown by the fact that a new colony was readily established from the material in the experimental group (wild) used in this study.

W. A. Rush

Table 1. Accumulation of newly molted adult O. vicarius experimental specimens, and mortality at the end of indicated time periods, 1978.

Weekly time period	COLONY STOCK				FIELD STOCK			
	Cumulative specimens introduced		Cumulative mortality[c]		Cumulative specimens introduced		Cumulative mortality[c]	
	♂	♀	♂	♀	♂	♀	♂	♀
3/26-4/3	1	1			1	1		
4/3-4/10	28	20			28	20		
4/10-4/17	52	38			52	38		
4/17-4/24	71	58			71	58		
4/24-5/1	79	70			79	70	1[b]	
5/1-5/8	85[a]	75[a]			85[a]	75[a]	1	
5/8-5/15			1[b]	1[b]			5	
5/15-5/22			1	3			5	
5/22-5/29			2	6			7	1[b]
5/29-6/5			3	17			7	6
6/5-6/12			6	42			7	9
6/12-6/19			13	57			8	11
6/19-6/26			32	72			9	14
6/26-7/3			49	75			12	25
7/3-7/10			65				21	49
7/10-7/17			70				36	62
7/17-7/24			73				50	65
7/24-7/31			77				66	69
7/31-8/7			82				75	75
8/7-8/14			85				81	
8/14-8/21							83	

[a]Final introduction of bugs.

[b]Earliest mortality.

[c]Number of specimens.

Table 2. Oviposition by matched groups of colony and field stock of O. vicarius, during indicated time periods, 1978.

Weekly time period	COLONY STOCK			FIELD STOCK		
	No. eggs produced	No. surviving females	Eggs per surviving female[a]	No. eggs produced	No. surviving females	Eggs per surviving female[a]
4/10-4/17	46[b]	20	2.3	0	20	0
4/17-4/24	159	38	4.2	9	38	0.2
4/24-5/1	385	38	6.6	49	58	0.8
5/1-5/8[c]	737	70	10.5	76	70	1.1
5/8-5/15	836	74	11.3	128	75	1.7
5/15-5/22	628	72	8.7	163	75	2.2
5/22-5/29	762	69	11.0	116	74	1.6
5/29-6/5	421	58	7.3	493	69	7.1
6/5-6/12	562	33	17.0	909	66	13.8
6/12-6/19	259	18	14.4	999	64	15.6
6/19-6/26	31	3	10.3	627	61	10.3
6/26-7/3		0		287	50	12.5
7/3-7/10				291	26	11.2
7/10-7/17				79	13	6.1
7/17-7/24				4	10	0.4
7/24-7/31				1	4	0.3
7/31-8/7					0	
TOTAL	4826			4231		
AVERAGE			9.4			5.5

[a]Calculated as the number of specimens present at end of time period, before introduction of new specimens.

[b]Earliest oviposition.

[c]5/8 is the date of last introduction of new specimens.

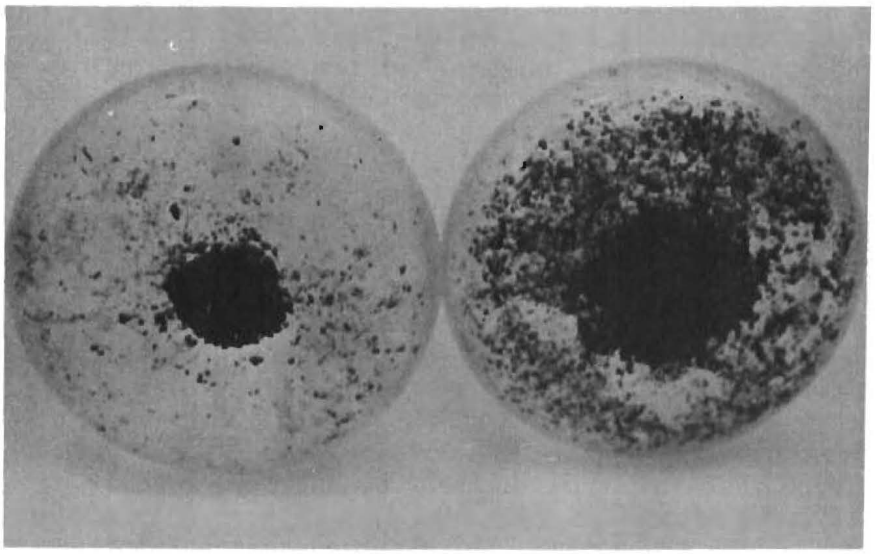


Fig. 1. Patterns of excrement on the bottom of flasks used to house experimental Oeciacus vicarius. Left: Contained 1st generation progeny of wild stock. Right: From a matched group of colony stock.

Seasonal Variation in Susceptibility of Swallow Bugs to Fort Morgan Virus, Related to Reproduction of Bugs

During initial studies of vector characteristics of Swallow Bugs with Fort Morgan virus, it was necessary to use field-collected bugs for experiments. Data from earlier experiments suggested that bugs collected in spring and early summer developed higher infection rates, and transmitted more readily by bite, than those collected later. A study was designed to explore this possibility.

Bugs were collected at approximately monthly intervals, allowed to feed on infected nestling sparrows, held for incubation, and tested in primary duck embryo cell culture for presence of virus in salivary glands and in the remainder of the body. A 300-bug sample was taken from each collection and tested for pre-existing infection. Periodic collections were also made of 100 male and 100 female bugs for oviposition studies. These specimens were held in the laboratory for 14 days with access to suckling mice. Records were kept of numbers of eggs produced.

Results are given in Table 1. Pre-existing infection was minimal (never >0.7%) and insufficient to influence the other data. Infection rates of whole bugs and whole bugs minus salivary glands were significantly higher (X^2 probability = <0.005) early in the season (40 to 93% in April and May) than later (5 to 18% in July and August). Virus could also be recovered from salivary glands more readily during April and May (8 recoveries from 40 bugs) than in July and August (2 recoveries from 120 bugs). The observed seasonal pattern appears to be independent of the titer of infective blood. Evidence for this may be found in the table, which shows a number of instances in which low late-season rates were obtained with relatively high infective titers, and the reverse.

There is a conspicuous reproduction pattern. The 14-day egg production by 100 females increased to a high of 971 in the group collected on May 11, and declined thereafter. The group collected June 28 deposited 158 eggs, and those collected in July and August, none. Virus susceptibility corresponded rather closely, then, to fecundity.

It is not clear what factors are responsible for these observations. The virus susceptibility pattern could be related to age of bug population (consisting early in the season of overwintered individuals), environmental conditions, or an inherent cycle possibly under hormonal influence. The oviposition pattern could be influenced by these same factors, plus greater access to hosts when nestling birds are present in the spring. Although heightened virus susceptibility and maximum reproduction occur concurrently, it is problematic at this point whether there is any other relationship between the two.

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Table 1. Seasonal Experimental Infection Rates, Egg Production, and Naturally-occurring Infection in Oeciacus vicarius

		SEASONAL EXPERIMENTAL INFECTION RATES Virus Recovery, no. pos/no. tested					SEASONAL EGG PRODUCTION		NATURALLY-OCCURRING INFECTION
		Lot 1		Lot 2					
Coll. date of bugs	Days inc. at 22C	Sal. glands[a]	Bugs less sal. glands	Intact bugs	Combined bugs[b]	Titer of infected blood[c]	Coll. date of bugs	14 day egg production by 100 ♀♀ + 100 ♂♂	No. pools pos./ No. tested[d]
3/8/77							3/8	54	0/12
4/8							4/8	288	0/12
4/27	20,21	4/20	14/20	12/20	26/40-65%	6.8	4/27		2/12 (0.7%)
4/27	20			8/20	8/20-40%	5.7			
5/11							5/11	971	2/12 (0.7%)
5/24	20,21	4/20	20/21	18/20	38/41-93%	6.2			
5/24	20			13/20	13/20-65%	4.1			
6/6							6/6	466	0/12
6/28		e	e	e	e		6/28	158	1/12 (0.3%)
7/18	20	0/20	1/20	1/20	2/40- 5%	4.8			
7/18	21	0/20	0/20	1/20	1/40- 2%	4.9			
7/18	20	0/20	5/20	4/30	9/50-18%	5.7			
7/18	22	0/20	4/20	4/30	8/50-16%	5.8			
7/29							7/29	0	
8/2	20	2/20	3/20	3/30	6/50-12%	7.5	8/2		0/12
8/2	21	0/20	3/20	3/30	6/50-12%	7.1			
8/2							8/2	0	
8/26							8/26		2/12 (0.7%)

[a] Salivary glands were dissected and tested separately in the bugs in Lot 1.

[b] Includes the total of bugs less salivary glands from Lot 1 plus the intact bugs from Lot 2.

[c] Log_{10} pfu/0.1 ml, Vero cc.

[d] 25 adult bugs per pool. Parentheses indicate minimum infection rates.

[e] No data available as donor nestling sparrow had concurrent naturally occurring WEE viremia.

REPORT OF THE ARTHROPOD-BORNE VIRUS RESEARCH UNIT,
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This report is the "Summary Statement" from a detailed research progress report that reviews field and laboratory studies on arboviruses and their vectors in California during the period May 1, 1977 through April 30, 1978. Extensive mailings have been made of the complete report. Copies are available upon request.

We have continued to monitor viral activity, vector populations and associated environmental factors in representative areas of the Central Valley of California. In Kern County, there was no evidence that WEE or SLE viruses were transmitted to any of 190 chickens that were distributed in 10 sentinel flocks. Turlock (TUR) viral transmission occurred in 7 flocks and 27 percent of birds were infected. Temperatures were above normal and rainfall was well below normal in this region. The seasonal average light trap index for female Culex tarsalis remained low through the season at almost all of 28 monitoring sites, including the sentinel flock locations. The levels of viral activity were consistent with earlier observations that TUR viral cycles are maintained by very low C. tarsalis populations and at levels where WEE and SLE cycles will be interrupted. No human or equine cases of WEE or SLE were detected in Kern County in 1977.

The parallel monitoring of viral activity in the Sacramento Valley in Butte, Glenn, Sutter, Yuba and Placer Counties revealed very similar findings to those in the San Joaquin Valley. There was no evidence of WEE or SLE viral infection in 254 chickens exposed in 12 sentinel flocks, and no cases of WEE or SLE were detected in human or equines. TUR virus was actively transmitted in 11 of 12 sentinel flocks, 32 percent positive. Both temperature and rainfall were below normal. The levels of C. tarsalis populations were considerably higher than in Kern County, even above the levels we believe could support transmission of WEE and SLE viruses if they were present or were introduced, but there was no resurgence of infection in 1977.

We began an intensive study of the genetics of C. tarsalis 4 years ago. We have isolated and characterized 15 spontaneous or induced mutations, have combined the mutations into 8 multiple marker lines, developed a new system for rapid identification of homozygote translocations and developed translocated homozygote and heterozygote lines for field trials. We wish to determine if translocated lines can be inserted into and modify natural populations. As a complimentary approach for genetic control, we have established a schedule for induction of sterility in C. tarsalis males by irradiation. Sterility curves have been established using doses of 5, 7, 9, and 11 krads. We will proceed with tests of mating frequency, competitiveness and life span of irradiated males. Progress has been made in developing techniques for preparation of polytene chromosomes from C. tarsalis. We will begin mapping of the polytene chromosomal patterns in the near future and then proceed to identify the site of chromosomal breaks in our induced translocation stocks.

Competitive mating trials between genetically modified and unmodified stocks are an important intermediate step between development of such stocks and field trials to determine their effectiveness as control agents. These competitive tests are done in relatively small cages in the laboratory and then in 4 large cages constructed on quonset hut frames in an outdoor environment. Tests to standardize data have revealed a difference in oviposition rates per female among the 4 outdoor cages. This variable must be taken into consideration in future tests. Populations derived from laboratory adapted colonies had higher

insemination rates than field populations in cage tests and field populations had lower oviposition rates than colony populations in small cage tests. Mating trials were done in which males from our car and ble mutant marker stocks and field collected males competed for mating with field collected females. The mutants were highly competitive. Males from a potentially lethal mutant, fringe wing (fr), were competitive in tests against males from our Knights Landing colony. Males from the heterozygous translocation T(1;2;3)1A were tested for competitiveness against wild males for mating with wild females. The results were disappointing as they indicated the translocated males might not be effective for genetic control. This was a reversal from a 1 to 1 or better competitive state in similar tests in 1976. The only known change was an additional year of maintenance as a colony. Finally, male hybrids from a cross between the lethal mutant fr and the T(1;2;3)1A translocation were tested in the quonset cages to determine if they would represent a "genetic time bomb". The translocation carried the expected reduction in fertility and there was an additive lethal effect as 33 percent of females were fr and 41 percent of these drowned during emergence. An added advantage of a mutant of this type for introduction into the field is that it would reduce female viability in subsequent generations. Studies have now been initiated to determine if females discriminate between males of different phenotypes and to develop more effective experimental designs for the quonset hut cage studies.

In the spring and summer of 1977, a major project was begun for the first mass release of a sex-linked double heterozygous translocated population of male C. tarsalis into the natural population at the Poso West field site. Previous reports provided a detailed description of this isolated field site, the establishment of population base-line data over a 2-year period for the area, and related activities. The objectives of the 1977 field tests were to ascertain if the translocation stock could be: mass-produced, inserted into the wild population, mate competitively, survive and be recovered after more than one generation.

The translocated stock was mass produced. Twelve production lines were initiated in January and pupae were transferred to field emergence ponds beginning in April 1977. An estimated total of 76,313 translocated males and 10,880 untranslocated females were released. This was fewer males than had been scheduled to be necessary in the computer model for a successful insertion. A portion of the translocated males, 11,583, were marked with fluorescent dust. Recoveries led to an estimated 72 percent mean daily survival rate. Mark-release-recapture studies through the summer established estimates of population size. A total of 31,853 C. tarsalis were marked and released; 154,692 mosquitoes were collected and examined for recovery; and 90 marked males and 1,121 marked females were recovered. Population estimates in 1977 surpassed those of either of the prior 2 summers and daily survival rates for females ranged from 54 to 67 percent in 1977 which was lower than in 1976. The release of translocated males did not affect the density of the native population and we did not recover the translocation from over 5,000 egg rafts derived from field collections. Studies of comparative survival of immature stages of the translocated and native stock indicated equal survival and developmental times. We concluded that the ratio of translocated males to the native population, which was less than 1:1, was inadequate for a successful insertion. We must develop improved techniques for a sexing procedure that will exclude females from future releases. We appear to have accomplished both of these objectives in a current field trial in 1978.

Aedes sierrensis, the Western tree-hole mosquito, is an important pest and potential vector of arboviruses and is difficult to control by conventional methods. The release of sterile males could be useful as a part of an urban-suburban control program. We have determined that irradiation with 7 krad under air will produce a sterile male that is competitive in mating trials against unirradiated males. Outdoor cage tests are scheduled for 1978 with reference to mating competitiveness and percent decrease in embryonation of eggs in females mated with irradiated males.

Some 4 years ago, we initiated studies to determine what structured and physiochemical factors control the competence of C. tarsalis and other mosquitoes to serve as efficient vectors of WEE, SLE, TUR and California encephalitis (CE) viruses. We detected major differences in the vector competence of field populations for WEE virus and proceeded to select subpopulations of C. tarsalis that were susceptible or resistant to WEE viral infection. Resistance was shown to be a polyfactorial recessive trait. The quantity of WEE virus that was ingested had a major influence on the proportion of a C. tarsalis population that could subsequently transmit infection. Resistance to organophosphorous insecticides, a genetic trait, could not be related to viral susceptibility of field collected or colonized C. tarsalis. Studies were initiated by electron microscopy on the basic ultrastructure of the mesenteron of C. tarsalis and on the nonspecific esterases and phosphatases of "normal" C. tarsalis. These studies have now been extended with the following findings.

There is a need for more sensitive and rapid systems to detect arboviral infections in individual mosquitoes. A specific and sensitive immunofluorescent antibody test has been developed for detection of SLE viral antigens in infected mosquito tissues. A similar test developed for WEE virus was specific but lacked some sensitivity possibly due to the diffuseness of viral antigens in infected cells.

It is difficult to calibrate and cross-interpret data from experiments where vectors are infected by inoculation, by feeding on viral suspensions or by feeding on viremic hosts. We have found that the level of sucrose in blood-virus suspensions that stimulated mosquito feeding without diversion of the meal into the ventral diverticulum was 1.25 - 2.5 percent (w/v) for C. tarsalis. Addition of adenine or cytidine nucleotides to blood-virus mixtures in place of sucrose did not stimulate C. tarsalis to take a meal.

Preliminary attempts to demonstrate multiplication of WEE virus in mosquito mesenterons maintained in vitro were relatively unsuccessful. It was hoped this approach could be used to study differences in receptor sites for viral adsorption between competent and incompetent vector populations.

We were disappointed to find that the droplet feeding technique failed to detect 18 to 20 percent of the female C. tarsalis capable of transmitting WEE virus to chicks after an extrinsic incubation period of 11 to 14 days.

It is possible that the inability of some infected female C. tarsalis to transmit WEE virus might be related to the ability of mosquitoes to modulate viral multiplication, possibly by genetically controlled mechanisms, if the concentration of ingested virus is not overwhelming. This possibility was suggested when we measured viral titers in F_1 females derived from reciprocal

matings between WS and WR parents. WEE virus multipled in mesenterons of a genetically selected WEE viral susceptible (WS) strain of C. tarsalis following either ingestion or intrathoracic inoculation of virus. In contrast, limited multiplication occurred in the mesenterons of a few WEE viral refractory (WR) females after virus was ingested but not after virus was inoculated intrathoracically. We have now identified strains of C. tarsalis that are good or bad transmittors of infection once they are infected. Our next objective is to extend the studies of mesenteronal barriers and to determine if there are also barriers to salivary gland infection.

The studies on vector competence for WEE virus have been extended into parallel studies with SLE virus. Female mosquitoes from 13 colonies, including 4 species, were evaluated for their ability to become infected after ingestion of SLE virus from pledgets and for their ability to transmit virus. Culex peus was found to be 100 to more than 1000 times more susceptible to infection than were Aedes dorsalis, Culex pipiens and 10 colonies of C. tarsalis. Infected females from the Ae. dorsalis colony and 1 colony of C. tarsalis, BFS-Winnipeg, failed to transmit virus after 14 days extrinsic incubation whereas 27 to 100 percent of the infected females from the C. peus, C. pipiens and other 9 C. tarsalis colonies were capable of transmitting virus. Mean viral titers in incompetent and competent viral transmitters were nearly identical. A mosquito species or strain that was competent to vector SLE virus was not necessarily competent to vector WEE virus.

Progress continued on the establishment of baseline physiological and physical factors that are genetically controlled and may effect vector competence. Optimal conditions were delineated for measuring nonspecific esterases and phosphatases in mosquitoes by electrophoretic and/or spectrophotometric techniques. Eighteen nonspecific esterases were revealed in whole body extracts of C. tarsalis by isoelectric focusing whereas only 5 distinct nonspecific esterases were observed by conventional electrophoretical methods. Whole body extracts of WEE resistant females contained 4 nonspecific esterases not found in WEE susceptible females.

Light and electron microscopic studies revealed that formation of the peritrophic membrane (PM) in adult female C. tarsalis might not begin until about 12 hours after ingestion of the bloodmeal. This suggests that the PM is not a critical factor in the mesenteronal barrier to infection of this mosquito species with arboviruses since viral adsorption to epithelial cells should occur prior to this time. Electron opaque material was observed between the mesenteronal epithelial cells of C. tarsalis 1 week after taking a bloodmeal. This suggests that viruses might gain access to the basal lamina of the mesenteron by an intercellular route, possibly allowing virus to enter the hemocoel without initial multiplication in the mesenteron.

Field observations have indicated there is an increase in susceptibility of C. tarsalis to WEE viral infection as the summer progresses and this correlates with temperature increases. We reared and maintained 3 C. tarsalis subcolonies at 18, 26, or 32C and 1 subcolony was reared as immatures at 32C and maintained as adults at 26C. At intervals of 2, 5, and 8 months after the colonies were initiated, adult females from all the lines were fed simultaneously on the same WEE viremic chicks. Rearing at a constant temperature of 18 or 26C did not

select for susceptible or refractory mosquitoes, the 32-26C line had developed some resistance by 5 months. Beginning at the 2 months interval the 32C line was resistant to viral infection and remained in that condition. These observations do not explain the increase in susceptibility of field populations as the season progresses, but have led us into further studies of the utilization of temperature as a selective factor in other vector competence and genetic studies.

Studies have been extended on the transovarial transmission of arboviruses in mosquito vectors. We demonstrated transovarial transmission of prototype CE virus (BFS 283) by experimentally infected Ae. dorsalis. As high as 13 and 24 percent of male and female progeny respectively were infected. Comparative tests were done with the protype viral strain and a selected ts mutant (CE-10-5). The 2 viral strains multiplied equally and infected equal proportions of ovaries but the ts mutant was not transmitted transovarially to progeny.

Tests for transovarial transmission of SLE and WEE viruses in experimentally infected Ae. dorsalis, Aedes albopictus, and Aedes triseriatus have all been negative. No virus has been isolated in tests of 6,646 adult or larval Ae. sierrensis originally collected as larvae or pupae in the field.

Aedes dorsalis cells were evaluated for their susceptibility to CE virus, a bunyavirus, and 13 other viruses (6 bunyaviruses, 5 togaviruses, 2 rhabdoviruses). Those viruses naturally vectored by mosquitoes (CE, Jamestown Canyon, Trivittatus, Turlock, Hart Park, WEE, and SLE) and by Culicoides (Lokern, Main Drain and Buttonwillow) grew well in Ae. dorsalis cells. A tick-borne virus (Powassan) and 2 nonarthropod-borne viruses (Modoc and Rio Bravo) failed to replicate. Two rhabdoviruses, Kern Canyon and Hart Park, displayed unusual growth patterns in that multiplication occurred only after a 3-day latent period. Kern Canyon virus (insect vector, if any, unknown) has not been previously reported to replicate in mosquito cells. None of the viruses that grew in the mosquito cells produced overt cytopathic effects. Two subpopulations of Ae. dorsalis cells were more susceptible than parental cells to CE virus by infectivity and immunofluorescent-antibody assays.

Serial undiluted passage of CE virus was performed simultaneously in vertebrate (Vero) and invertebrate (Ae. dorsalis)cell cultures at 30C. Passage of virus in Ae. dorsalis cells resulted in a rapid appearance of small-plaque, temperature-sensitive (sp-ts) mutants and within 6 passages the viral population consisted exclusively of sp-ts mutants. However, mutants were not detectable after 12 serial passages of virus in Vero cells. Thus, the host cell and low temperature apparently influenced the evolution of sp-ts mutants. Eighteen sp-ts mutants were cloned after 6 to 12 passages in Ae. dorsalis cells. One stable mutant (CEV-10-5) was randomly selected for comparative studies with wild type virus.

Our long-term studies on persistent infections of vertebrate and invertebrate hosts with WEE virus have led to the development of ts mutants. Eleven relatively stable ts mutants have been isolated and partially characterized as to their RNA phenotype and they have been classified with 6 complementation groups based on growth of various pairs at 41.5C. Thermal inactivation rates were determined at 56C. It appears that all of the mutants have altered structural or nonstructural proteins. We propose to select representative mutants and evaluate their capacity to persist as in vivo-viral infections.

There was a need to establish further baseline data on the survival rates and timing of gonotrophic cycles of C. tarsalis under relatively controlled conditions. Populations of known numbers of males and females were introduced into quonset hut cage units and observed and sampled daily. The daily survival rates were 70 percent for males and 69 percent for females. Gravid females first appeared on the third day. An average of 50 percent of the population was gravid from days 6 through 10. Oviposition began on the 4th day and peaked on the 7th day. These data are surprisingly similar to observations made on field populations.

There is a need for information that will allow data on indices of adult mosquito populations obtained from operation of CDC/CO_2 light traps to be converted for comparison with indices obtained from standard New Jersey light traps. Carefully controlled comparison trap runs were done in various habitats by the Sutter-Yuba Mosquito Abatement District in 1977 and 134,485 mosquitoes were collected. Our analyses indicate that the CDC to NJ mean trap ratios for female C. tarsalis ranged from 3 to 1, to 12 to 1 in different habitats. The ratio for male C. tarsalis averaged 10 to 1 or lower for NJ over CDC traps. The ratio for female Anopheles freeborni was 3 to 1 for NJ over CDC traps in rural areas. We believe that with further experience, ratios could be standardized between the several types of traps when operated in various types of habitats.

Data analyses have been completed for 1953-1973 for the entire state of California on mosquito population indices, WEE and SLE viral activity in basic cycles and the incidence of WEE and SLE in humans. The purpose was to determine if the indices of C. tarsalis female populations in urban areas could be related to viral activity and case incidence in the surrounding areas. Correlations were established and appear to identify vector light trap indices that have predictive values.

(William C. Reeves, James L. Hardy, S. Monica Asman)

REPORT FROM THE VIRAL AND RICKETTSIAL DISEASE LABORATORY
CALIFORNIA DEPARTMENT OF HEALTH SERVICES, BERKELEY, CA

Arbovirus Surveillance, 1978 (Preliminary Report)

Despite an unusually heavy Sierra snowpack and anticipated extensive flooding and excessive surface water in the San Joaquin and Sacramento Valleys particularly, with attendant "bumper crop" of mosquitoes, an epidemic of arbovirus encephalitis has not materialized. Thus far, no human cases of SLE or WEE have been detected or reported to the State by our Laboratory or the local health department laboratories which do these specific tests. Three equine cases of WEE have been confirmed, from Riverside County (June), Butte County (July) and Yuba County (June). Mosquito testing (suckling mouse inoculation) has thus far yielded 62 isolates of WEE virus, from late May through early August, largely from Imperial and eastern Riverside Counties and a few strains have been isolated from San Bernardino, Tulare and Sacramento Counties. Ten strains of SLE virus have been isolated, from Yuma County, Arizona, Riverside, and Imperial Counties, in late June and early July. All these isolates were from Culex tarsalis mosquitoes except 2 WEE viruses from Culex erythrothorax and 1 from Culex pipiens; and 1 SLE virus from Culex erythrothorax. At least 28 strains of Turlock and 17 of Hart Park virus have also been isolated, as well. Seroconversion of sentinel chickens to SLE and WEE infection has also been shown (collaborative study with Dr. W.C. Reeves) and a detailed annual report will be prepared, as usual, at the end of the calendar year. A number of the apparent WEE isolates from Imperial County have been confirmed by neutralization test as well as by the direct FA test, to be sure they were not actually VEE virus "masquerading" as WEE, in view of the recent reports of VEE virus isolates in Texas.

At least 5 cases of Colorado tick fever have been confirmed thus far, by virus isolation, fluorescent antibody staining of blood smears, or serologic response.

One imported case of dengue from exposure in Puerto Rico has been confirmed (indirect FA antibody response), plus 7 confirmed from exposure in Tahiti and 3 or more probable cases still to be confirmed. Antibody levels for dengue types 1-4 will be determined using CF, IFA, HAI and plaque-reduction neutralization tests, and a more complete report will follow.

(R. W. Emmons)

Studies on Possible Hybridization of Viruses, e.g., WI-38 Cell Culture Rabies Vaccine and Mokola Virus (Dr. Harald N. Johnson)

In papers by Wiktor and Koprowski, (Int. Symp. on Rabies, Talloires, 1965, Karger, Basel, 1966) the lytic effect and the 100% FRA positive cells were attributed to the rabiesvirus alone. A high antibody titer in monkeys was reported following with one injection of vaccine, reminiscent of the Salk polio vaccine which had live virus in it. In the rabies vaccine trial in Iran (Bahmanyar et al), the vaccine was produced using the Wistar rabies virus strain PM (Pitman Moore) WI-38 1503-3M. In the study by Wiktor, Kaplan and Koprowski, (Ann. Med. exp. Fenn., 1966, 44: 290) the Pitman Moore virus had LCM virus in it too, at least from the 10th passage in WI-38 cells. They showed that LCMV and rabiesvirus were selected out by cloning at the 50th passage level. Apparently the cloned 50th passage (PM WI-38) was used to make the vaccine. This could have contained a hybridized LCMV-RV clone.

Studies of this potentially significant problem should be pursued, e.g., with the WI-38 PM virus vaccine, before and after vaccination, for antinuclear antibody and for antibody to LCM virus. Sera of several people immunized with the experimental human diploid cell culture rabies vaccine (Wyeth) in California were tested in this Laboratory for IFA antibody to LCM virus, with negative results, but this study should be amplified.

The circumstances of the isolation of Mokola virus and the relationship described to two viruses isolated from arthropods, stimulated us to examine some of the characteristics of this virus. Contrary to published reports we could not demonstrate the virus in the blood or liver of infected mice. By the fluorescent rabies antibody (FRA) test, Mokola virus is rabiesvirus. No associated virus was demonstrable by neutralization "breakthrough" tests with rabies immune serum. The cultural characteristics of Mokola virus in established cell lines of hamster kidney and bovine kidney and in chicken embryos were similar to that of rabiesvirus. However, in the serum-virus neutralization test, using high titer rabies immune serum, we could not demonstrate sufficient neutralization of the virus to confirm that it was rabiesvirus. By the cross protection test, mice immunized by ic inoculation with the HEP Flury canine kidney cell vaccine virus, though completely resistant to subsequent ic challenge with the CVS rabiesvirus, showed no immunity to Mokola virus when challenged by ic inoculation. The vaccine-challenge protection test has been considered the ultimate test for the identification of a virus. Cross protection can be demonstrated between members of a virus group that show only minimal relationship by NT, CF and HI tests. This causes one to question the validity of the FRA test for the identification of new strains of rabiesvirus. There is good evidence that the nucleoprotein core of Mokola virus is very similar or identical to that of rabiesvirus. It is the glycoprotein of the viral envelope that seems to be different from rabiesvirus or at least a part of the viral envelope contains antigenic determinants from another virus. In view of the published information on hybridization of viruses, especially VSV and SV40, we must consider the possibility that Mokola virus is a hybrid of rabiesvirus and some other virus. We must be alert to the danger of working with Mokola virus because

it is evident that immunization against rabiesvirus will not protect against infection with Mokola virus.

The purpose of presenting the results of the Mokola virus studies at this time is to call attention to the unusual serological relationships that have been reported in the arbovirus studies around the world and to raise the question as to whether some of these are the result of hybridization. The use of the mouse as a test animal makes it possible for hybridization to occur in the brain if two or more viruses are there at the same time. The most common virus encountered in mouse passage work is LCM virus. It will be present in almost 100% of the brain cells in a tolerant infection in mice. In tissue culture systems there are many opportunities for hybridization. Parainfluenza viruses have been found in bovine blood serum and in established lines of pig kidney cells. The corona virus of swine and the bovine diarrhea virus have been found as contaminants in cell cultures. The LCM virus has been the most frequent viral contaminant in isolation studies of rabiesvirus. LCM virus has gained entry into human cell culture systems in a yet unexplained manner. The important thing to remember is that cloning will not change the character of a hybrid virus, i.e., the antigenic determinants derived from a second virus will be carried along with the nucleoprotein core. The hybrid virus may be more invasive and dangerous than the natural virus. Newer results such as oligonucleotide "fingerprinting" might be helpful in further studies of this problem.

CROSS PROTECTION TEST - MOKOLA VIRUS AND RABIESVIRUS

Rabies immunization: HEP Flury virus canine kidney cell origin

Challenge virus inoculation: One month after vaccination

Vaccine Virus (infant mice)		Vaccinated Mice (adult mice)	CVS Rabiesvirus 400 LD_{50} ic		Mokola Virus 200 LD_{50} ic	
			vaccinated	control	vaccinated	control
undiluted	-	0/12	0/6**	5/5	6/6	5/5
10^{-1}	6/6*	0/12	0/6	"	6/6	"
10^{-2}	6/6	0/12	0/5	"	6/6	"
10^{-3}	5/6	0/12	0/6	"	6/6	"
10^{-4}	5/6	-	-		-	
10^{-5}	1/6	-	-		-	

* Kidney pool of 6 mice killed when sick on day 6 and 7, positive for rabiesvirus
** Mortality ratio

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY,

UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, B.C., V6T 1W5, CANADA

In the Yukon Territory, Canada at latitude 61 to 62°N, longitude 134 to 136°W, 3 isolates of California encephalitis (CE), snowshoe hare subtype, have been recovered from 2349 unengorged female Aedes communis mosquitoes which were collected at 5 locations between 12 June and 5 July 1978. An additional 673 Culiseta inornata mosquitoes collected at these locations did not yield virus after intracerebral injection of suckling mice. At the one virus positive location, the minimum field infection rate for A. communis was 1:159. Also 46 pools of larval Aedes sp. mosquitoes (approximately 100 larvae per pool) collected in the same localities 1 to 5 June did not yield virus.

Coupling of anti-rabbit immunoglobulin (Ig), to glucose oxidase was employed for the first time to detect the presence of antigens of CE and Northway (NOR) viruses in salivary glands of domestic A. aegypti and wild-caught Culiseta inornata during 1978, in parallel with the immunofluorescence test, using the indirect (antibody sandwich) technique for each procedure. Although infectivity titrations in BHK-21 cells (plaque technique) revealed NOR virus replication in 85 to 95% A. aegypti which were injected intrathoracically with NOR virus after incubation at 13 or 23°C, antigen was detected in 40% of glands by glucose oxidase and in 35% of glands by immunofluorescence. In Cs. inornata, NOR virus replication was detected regularly 2 weeks after intrathoracic injection and incubation at 13°C, but only after 4 weeks incubation at 0°C; but after virus was fed to mosquitoes, replication was found irregularly only after 3 weeks incubation at 13°C, and not at 0°C. Similarly CE virus replication was found regularly after incubation of Cs. inornata for 2 weeks at 13°C after intrathoracic injection, and after 4 weeks incubation at 0°C, but virus replication has not yet been demonstrated after 4 weeks incubation at 0 or 13°C following feeding. Both glucose oxidase and immunofluorescence were less reliable than infectivity determinations for detection of virus in mosquito salivery glands.

(D. M. MCLEAN)

REPORT FROM THE DIVISION OF LABORATORIES
HEALTH BRANCH, DEPARTMENT OF HEALTH, VANCOUVER
BRITISH COLUMBIA, CANADA

No human cases of arthropod-borne infection have been identified yet this year in British Columbia. Nor have veterinarians identified western equine encephalitis or other arthropod-borne infection in equine animals or other species.

(G. D. Kettyls)

REPORT FROM THE PACIFIC RESEARCH SECTION, NIAID, NIH

HONOLULU, HAWAII

Following our recent demonstration of experimental transovarial transmission of Japanese encephalitis (Rosen, L et al., Science 199:909, 1978) and yellow fever (Aitken, T et al., Am J Trop Med Hyg in press) viruses in mosquitoes, we have examined a number of other mosquito-borne flaviviruses to see if they might also be vertically transmitted. To date we have obtained transovarial transmission of dengue (types 1, 2, 3 and 4), Kunjin, Kokobera, Ilheus, Banzi and Bussuquara viruses in experimentally infected Aedes albopictus. While our results indicate that a number of flaviviruses can be transovarially transmitted in mosquitoes, the filial infection rates with these agents are low (ranging from about 1:100 to 1:6,000). We are currently investigating whether higher infection rates might occur under certain field conditions or with other combinations of mosquito species and virus strains. However, from the available data, it appears that transovarial transmission alone could not sustain these agents in the insect population indefinitely. Therefore the principal cycle for both maintenance and amplification of these viruses in nature probably involves adult mosquitoes and vertebrates. Nevertheless, during adverse climatic conditions (winter or dry season) or in the absence of susceptible vertebrate hosts, theoretically these viruses could be maintained solely by vertical transmission in the insect population for 1 or 2 generations until environmental conditions were favorable for reestablishment of the primary mosquito-vertebrate cycle. We suspect that transovarial transmission may represent an important alternative mechanism for biological survival of mosquito-borne flaviviruses in nature. In addition to our laboratory work designed to elucidate the mechanism of transovarial transmission of arboviruses in mosquitoes, we are also planning field studies to attempt to isolate selected flaviviruses from wild-caught mosquito larvae.

(Robert B. Tesh and Leon Rosen)

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUT PASTEUR DE NOUMEA - NEW CALEDONIA

Dengue epidemic in New Caledonia

From the beginning of the year to June 30, 1978, it was obvious that dengue 1 virus was still transmitted in Nouméa, the main town, and in Ouvéa (Loyalty Islands).

1/ Sero-diagnosis

Sera from 626 suspected cases were tested in hemagglutination inhibition reaction, using dengue 1 antigen prepared from prototype Hawaian strain (Institut Pasteur de Paris, Dr. Sureau).

Results were as follows :

	tested	positive	per cent
Paired sera	209	38	18,2
One serum sample	417	12	2,9
Total	626	50	8

Positive tests, interpreted as indicated by WHO Technical Advisory Committee on dengue (1975), showed 22 (44 %) primary responses, 9 (18 %) secondary responses and 19 (38 %) presumptive recent infection on the total of 50.

Monthly records of serological results reveal that from March to May the number of suspected cases rose sharply but not the number of positive dengue sero-diagnosis. Tests using influenza and adenovirus antigens gave evidence that viruses other than dengue were responsible in many cases with similar symptoms, especially influenza B :

	JAN	FEB	MAR	APR	MAY	JUN	Total
Dengue primary responses	3	6	3	5	1	4	22
secondary responses	4	2	0	1	1	1	9
presumptive	4	3	4	6	1	1	19
total	11	11	7	12	3	6	50
Total suspected cases	72	45	94	218	161	36	626
Influenza positive	3	4	21	40	15	2	85
Adenovirus positive	1	0	1	1	0	0	3

2/ Virus isolation

Sera taken in acute phase from 176 patients were tested by intra-cerebral inoculation of baby mice (24 hours old), giving 17 strains (9,7 %). Two were obtained after two blind passages ; 12 originated from patients with positive sero-diagnosis.

Intra-thoracic inoculation of Aedes aegypti mosquitoes was employed for 71 samples, giving 20 strains ; 19 paralyzed baby mice when the suspension of ground infected mosquitoes was inoculated intra-cerebrally ; 1 produced only anti-dengue 1 HI antibodies as immune response to inoculation.

Research on transovarian transmission of dengue, in natural conditions

Larvae and adults emerged from pupae caught in premises where dengue cases occured were frozen and ground in phosphate buffered saline containing 20 % decomplemented normal rabbit serum, then inoculated intra-cerebrally into baby mice. No strain was recovered from Aedes aegypti and Aedes vigilax specimens collected in Nouméa :

	Nº of pools	Nº of specimens	strains
Aedes aegypti larvae	4	207	0
adults (ex nympha) ..	2	6	0
Aedes vigilax larvae	1	3	0
Total	7	216	0

Research on natural arbovirus infections

1/ Mosquitoes

No strain was isolated from 1575 specimens caught on human bait and by light traps, in localities out of Nouméa. Species tested were :

	Nº of pools	Nº of specimens	strains
Aedes aegypti	29	272	0
nocturnus	4	34	0
notoscriptus	4	34	0
vigilax	18	856	0
Culex annulirostris	3	66	0
pipiens fatigans	9	213	0
sitiens	1	1	0
Coquillettidia xanthogaster	3	69	0

2/ Birds

Puffinus pacificus (brain)		6	0
(liver)		13	0
Pterodroma leucoptera (brain)		7	0
(liver)		9	1
nigripennis (brain) ...		2	0
rostrata (brain)		1	0
Cygnus olor (captive)		1	0

3/ Mammals

Equus caballus		9	0
Bos taurus		3	0

The strain AN NC 28 from Pterodroma leucoptera paralyses the baby mice inoculated intra-cerebrally, after 6 days, at the first passage ; it is ether sensitive and its size estimated by membrane filtration is between 50 and 100 nanometers.

Pierre Fauran and Georges Le Gonidec

QUEENSLAND INSTITUTE OF MEDICAL RESEARCH AND

DEPARTMENT OF SOCIAL AND PREVENTIVE MEDICINE,

UNIVERSITY OF QUEENSLAND

The following summary of work in 1977-78 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 1978.

Epidemiology of arboviruses

There was little evidence this year of infection with Murray Valley encephalitis or Sindbis viruses in sentinel chickens held near Charleville. No results are available for northern Queensland, as the Flinders River basin experiment was closed down.

Much effort went to identification of a large number of virus strains either isolated in the Institute's field program or submitted by collaborating scientists to the Institute as a Collaborating Centre for Arbovirus Reference and Research.

Strains Ch19520 and Ch19546, both isolated from Cx annulirostris from Charleville, appear to be arboviruses previously unrecognized in Australia, and have been characterized and sent to the International Reference Centre, Yale. Ch19520 was also isolated from Australian pratincoles collected in south-west Queensland. The CSIRO series has been productive.

Strain CSIRO-79 was isolated from Lasiohelea spp. collected at Beatrice Hill, and is identical to a strain from Culicoides marksi also collected there. This virus is also newly recognized in Australia, and has been sent to Yale for identification.

It is interesting that two strains of D'Aguilar virus were isolated from Culicoides schulzei and two of Corriparta virus from Cx annulirostris came from Beatrice Hill.

Ixodes uriae ticks from Macquarie Island provided 23 isolations in the latter part of the series, studied this year. A group of seven strains, with CSIRO-122 as prototype are of Group B related to Saumarez Reef virus, but distinguishable from it. Six strains were identified as Taggert virus, nine strains were identical to MI19334 virus, and one Nugget virus strain was recovered.

CSIRO-19 was identified as a bluetongue virus at the International Reference Centre, an enormously important finding which has led to extensive research by veterinary departments and to much public discussion. The Australian bluetongue virus appears to be antigenically distinct by neutralization test from the 20 known types; the distribution may be compatible with Culicoides schulzei as vector.

Two experiments with native birds infected with MVE virus in the laboratory have demonstrated low level viraemia of short duration. Persistent haemagglutination inhibiting and neutralizing antibody responses followed in egrets, but in galahs titres declined to undetectable levels by 60 days. Population studies of waterbirds in south-west Queensland continued, with very little nesting observed.

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

Entomology

Mosquito biology and systematics.- Mosquito problems at Darwin and Gladstone were examined and reported on. Taxonomic studies were concerned principally with Culex and Aedes and with work for a checklist of Australasian Culicidae, for which also a history of Australian mosquito research was completed. Aedes imprimens was newly recorded from south Queensland. Specimens received for identification provided new distribution records for other states.

(Elizabeth N. Marks).

Mosquito ecology and mosquito-virus relationships.- This year saw the completion of seven papers, a book chapter and a Ph.D. thesis while a further four papers reached semi-final stage. Most of this work relates to the investigation of why the mosquito Culex annulirostris is the major vector of arboviruses, including Murray Valley encephalitis virus, in Australia. Another paper provided information on the blood feeding patterns of biting midges at Kowanyama, north Queensland.

Two established field projects at Charleville (host-feeding patterns of mosquitoes) and at Lake Bullawarra near Thargomindah (epidemiological studies of bird-mosquito-arbovirus interactions) continued satisfactorily. The Charleville project is also providing useful data on diurnal resting habits.

Perhaps the most exciting event of the year was the receipt from Victoria of the only colony of Cx annulirostris. Detailed biological, physiological and virological studies are underway. A part-time Ph.D. student will also utilize the colony in a study related to the control of larval Cx annulirostris. Although the insectary humidification system is still unsatisfactory, some experimental virus studies were completed.

(B.H. Kay).

Arbovirus immunology

Investigation of Ross River virus infection in the mouse suggests that the virus replicates in the macrophage and possibly the B lymphocyte. Both humoral and cell-mediated responses to the infection can be detected but cell-transfer experiments suggest that it is antibody alone which prevents spread of virus from the blood to the tissues. In vitro the leucocyte-mediated cytotoxic response has both a virus-specific and a nonspecific facet. The nonspecific facet may contribute to the rapid clearance of viraemia. It has also been demonstrated that Ross River virus crosses the placenta in mice

causing limited fatalities in utero.

The development of a rapid immunofluorescence assay for IgM antibody to Ross River virus has enabled us to collect lymphocytes from cases of acute polyarthritis to study aspects of cell-mediated immunity to this virus. A lymphocyte transformation assay appears to detect reactivity in the early weeks following onset of symptoms. The absence of lymphocyte-mediated cytotoxicity in vitro is believed to be due to differences in HLA type between lymphocytes and target cells.

An indirect immunofluorescence assay for antibody to bluetongue virus (CSIRO-19) was effective with fresh sera but less so with stored sera.

(J.G. Aaskov).

Laboratory studies of arboviruses

Structure and genetics of orbiviruses.- A high titre variant of the CSIRO-19 isolate of bluetongue virus has been isolated, allowing analysis of the electrophoretic migration pattern of the genome RNA. The genome diaplays little similarity to two other distantly related Australian orbiviruses, Eubenangee and Tilligerry viruses.

Electrophoretic analysis of the genome RNA of laboratory-induced recombinant viruses has continued. Further evidence for the genome segment reassortment mechanism of genetic interchange has been obtained.

A new method for detecting genetic relationships between double-stranded RNA genome segments has been applied. Genome segments of Wallal and Mudjinbarry viruses have been compared in this manner. The method also allowed closer examination of the genomes of recombinant orbiviruses.

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

Biochemistry of arboviruses.- Studies of Leanyer virus indicate that it is not a member of the alpha- or flavivirus groups. The proteins of two strains of Ross River virus (T48 and NB5092) were indistinguishable in polyacrylamide gel electrophoresis.

(M.H. Symons).

Acarology

Good progress in the classification of mite parasites of Australian vertebrates was made in several groups, especially the Dermanyssidae. Two large manuscripts were completed, one detailing the blood-sucking rhinonyssines of birds collected on recent field expeditions, the other an important circum-Australian collection from rodents. Two lesser papers involved new species of Andreacarus from Tasmanian rodents and a new genus of interestingly modified mites from the ear canal of marsupials. A large collection comprising three dermanyssoid groups from the Kimberley was examined. The first two (dermanyssines from birds and spinturnicids from bats) were described; the third (laelapines from rodents) is under study.

Miscellaneous topics included blood-sucking paramegistids from repiles, subcutaneous hypoderids of birds and the first Australian records of the cat fur-mite.

(R. Domrow)

REPORT FROM 'ATTWOOD' VETERINARY RESEARCH LABORATORY,

MELBOURNE, AUSTRALIA

Murray Valley Encephalitis (MVE) Virus

Sentinel chicken flocks have been maintained on the River Murray or its tributaries since the 1974 human epidemic of Murray Valley Encephalitis. Between November and April serum samples are collected weekly from 10 flocks of 20 birds each at locations ranging from Mildura in the north west to Wodonga in the north east of Victoria. No HI antibody to M.V.E. virus has been present in any of these sera.

Group A HI Antibody

Testing of the above sera for Group A HI antibody has shown the presence of a Group A virus in the areas indicated.

TABLE 1. Group A HI Seropositive Chickens 1975-1978

YEAR*	LOCATION	NO POSITIVE**	DATE OF 1ST SEROCONVERSION	DATE OF LAST SEROCONVERSION
1975	SWAN HILL	5	13 JAN	20 JAN
	MILDURA	8	13 JAN	3 MAR
	ECHUCA	3	4 FEB	11 FEB
	WODONGA	6	27 JAN	2 MAR
1976	KERANG	7	5 JAN	9 MAR
	MILDURA	16	20 JAN	9 MAR
	SWAN HILL	6	10 FEB	23 FEB
1978	MILDURA	6	13 FEB	6 MAR

* No seroconversions took place in any of the birds during the summer of 1977.

** Out of a total of 20 tested.

The sentinel chicken project is part of a collaborative study with the Victorian Department of Health. It will be repeated in the coming season and will be supplemented by mosquito trapping and virus isolation attempts.

Penguin Arboviruses

Sera from penguins on Macquarie Island, south of New Zealand, were collected late in 1976 and early 1977. No Group A HI antibody

was present in any sample.

TABLE 2. Group B HI Antibody in Macquarie Island Penguin Sera

Penguin Species	No. Tested	No. Positive
ROYAL	384	9
KING	218	7
GENTOO	85	NIL
ROCKHOPPER	101	7
TOTAL	788	23

Of 120 sera from Fairy penguins collected at 2 sites on the coast of Victoria, 34 contained Group B HI antibody. No Group A HI antibody was present.

Homogenates from 23 of 45 pools of Ixodes Uriae ticks from Macquarie Island caused paralysis and death in suckling mice on the first passage of the material. Second and subsequent passages caused similar signs. Identification of these isolates is in progress.

(J. Campbell and I. Morgan, 'Attwood' Veterinary Research Laboratory, Department of Agriculture, Mickleham Road, Westmeadows, Victoria, 3047, Australia.)

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

The table shows the current state of examination of 208 virus isolates.

Since our last report, Western Australia has experienced its first outbreak of Australian arbovirus encephalitis. The clinical cases and the associated epidemiological and field and laboratory work will be reported elsewhere.

The observations are summarized briefly as follows:

Of 8 clinical cases, 7 were in the Kimberley and 1 at Port Hedland in the Pilbara (see figure). The latter is the first sero-conversion and clinical case recorded in the arid area of Western Australia and many miles south-west of the Kimberley, where MVE is enzootic. Serological studies with neutralization tests and IgM H-I tests confirm that 4 cases appear to be MVE, 1 definite and 1 probable Kunjin, and in the remaining case the serology is not clear, although MVE antibodies are present.

In a field trip designed to collect and identify mosquitoes, collect blood and follow-up contacts, the Western Australian team were joined by Dr. Telford H. Work and Dr. Martine Jozan from the University of California, Los Angeles, Dr. Ian Marshall from John Curtin School of Medical Research, Australian National University, and Mr. Peter Whelan, an entomologist from the Northern Territory. Mr. G. Harnett and Mr. W. Jolly of the State Health Laboratory Services are involved in entomological and serological work respectively.

More than 40,000 mosquitoes were collected using light traps (loaned by the California Department of Health Vector Control) and locally made bait traps using one rabbit and one chicken per cage. Further reports will follow on the total and differential counts and the virus isolates made from the two types of traps under these well-defined conditions. Although *Culex annulirostris* still continues to be the overall predominant mosquito collected by either method there appear to be a few foci where *Culex fatigans*, *Aedes* or *Anopheles* sp. predominate.

To supplement the earlier serological studies about 600 sera were collected from human case contacts, passerines, other birds, horses, cattle and crocodiles. The results of virus isolations and serological studies will be reported later.

The outbreak has been associated with unusually heavy rain in the West and South Kimberley and the Northern Pilbara and MVE has jumped the desert barrier in Western Australia. This is an important and significant event. Using the human cases cited above, as sentinels of great enzootic activity, geographical sites were selected for intensive investigation. This has spanned the entire area of North-west Australia. It will be of considerable interest to apply methods similar to those used in the studies of the arbovirus encephalitides in Western U.S.A. in an endeavour to answer some of the critical questions concerning the enzootic sources of the viruses and by what mechanisms the enzootic cycles are maintained. In view of our isolation of MVE from *Culex fatigans* and the recent activity of this mosquito in the Northern Pilbara, its role, if any, must be examined alongside that of *Culex annulirostris*. In addition, it seems highly likely that Kunjin virus may produce a clinical picture in man indistinguishable from that of MVE.

(N.F. Stanley, S. Anderson, A. Wright; joined in June 1978 by *P. Liehne, P. Pihu, T.H. Work, M. Jozan* and *S. Roberts.)*

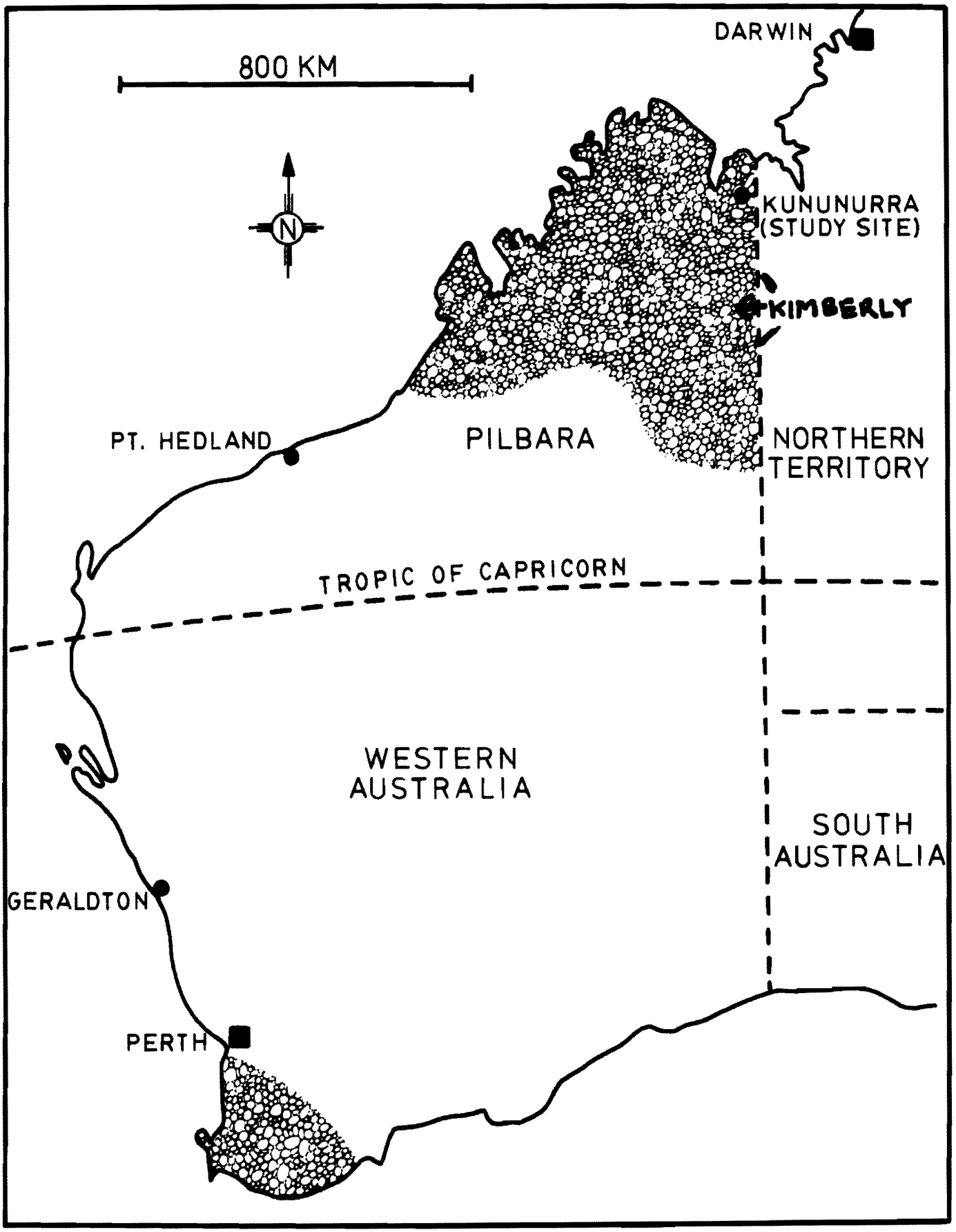
VIRUS ISOLATIONS FROM KIMBERLEY (ORD RIVER AND DERBY) MOSQUITOES :
CURRENT IDENTIFICATION OF 208 ISOLATES FROM 5 VECTORS.

VECTOR	VIRUS GROUPS																
	FLAVIVIRUS B			ALPHAVIRUS A		KOONGOL			COR	UNGROUPED RHABDOVIRUS			ANOPH A/B.	UNKNOWN † UNGROUPED			UNCLASSIFIED	TOTAL
	MVE	KUN	KOK	SIN	RR	KOO	WON	NON HA	COR	KNA* OR 194	PC* OR 189	KIM* OR 250	OR 540	I OR 379	II OR 512	III OR 869	WGR	
CULEX ANNULIROSTRIS	32	20	2	13	1	4	12	64	1		3	1	6	2	2		3	166
AEDEOMYIA CATASTICTA									32	1						4		37
AEDES TREMULUS		1																1
AEDES NORMANENSIS				2													1	3
CULEX FATIGANS	1																	1
TOTAL	33	21	2	15	1	4	12	64	33	1	3	1	6	2	2	4	4	208

* NOW RECOGNISED AS NEW TYPES

† TESTED AGAINST WORLD GROUP ANTISERA AND AUSTRALIAN ANTISERA AND FOUND TO BE NON – REACTING

MVE = MURRAY VALLEY ENCEPHALITIS : RR = ROSS RIVER : KNA = KUNUNURRA : PC = PARRY'S CREEK :
KIM = KIMBERLEY: KUN = KUNJIN : SIN = SINDBIS : KOK = KOKOBERA : WON = WONGAL ;


DARWIN
800 KM
N
KUNUNURRA
(STUDY SITE)
KIMBERLY
PT. HEDLAND
PILBARA
NORTHERN
TERRITORY
TROPIC OF CAPRICORN
WESTERN
AUSTRALIA
SOUTH
AUSTRALIA
GERALDTON
PERTH

LABORATORY SERVICES BRANCH, ONTARIO MINISTRY OF HEALTH, TORONTO; HEALTH AND WELFARE CANADA, OTTAWA; AND TORONTO GENERAL HOSPITAL, TORONTO, ONTARIO, CANADA

FLAVIVIRUS INFECTION IN A SUSPECT CASE OF LASSA FEVER (LF)

Case History: On July 11, 1978, a 30 year old woman presented herself to the Tropical Diseases Clinic of Toronto General Hospital. Her chief complaint was fever.

She has lived in Ife, Nigeria, since 1973. She and her husband both lectured at the University of Ife. They have two daughters and lived in a modern three bedroom home. Three weeks prior to her illness, she had remained on campus. About two weeks before becoming ill, the patient had visited with her family "an experimental" farm which was also located on campus.

On July 6, the whole family departed from Lagos, Nigeria, by plane to Toronto. During the flight, the patient experienced symptoms of fever, malaise, myalgia, backache, headache and a sore throat. On July 7, she finally arrived at Toronto and stayed with her parents. That evening her symptoms had worsened slightly and she noticed on her upper trunk "heat rash" which gradually disappeared after three

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LF = Lassa Fever
EDCD = Exotic Dangerous Communicable Diseases
CPHL = Central Public Health Laboratories, Toronto
CDC = Center for Disease Control, Atlanta, Ga., USA

days. The patient had experienced malaria in 1973 and 1975. On July 8, she had fever (38.9°C) and shaking chills, she took chloroquine tablets and completed a therapeutic course by July 10. This measure failed to resolve her symptoms by July 11.

Clinical Examination and Differential Diagnosis: The initial clinical findings for this patient when seen at the Clinic on July 11 (J.S.K.) showed signs of pharyngitis, a relative bradycardia and fever of 38.2°C. "Routine" laboratory tests done July 11 at Toronto General Hospital showed 2+ albumenuria. Blood smears had 47% neutrophils, 44% lymphocytes, 9% monocytes and a marked leukopenia with 2400 WBCs per cu.m.m. Malarial parasites were not seen on the smear.

Due to these laboratory results, the following diagnoses were considered likely (in order of "index of suspicion"): (1) Tyhpoid fever, (2) Dengue-like viral illness, (3) Lassa fever.

Malaria was still a remote possibility. However, it was ruled out, especially since the patient had taken an adequate course of chloroquine. This impression was futher confirmed on July 14 (Table 2). Although LF had occurred in other parts of Nigeria, none has been reported in Ife. Active surveillance, however, for LF is not being carried out in Nigeria. Arboviral infections are prevalent in that area and Woodruff et al[1] identified infection with arboviruses in 15 out of 86 travellers from tropical Africa.

Thus, LF could not be considered as having a "high index of suspicion" but neither could it be excluded.

Isolation of Patient and Surveillance of Contacts: Since LF was a possible diagnosis, the attending physician alerted the Provincial Epidemiologist on the evening of July 11. The "Canadian Contingency Plan" for "Exotic Dangerous Communicable Diseases"[2] was then implemented that evening. This case became the seventh "LF alert" in Canada.[3]

Since the patient had been sent home with her mild illness, she was placed in strict isolation with her family. Had her condition deteriorated, she would have been transported by air to Ottawa in a "transit isolator" where she could get medical care in proper isolation facilities.

The local medical officer of health was responsible for isolation procedures. The attending physician continued daily medical supervision, primarily by telephone. To minimize further exposure, all contacts for patient's care (e.g. blood samples, etc.) were limited essentially to these two men. Close contacts of the patient were placed under active surveillance and included her family, parents, a cousin and an aunt who visited July 9; attending physician, his resident and support staff at the Clinic; and the local medical officer of health. Tracing of other contacts or notification procedures were not pursued.

Clinical Course: The patient improved rapidly and spontaneously recovered by July 13. Besides a transient erythematous macular exanthem on her trunk and extremities, which appears to be of allergic etiology related to bath oils, her clinical course was uneventful.

Laboratory Investigations: Although LF was not given a high index of suspicion, all laboratory tests had to be done under the highest containment facilities available to us. All samples were transferred, therefore, to the Central Public Health Laboratory, Toronto, on the evening of July 11. The high index of suspicion for typhoid made bacteriological studies a priority. By July 13, typhoid fever and tonsillitis could be ruled out (Table 1), especially since the patient became afebrile.

Tests for LF virus were important both to diagnosis and to public health measures. Negative results of LF would have helped to discontinue isolation procedures and the "state of emergency". Consultation with CDC, Atlanta, pointed that there was no strong epidemiological evidence implicating LF. This possibility, however, had to be ruled out. Arrangements were made, therefore, with CDC to perform tests for LF virus and antibodies.

On July 16, specimens (Table 3,4) were taken to CDC. Presumptive results were negative for LF virus and antibodies and were finally confirmed on July 24 (Table 3,4).

Both patient's isolation and surveillance of contacts were thus dicontinued on July 24. Virological tests were then pursued at CPHL, Toronto. On July 28, 1978, serological results indicated a flavivirus infection (Table 5). A serum sample from the patient's husband had no antibodies either to LF virus or to the arbovirus listed in Table 5.

Discussion: The patient's disease is not caused by malaria, typhoid or LF. She had no detectable antibodies to three alphaviruses: chikungunya, eastern and western equine encephalitis. Her illness may be either caused by or associated with flavivirus infection(s) since she has had seroconversion to several flavivirus antigens. The broad antibody response and the high titres suggest that in addition to her immunization against yellow fever (March 1973) she may have experienced, prior to this illness, another flavivirus infection. Fifteen out of 86 (17.4%) travellers to Britain who had suffered from fever while in tropical Africa, had antibodies to different arboviruses. Tests for antibodies to 57 "African" viruses by haemagglutination inhibition, complement fixation and immunofluorescence techniques indicated past infections with one or more of ten viruses. The most important of these viruses were: O'Nyong-nyong, dengue, chikungunya and Ntaya. Eight of these 15 patients have either visited or been infected in Nigeria. In our case, the identity of the flavivirus is not yet determined and we hope to resolve it.

It is important that tests in suspect cases are conducted for possible infection with these or other arboviruses known to exist in the area. It is also important to note that an arboviral or other microbial or parasitic infections do not exclude the possibility of concurrent infection with LF or other EDCD. This possibility should be considered both in performing laboratory tests, in patient's management and in public health measures.

This recent episode raises several important issues regarding the differential diagnosis and management of suspected Lassa fever cases.

Two previous episodes in Ontario provided the impetus to formulate "The Canadian Contingency Plan for Exotic Dangerous Communicable Diseases". However, this recent episode has apparently contributed little to our "diagnostic acumen" for Lassa fever.

Literature indicates that diagnosis of LF on clinical grounds is almost impossible. Diagnosis is best substantiated with epidemiological criteria, especially since infection may lead to mild disease or even be inapparent. Epidemiological evidence for LF in this case appears to have been weak indeed and favoured the possibility of an arboviral infection.

Several problems were demonstrated in this case. The Tropical Diseases Clinic will continue to be challenged with diagnosing exotic dangerous communicable diseases and

will request some "routine" tests from the hospital laboratory. The handling of potentially dangerous specimens should be done under the strictest containment facilities available to the hospital and would seem to be supported by common sense and by some literature reports. However, this approach is not yet an accepted policy in Canada.

Conclusions: Recent experience with a case of suspected LF served as a reminder of the potential threat posed by EDCD. It also emphasized the need for safe and practical facilities to meet this challenge. The "Canadian Contingency Plan" proved its worth in providing a basis relative to policies for public health measures dealing with EDCD. This case was negative for LF but had a flavivirus infection.

Consideration should be given therefore to the following issues in order to prepare for future challenges:

(1) How can determining the "Index of Suspicion" for LF be improved, especially since it is the "trigger" mechanism of alert?

(2) Is "Home Isolation" an acceptable public health policy for LF and other EDCD? If so, what are the operational details (e.g. contacts, concurrent and terminal disinfection)?

(3) What is the contingency plan for Tropical Diseases Clinics for detecting suspect cases of EDCD?

(4) How can the protocol for performing "routine" tests to aid in the laboratory the recognition of suspect cases of EDCD be realistically improved?

(5) How can the biohazard containment facilities be improved to test for other possible viruses or antibodies in specimens from travellers with suspect disease?

ACKNOWLEDGEMENT: All laboratory tests for LF virus and antibodies were done at Special Pathogens Branch, CDC, Atlanta. We are grateful to Dr. Karl Johnson, Chief of this Branch, for his continuous support and valuable advice.

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LABORATORY TESTS ON SUSPECT CASE OF LASSA FEVER

BACTERIOLOGY (Table 1)

Specimen No.	Type	Date Obtained	Results
SP8-5	Blood	11/7/78	No Salmonella was isolated
SP8-6	Blood culture	11/7/78	No Salmonella was isolated
SP8-7	Blood culture	11/7/78	No Salmonella was isolated
SP8-8	Throat swab	11/7/78	No bacterial pathogens were isolated
SP8-9	Throat swab	11/7/78	No bacterial pathogens were isolated
SP8-10	Urine	11/7/78	No Salmonella was isolated
SP8-11	Urine	11/7/78 (11 pm)	No Salmonella was isolated
SP8-12	Urine	12/7/78	No Salmonella was isolated
SP8-13	Stool	12/7/78	No Salmonella was isolated
SP8-3	Blood/serum	11/7/78	Widal Agglutination test - Negative
SP8-14	Blood/serum	13/7/78	Widal Agglutination test - Negative

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PARASITOLOGY (Table 2)

SP8-14a	Blood, thin film	13/7/78	Negative for Malaria parasites
SP8-14b	Blood, thick film	13/7/78	Negative for Malaria parasites

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VIROLOGY - a. Virus Isolation (Table 3)

SP8-3	Blood/serum	11/7/78	Inoculation of duck embryo cell cultures (Central Public Health Laboratories) and of Vero Cell cultures (Center for Disease Control, Atlanta) failed to reveal the presence of Arbo or Arenaviruses.
SP8-14	Blood/serum	13/7/78	
SP8-15	Blood/serum	16/11/78	
SP8-8	Throat swab	11/7/78	
SP8-9	Throat swab	11/7/78	
SP8-10	Urine	11/7/78	
SP8-11	Urine	11/7/78 (11 pm)	
SP8-12	Urine	12/7/78	
SP8-16	Urine	16/7/78	

VIROLOGY - b. Serological Tests for Antibodies (Table 4)

Specimen No.	Date Obtained	Test	Results
SP8-3	11/7/78	IF/LF*	Negative at 1:4 dilution
SP8-14	13/7/78	IF/LF	Negative at 1:4 dilution
SP8-15	16/7/78	IF/LF	Negative at 1:4 dilution
SP8-17	27/7/78	IF/LF	Negative at 1:4 dilution

*IF/LF: Immunofluorescence tests for Lassa fever antibodies were done at the Center for Disease Control, Atlanta, Georgia, USA. Dr. Karl Johnson, Chief, Special Pathogens Branch.

VIROLOGY - b. Serological Tests for Antibodies (Table 5)

TEST 1,4

Specimen No.	Date Obtained	Haemagglutination Inhibition				Complement Fixation			
		POW[2]	SLE	DEN2	BAN	POW	SLE	DEN2	BAN
SP8-4	11/7/78	1280	2560	640	10240	128	256	128	N/A[3]
SP8-14	13/7/79	2560	20480	1280	81920	256	512	256	"
SP8-15	16/7/78	10240	20480	2560	40960	512	1024	256	"
SP8-17	27/7/78	5120	10240	2560	10240	256	512	512	"

1. *Done at Central Public Health Laboratories, Toronto, and confirmed by the National Arbovirus Reference Service, Toronto: Drs. L. Spence and H. Artsob.
2. POW = Powassan; SLE = St. Louis Encephalitis; DEN[2] = Dengue Type 2; BAN = Banzi.
   Results are expressed as reciprocal of antibody enpoints.
   Tests for Ntaya, Yellow fever and some suspect arboviruses are in progress.
3. N/A = Not available.
4. All specimens had no detectable Hl or CF antibodies to the following alphaviruses: eastern equine, western equine encephalitis or chikungunya viruses.

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