ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 31 September 1976

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COMMENTS FROM THE EDITOR

The response to my request for increased collaboration has been gratifying. You will note that Issue No. 31 contains a number of very interesting reports from research groups who have not before been represented or who have been rarely heard from.

Those of you who will be able to attend the joint meeting of the American Society of Tropical Medicine and Hygiene and the Royal Society of Tropical Medicine and Hygiene at Philadelphia, November 3-5, will be pleased with the program. The open meeting of the American Committee on Arthropod-borne Viruses will occupy the morning of Wednesday, November 3; and a symposium on flaviviruses is scheduled for that evening. Many other reports on viruses, mainly arboviruses, will be presented at other sessions.

The deadline for reports for Information Exchange Issue No. 32 is March 1, 1977. Please mark your calendars and send your reports early to allow for possible slow mail service.

Send your reports to:

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PROGRAM OF THE AMERICAN COMITTEE ON ARTHROPOD-BORNE VIRUSES, PHILADELPHIA, PENNSYLVANIA, NOVEMBER 3, 1976

The sessions of the American Committee on Arthropod-borne Viruses, which are held annually in conjunction with the meeting of the American Society of Tropical Medicine and Hygiene, are scheduled for the AM and evening of Wednesday, November 3. This year they are held in Philadelphia, Pennsylvania.

The proposed sessions of the program are as follows:

17TH ANNUAL OPEN MEETING OF THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

A. Business Meeting (one hour)
James L. Hardy, Chairman

(15 min. break)

B. Scientific Session (two and one-half hours)
(each 25 min. + 5 min. discussion)

1. MOLECULAR ASPECTS OF COMPARATIVE ARBOVIROLOGY. P.K. Russell, J.M. Dalrymple and D.W. Trent. Walter Reed Army Medical Center, Washington, D.C.; and Vector-Borne Diseases Division, Center for Disease Control, Fort Collins, Colorado.

2. RELATION OF VIRION SURFACE CHARGES AND IN VIVO CLEARANCE TO VIRULENCE OF ALPHAVIRUSES. P.B. Jahrling and G.A. Eddy. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland.

3. CURRENT STATUS OF ALPHAVIRUS ISOLATIONS FROM SWALLOWBUGS. T.P. Monath, D.B. Francy, G.S. Bowen, W.A. Rush, G.S. Smith, C.H. Calisher, J.S. Lazuick, D.W. Trent, G.E. Kemp and R.O. Hayes. Vector-Borne Diseases Division, Center for Disease Control, Fort Collins, Colorado.

4. BACKGROUND AND EPIDEMIOLOGY OF THE 1975-76 DENGUE EPIDEMIC IN PUERTO RICO. B.L. Cline, G.S. Sather, R. Lopes-Correa, J.P. Woodall, C.G. Moore, E. Ruiz-Tiben, G. Kuno and M. Oberle. San Juan Laboratories, Center for Disease Control, San Juan, Puerto Rico.

5. A NOSOCOMIAL OUTBREAK OF VIRAL HEMORRHAGIC FEVER IN PAKISTAN, JANUARY 1976, CAUSED BY CHF-CONGO VIRUS. M.I. Burney, P.A. Webb, G.H. Tignor and S.M. Buckley. National Health Laboratories, Islamabad, Pakistan; Viral Pathology Branch, Virology Division, Center for Disease Control, Atlanta, Georgia; and Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University, New Haven, Connecticut.

SYMPOSIUM PROGRAM

Recent Group B Arbovirus Encephalitis Epidemics in the Americas

Convenor: T. M. Yuill, University of Wisconsin, Madison

St. Louis Encephalitis, A Continuing Public Health Problem

CURRENT STATUS OF EPIDEMIC ST. LOUIS ENCEPHALITIS. T. P. Monath, CDC, Ft. Collins, Colorado.

RECENT STUDIES ON ST. LOUIS ENCEPHALITIS VIRUS VECTORS. D. B. Francy, CDC, Ft. Collins, Colorado.

WILDLIFE AS RESERVOIRS OF ST. LOUIS ENCEPHALITIS VIRUS. J. L. Hardy, University of California, Berkeley.

ARE ST. LOUIS ENCEPHALITIS VIRUS EPIDEMICS PREDICTABLE? W. C. Reeves, University of California, Berkeley.

RECENT ADVANCES IN VECTOR CONTROL. D. A. Eliason, CDC, Atlanta, Georgia.

ROUND TABLE. Discussants:
G. S. Bowen, CDC, Ft. Collins, Colorado.
D. W. Trent, CDC, Ft. Collins, Colorado.
P. K. Russell, Walter Reed Army Institute of Research, Washington, D.C.

Emergence of a Newly-Recognized Group B Encephalitis Virus in Brazil

AN EPIDEMIC IN SAO PAULO STATE AND SOME CHARACTERISTICS OF THE VIRUS. O. S. de Souza Lopes, Instituto Adolfo Lutz, Sao Paulo, Brazil.

DISCUSSION. C. Calisher, CDC, Ft. Collins, Colorado.

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY, FACULTY OF MEDICINE

THE UNIVERSITY OF BRITISH COLUMBIA

VANCOUVER, CANADA

Replication of a Yukon isolate of California encephalitis (CE) virus (snowshoe hare subtype) in its first mouse passage was demonstrated in salivary glands of wild-caught Culiseta inornata mosquitoes from the western Canadian Arctic, both after 100 mouse LD_{50} was fed to or injected into them, when mosquitoes were incubated at $0^{o}C$ or $13^{o}C$ for 13 to 20 days. By inducing individual mosquitoes to imbibe defibrinated sheep blood whilst restrained in cages 2 cm diameter, virus transmission occurred after 20 days incubation at $13^{o}C$ following intrathoracic injection. The minimum infectivity dose for this species was less than 0.1 mouse LD_{50}. This CE isolate replicated readily following injection of wild-caught Aedes communis with doses ranging from 100 to 0.01 mouse LD_{50} after incubation at 0, 13 and $23^{o}C$. Virus transmission occurred after incubation for 13 to 20 days at 0 and $13^{o}C$ following injection of 1 to 0.01 mouse LD_{50}, and after 20 days incubation at $13^{o}C$ following imbibing of 100 mouse LD_{50}. Immunofluorescence was detected in cytoplasm of salivary gland acinar cells in both species of mosquito 13 to 20 days after injection with 10 or 100 mouse LD_{50}, regularly following incubation at $13^{o}C$ and less frequently after incubation at $0^{o}C$.

(D. M. MCLEAN)

Surveillance for St. Louis Encephalitis in the United States

St. Louis encephalitis was epidemic in the United States during the 1974 and 1975 summer seasons. The epidemics in 1975 resulted in 1,995 confirmed and presumptive cases and 127 deaths, of which at least 1,858 cases and 125 deaths occurred in areas east of the Mississippi River, where Culex pipiens was the probable vector species of major importance. Outbreaks occurred over a geographically wide area from Houston, Texas, to Ontario, Canada.

In order to assess virus activity prior to the appearance of human cases, a number of local and state health departments have either expanded existing surveillance programs or initiated new ones. The primary focus has been on rapid determination of the prevalence of HI antibody in the sera of hatching-year wild birds. Additionally, sentinel chicken flocks and virological tests of Culex mosquitoes are being used in some localities. Hemagglutination inhibition tests on bird sera are performed in laboratories at various county and state health departments and at the Vector-Borne Diseases Division, Fort Collins. The data presented below has kindly been made available by the respective county and state health departments.

Nearly 10,000 avian sera have been tested in 1976. Data from individual localities are examined and analyzed on a weekly or biweekly basis, but, for the sake of simplicity, Table 1 shows prevalence data on a monthly basis by state.

In Mississippi, virus activity was first indicated by tests on bird sera collected July 6-12. Four confirmed and presumptive human cases have subsequently occurred in the State, with onset dates of July 12, 16, 21, and 22 (Dr. Kenneth Powell, personal communication).

In Memphis, Tennessee, the prevalence of HI antibody in wild birds increased sharply in the last week of July, in concert with the appearance of virus in Culex pipiens quinquefasciatus. No human cases have been recognized to date (August 10).

In Houston, Texas, the percentage of HI positive birds increased significantly in the last 2 weeks of July. Five strains of SLE virus have been isolated from Culex tested between July 9-23. No human cases have been recorded to date.

On the basis of avian, vector, and human surveillance, SLE virus activity appears low elsewhere in the eastern United States. However, the risk of epidemic SLE in Mississippi, Memphis, and Houston is recognized.

(Thomas P. Monath)

TABLE 1. SEROLOGICAL SURVEILLANCE, BIRDS, UNITED STATES, 1976

Locality	Age	No. (%) Positive[1]/No. Tested by Month of Collection and Age				
		April	May	June	July	August
Mississippi (~20 locations)	Juvenile		1/169 (0.6)	5/508 (1)	24/602 (4.0)	
	All ages		14/1003(1.4)	16/954 (1.7)	44/878 (5.0)	
Louisiana (~10 locations)	Juvenile		0/87	6/365 (1.6)	3/388 (0.8)	
	All ages		4/343 (1.2)	12/592 (2.0)	13/738 (1.8)	
Tennessee (Memphis)	Juvenile		0/137	5/402 (1.2)	12/428 (2.8)	
	All ages		0/173	6/543 (1.1)	22/592 (3.7)	
Alabama (Birmingham)	Juvenile			20/297 (6.7)	1/82 (1.2)	
	All ages			-	1/183 (0.6)	
Illinois (9 locations)	Juvenile		0/30	3/84 (3.6)	1/437 (0.2)	
	All ages		4/131 (3.1)	13/362 (3.6)	4/741 (0.6)	
Ohio (22 locations)	Juvenile		0/36	0/274	1/226 (0.4)	
	All ages		2/56 (3.6)	1/336[2] (0.3)	2/290[2] (0.7)	
Florida (7 locations)	All ages				4/539 (0.7)	
Texas (Houston)	Juvenile			6/345 (1.7)	21/334 (6.3)	
	All ages			19/589 (3.2)	25/480 (5.2)	

[1]--SLE HI ≥20

[2]--Includes some chicken sera

Acknowledgements: Mississippi--Ms. Emily Jumper & Dr. Kenneth Powell, Miss. Dept. of Public Health, Dr. G. Middlebrook, University of So. Miss., Mr. C. Crosby, Gulf Coast Mosquito Control Commission; Louisiana--Mr. George Carmichael, New Orleans City Health Dept., Mr. G. Roy Hayes and Dr. George H. Hauser, La. State Dept. of Health; Tennessee--Mr. I. K. Moseley, Memphis-Shelby Co. Health Dept., and Tennessee Dept. of Public Health; Alabama--Dr. H. Michael Maetz, Jefferscn Co. Health Dept.; Illinois--Dr. Gary Clark, Mr. Harvey Pretula, and Mr. Richard Morrissey, Div. of Public Health Labs., Illinois Dept. of Public Health; Ohio--Margaret A. Parsons, Barbara J. Weigert, Richard L. Berry, Howard Stegmiller, Judy Lebio, and Cecilia Sipos, Dept. of Health-Ohio; Florida--Ms. Elsie Buff, Dr. Nathan Schneider, and Dr. Gerald Hoff, Fla. Div. of Health; Texas--Dr. Lois Leffingwell, State Dept. of Health, Drs. A.G. Randall and R. A. MacLean, Houston City Health Dept., Mr. R. E. Bartnett, Harris Co. Mosquito Control District.

Laboratory Inoculations of North, Central and South American strains of St. Louis Encephalitis Virus in House Sparrows

There has been a recent upsurge in St. Louis encephalitis virus activity manifested by human epidemics in 1974 and 1975 in the United States. St. Louis encephalitis (SLE) virus has also been found in the Caribbean, Central and South America, but large urban outbreaks have not occurred. Both field and experimental studies by several authors have indicated that the house sparrow (Passer domesticus) is an important amplifying host species for SLE virus. This is an exotic species introduced into the eastern U.S. before 1900 which has only relatively recently spread into Central and South America.

These studies were designed to test the hypothesis that a difference in the ability of South and North American strains of SLE virus to infect and produce viremia in house sparrows might partially explain the lack of large SLE outbreaks in South America. Therefore, three strains of SLE virus from North America, three from South America, one from Guatemala, one from Jamaica, and one from Trinidad were inoculated subcutaneously into 7-11 day old nestling and adult house sparrows. Each bird received 1000-10,000 SMICLD50/ml of virus. Viremia titers were determined as plaques on duck embryo cell culture. The results are shown in the accompanying (Table 2).

The preliminary results show a marked difference among SLE strains in their ability to infect and produce viremia in house sparrows. Strains from Jamaica, Trinidad, Sao Paulo, Brazil and an arthropod strain from Belem, Brazil produced a low percentage of infected birds and short, low titered viremias. Three North American strains, a strain from Guatemala and a human strain from Belem, Brazil produced a high percentage of infected birds and moderate to high titered viremia for several days.

Several possible explanations for the above differences are:

1. The house sparrow is a better host for North American than South American SLE viruses perhaps because of longer mutual adaptation. Three of 3 North American, but only 1 of 3 South American (1 of 4 if nearby Trinidad is included) strains produced good viremias of several days duration. The only exception was a human isolate from Belem, Brazil which might be more pathogenic than other strains.

2. The three North American strains used were isolates obtained during the major epidemic year 1975 from several sources. Perhaps these strains are atypical and different results would be obtained with other isolates from non-epidemic situations.

3. Differences in Passage history. The 3 North American and the Guatemalan strain were used at the 2nd or 3rd mouse passage. The strains from Trinidad and Jamaica have been passed at least 11 times in mice. The strains from Brazil were passed at Yale or Ft. Collins only 2 or 3 times, but an unknown number of times previously.

Further studies with non-epidemic North American strains and with higher passage material of the North American strains used above should further clarify the above possibilities.

(G. Stephen Bowen, Thomas P. Monath, Graham E. Kemp)

TABLE 2. Results of Experimental St. Louis Encephalitis Virus Infections in House Sparrows

Locality & Source of Virus (If known)	Passage Level	Age of Birds	No. Viremic / No. Inoculated	Duration of Viremia Range	Duration of Viremia Mean	Peak Viremia (Log_{10} DECC PFU/ml) Range	Peak Viremia (Log_{10} DECC PFU/ml) Mean
Culex pipiens quinquefasciatus	2	N*	5/5	3-5	3.6	3.3-5.6	4.5
Memphis, Tenn. 1975		Ad**	6/6	1-2	1.7	3.0-4.5	3.5
Culex salinarius	2	N	5/5	4	4.0	4.4->6.2	5.0
Chicago, Ill. 1975		Ad	6/7	1-3	2.5	3.0-4.0	3.7
Nestling House Sparrow	2	N	6/6	2-5	3.8	4.0-5.6	4.9
Indianola, Miss. 1975		Ad	5/15	1	1.0	2.5-4.3	3.6
Culex nigripalpus	3	N	5/5	3-4	3.2	3.6-4.8	4.2
Guatemala		Ad	6/6	1-3	1.7	2.3-3.3	2.9
Trinidad	11	N	3/5	1-2	1.3	2.3-3.0	2.8
		Ad	2/6	1	1.0	2.3-2.5	2.4
Jamaica	11	N	0/5	-	-	-	-
		Ad	2/6	1-4	2.5	3.3-4.2	3.8
Human	2 +	N	5/5	2-4	3.0	3.6-4.5	4.1
Belem, Brazil		Ad	5/6	2	2.0	3.2-3.8	3.5
Arthropod	3 +	N	0/5	-	-	-	-
Belem, Brazil		Ad	3/6	1-2	1.7	2.3-3.7	3.2
Sao Paulo, Brazil	2 +	N	2/5	2-3	2.5	3.0	3.0
		Ad	0/5	-	-	-	-

*Nestling

**Adult

+Unknown number of additional passages in South America

Serologic survey for antibody to Rio Grande virus, a new PHL group virus from south Texas

As reported in the Arbo Info. Exch. 30:105-108, 1976, a new Phlebotomus Fever group virus, tentatively designated as Rio Grande virus, was isolated from pack rats (Neotoma micropus) captured near Brownsville, Texas in 1973 and 1974. We have recently completed an antibody survey by neutralization testing of sera collected mainly during the 1973-74 studies. Human and equine sera were collected in 1971 during the VEE epizoodemic in south Texas. All sera were heated at 56°/30 mins and screened at a final 1:10 dilution with 100 PFU of Rio Grande virus in double-overlayed Vero cells grown in 1-oz. bottles. The results are shown in Table 3.

The preponderance of antibody found in N. micropus did not reflect the true antibody rate as there were fewer than 241 individuals tested. The disparity was due to a high recapture rate during the months of December, 1973 and January, February, March, April and May, 1974. Of 101 individuals, from which 267 serum samples were obtained, 210 were available for testing. To summarize the results:

a) Serologic conversions (<1:10 to ≥1:10) were noted in 21 of 50 individuals for which 2 or more specimens were available.

b) One of 2 bled between December, 1973 and February, 1974, 2/5 bled Jan. and Feb., 1/4 bled Jan.-March, 1/10 Jan.-April, 5/9 Feb.-Mar., 7/15 Feb.-Apr., 3/4 Mar.-Apr. and 1/1 Apr-May seroconverted.

c) 26 individuals had no antibody in single specimens.

d) 26 individuals had antibody in all (1 or more) specimens tested.

e) Seroconversion (February <1:10, April 1:80) was demonstrated in one N. micropus from which the virus was isolated in March and antibody in one bled in January, 1974, from which another isolate of this virus had been obtained in December, 1973.

Apparently virus is present and transmission occurs at least from December to May, the period of these studies.

From the isolation and serologic studies, it appears that the natural history of Rio Grande virus is fairly well restricted to a vector (as yet undetermined) and N. micropus. The absence of antibody in 314 humans suggests that this virus has little or no significance for man under natural conditions. Ten laboratory and field workers with possible if problematic exposure to the virus had no antibody.

(Charles H. Calisher)

TABLE 3. Results of Rio Grande Virus N Tests with Human and Animal Sera from South Texas.

Genus	Species	(≥1:10) Positive/Tested	% Positive
Neotoma	micropus	151/241	62.7
Didelphys	marsupialis	15/75	20.0
Gopherus	berlandieri	20/217	9.2
Equus	caballus	4/45	8.9
Reithrodontomys	fulvescens	1/19	5.3
Peromyscus	leucopus	5/113	4.4
Ovis	sp.	1/27	3.7
(birds)		5/256	1.9
Homo	sapiens	0/314	0
Spermophilus	mexicanus	0/30	0
Sigmodon	hispidus	0/98	0
Bos	sp.	0/50	0
Silvilagus	floridanus	0/18	0
Onychomys	leucogaster	0/10	0
Crotalus	sp.	0/4	
Oryzomys	couesi	0/3	
Rattus	rattus	0/2	
Perognathus	hispidus	0/1	
Dasypus	novemcinctus	0/1	
Phrynosoma	cornutum	1/1	
Pituophis	catenifer	0/1	
Thamnophis	sp.	0/1	
TOTAL		203/1527	13.3

Effect of feeding on different hosts on the reproductive capacity of swallow bugs

Attempts to colonize swallow bugs (Oeciacus vicarius) have been unsuccessful to date because of our inability to induce the bugs to lay eggs as needed. Engorged swallow bugs collected during the late summer have been held in the laboratory and have failed to oviposit. Further, bugs collected during fall, winter, and early spring and given blood in the laboratory also did not oviposit. In contrast, engorged bugs collected in May, 1976, and brought to the laboratory for holding, readily oviposited.

As part of a systematic inquiry into the factors affecting oviposition, a preliminary experiment was done using 3 different avians as hosts for blood feeding of bugs. A sample of adult bugs was collected from cliff swallow nests at a bridge near Ft. Collins, Colorado, where cliff swallows had failed to return in 1976. Bugs from these nests displayed the classically described behavior of concentrating around the conical neck portion of the cliff swallow mud nests. Results of the host feeding experiment are shown in Table 4. Since cliff swallows cannot be reliably sexed from external characteristics, the birds were dissected following completion of feeding to determine sex.

There did not appear to be a difference in egg deposition by swallow bugs fed on male and female swallows; therefore, the results from the males and females were combined for statistical comparison with house sparrows and chickens. There was a significant difference ($P < 0.05$) between the average number of eggs from engorged female swallow bugs which fed on cliff swallows and those which fed on either chickens or house sparrows.

The result of the experiment reported herein, along with the observations made on swallow bugs collected in the field, corroborates the close host association that has apparently evolved between the swallow bugs and cliff swallows. This is shown by the greater number of eggs from swallow bugs fed on cliff swallows and also by evidence from laboratory held field specimens that oviposition is seasonal, and occurs in association with the return of cliff swallows to their nesting sites.

(Vector Ecology Branch)

TABLE 4. Ovipositing from female swallow bugs (Oeciacus vicarius) fed on three different avian hosts

Lot #	Host	No. engorged females	No. of eggs*	No. of eggs per female
1	chicken	74	47	0.64
2	chicken	77	56	0.72
3	sparrow	99	80	0.81
4	sparrow	106	58	0.55
5	swallow, male	112	167	1.49
6	swallow, male	99	97	0.98
7	swallow, female	113	194	1.72
8	swallow, female	100	202	2.02
9	swallow, female	82	160	1.95
10	swallow, female	100	95	0.95

* Number of eggs deposited during the first 9 days after engorgement, at which time oviposition was virtually complete.

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

I. Venereal Transmission of La Crosse Arbovirus in Aedes triseriatus:

Demonstration of horizontal transmission of La Crosse (LAC) arbovirus from male to female Aedes triseriatus in mating cages and visualization with fluorescent antibody technique of large amounts of antigen in reproductive tract tissues (especially male accessory sex organs) prompted investigation of venereal transmission.

To eliminate possible modes of horizontal virus transmission other than venereal, the limited contact technique of induced insemination was used for mating.

Males were infected with LAC virus by intra-thoracic inoculation 15 or 16 days prior to mating. LAC antigen was routinely observed by FA in many tissues including accessory sex glands when males were dissected after use for induced insemination.

After mating, females were individua lly maintained in cartons for selected extrinsic incubation periods from 1 to 14 days, after which female abdomens were dissected and processed for FA visualization of LAC antigen in various organs. Remnant portions were individually stored frozen for later virus isolation studies.

LAC viral antigen was detected by FA and photographed in the accessory sex glands of males and in semen extruded from the ejaculatory duct, in bursal contents and mating plugs from recently-mated females from two otherwise LAC virus free colonies. Disseminated infections in venereally infected females into organs and other tissues other than those of the lower reproductive tract (including heart, ganglia, ovaries, gut, salivary glands and/or fat bodies) were detected in 13 of the 373 females dissected up to 14 days after insemination.

Isolations of LAC virus were obtained by intracranial inoculation into suckling mice of 1) remnants of males in which antigen had been visualized in accessory sex glands; 2) each of 2 pools of bursa from females dissected 24 hours after induced insemination, and 3) remnants from 6 of the 13 females in which disseminated viral antigen had been observed by FA in non-genital organs and tissues. Transovarially infected males have also been shown capable of venereally infecting females.

II. Fluorescent Antibody Studies of the Development of La Crosse Virus in Aedes triseriatus infected by Virus in Blood Meals:

Virus typically replicated to large amounts in the midgut and foregut, followed by disseminated infection into tissues or organs other than those of the alimentary tract in 75% of the mosquitoes by 9 days following infection by virus in blood meals. There was much individual variability

in mosquito-host response to infection. Individuals with highly infected midguts but no evidence of disseminated infection were still being detected as late as 16 days extrinsic incubation.

Ovarian or reproductive tract tissues, abdominal ganglia, fat body, and pericardial cells were typically the first disseminated organ system infections observed. There did not seem to be a predilection for any particular one of these systems. First appearance of virus seemed randomly divided among the before mentioned organs, supporting a hemolymph spread of LAC virus in Aedes triseriatus.

(Wayne Thompson and Barry Beaty)

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

THE ROLE OF WILDLIFE HOSTS IN THE SELECTION OF LA CROSSE VIRUS VARIANTS

Studies designed to determine the role of natural wildlife hosts in the selection of La Crosse (LAC) virus plaque size and antigenic variants continue. Attempts were made not only to detect variation in the laboratory but to seek evidence for similar variation in nature.

La Crosse Virus Plaque Variants

Two experimental approaches were used; first, prototype LAC virus was plaque purified into a population of either predominantly large (LP) or small (SP) plaque type virus for natural host inoculation studies. Second, serial passage of field origin LAC virus to chipmunks (Tamius striatus) and gray squirrels (Sciuris carolinensis) by A. triseriatus was accomplished in the laboratory to determine if plaque or antigenic variants emerged.

Comparison of LP and SP replication rates in Vero cells, thermal inactivation rates (Fig. 1) at three temperatures (38° C, 24° C, and 17°C), and antigenic characteristics by the plaque reduction neutralization test (PRNT) demonstrated that both plaque types were similar to each other and to the prototype LAC virus from which they were derived.

The small size of SP variant and prototype LAC virus plaques was due to a component(s) present in the gum tragacanth overlay. The action of the component(s) was prevented by use of diethylaminoethyl dextran (polyanion) but not dextran sulfate (polycation) suggesting that the gum component(s) had a positive charge (Table 1).

Partial virus attenuation due to Vero cell passage occurred for chipmunks; gray squirrels were refractory to infection. Additional chipmunk passage increased virus infectivity for that species. Inoculation of gray squirrels with virus recovered following one chipmunk passage resulted in viremias of low titer and short duration in one of the three gray squirrels inoculated with the SP variant and both of those inoculated with the LP variant. The other gray squirrels did not develop viremias or an antibody response. A second passage of the SP variant in gray squirrels resulted in viremias in two of three animals inoculated, but with titers lower than the first passage. Thus, one chipmunk passage greatly increased virus infectivity for the same species but only partially for gray squirrels.

Serial chipmunk and gray squirrel passage of inoculated virus did not select for LAC virus of a specific plaque size or antigenic characteristic (Table 2). This result was partially confirmed by a mixed plaque infection experiment where each plaque type replicated and appeared independently on consecutive days of the chipmunk viremia.

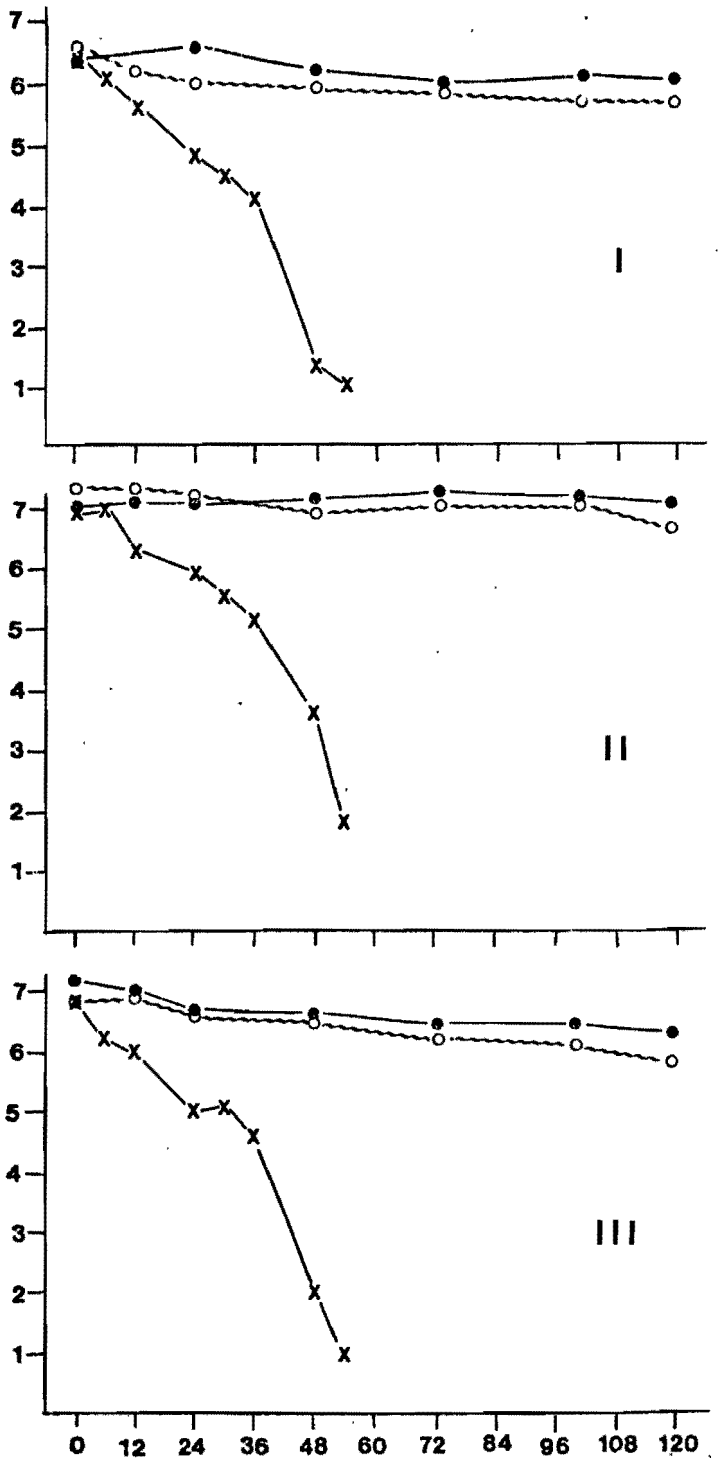

Figure 1. Thermal inactivation of prototype (I) and plaque purified large (II) and small (III) plaque type La Crosse virus at 17° C (●), 24°C (o), and 38°C (X). abscissa: hours of incubation; ordinate: virus titer in log PFU/ml.

Table 1. Effect of DEAE-dextran or dextran sulfate on the plaque size of prototype, large, and small plaque variant La Crosse virus

La Crosse virus	Overlay	Number of plaques	Plaque size in ocular units		
			Range	Mean	SD ± 1
Prototype	N	127	10-190	49	39.0
	D	120	60-200	161	22.5
	S	135	10-190	57	46.2
Large	N	40	80-210	166	28.4
	D	30	90-200	170	22.4
	S	27	140-210	176	20.6
Small	N	101	10-70	33	8.8
	D	120	30-180	142	25.3
	S	83	10-60	34	9.1

Symbols used include: N = normal overlay; D = 25 ug DEAE-dextran/ml; S = 25 ug dextran sulfate/ml. of overlay; SD = standard deviation.

Table 2. Comparison of plaques produced by prototype La Crosse virus to large and small plaque variant virus before and after passage in chipmunks and gray squirrels

Plaque diameter in ocular units	Prototype*	La Crosse virus plaque variant serial passage in one chipmunk and one gray squirrel@					
		Large			Small		
		Stock	CM	SQ	Stock	CM	SQ
≤5-15	7	0	0	0	0	2	7
16-25	45	0	0	0	23	52	67
26-35	97	0	0	1	67	108	134
36-45	35	1	3	2	29	54	43
46-55	4	2	6	1	2	1	3
56-65	1	3	4	5	0	3	2
66-75	1	2	10	7	0	0	0
76-85	2	3	19	23	0	0	0
86-95	1	7	33	33	0	0	0
96-105	4	16	65	62	0	0	0
106-115	2	14	45	39	0	0	0
116-125	1	9	17	29	0	0	0
126-135	1	4	3	6	0	0	0
136-145	0	1	0	0	0	0	0
146-155	0	0	1	0	0	0	0
Total plaques assayed	201	62	206	208	121	220	256
Mean plaque size	34	100	97	99	31	30	29
Standard deviation (±)	18.9	21.2	18.0	17.3	7.0	8.1	8.1

* Prototype La Crosse virus after five passages in suckling mouse brain.

@ Virus used was first SMB passage of tissue culture reference stocks and whole animal blood.
Symbols used include: CM = chipmunk; SQ = squirrel.

A biological transmission cycle was established under laboratory conditions using colonized A. triseriatus to infect chipmunks and gray squirrels with a LAC virus pool containing 5 field isolates from adult A. triseriatus. These natural hosts maintained LAC virus through three serial transmission cycles. After three serial transmission cycles in either vertebrate, results suggested that neither chipmunks nor gray squirrels selected for virus producing a specific plaque size, nor were changes in viral antigens detected. These results are in contrast to earlier work in our laboratory with prototype LAC virus. Passage of the prototype virus in squirrels rapidly selected for a small plaque variant, and passage in chipmunks a large plaque variant. These variants showed slight antigenic differences when compared in the PRNT. The difference may have been due to inherant or passage-induced differences between the strains of LAC virus.

Our results suggest that A. triseriatus, chipmunks, and gray squirrels are closely associated with the summer amplification cycle of LAC virus in Wisconsin. None of these hosts selectively replicated virus to produce a specific plaque size. Therefore, the importance of predominantly LP types in LAC virus field isolates is still uncertain. The antigenic stability of LAC virus following passage in these same natural hosts may reflect a long association with this vector and these vertebrates.

(W. R. Hansen, T. M. Yuill, G. R. Defoliart)

Squirrel and chipmunk sentinels of La Crosse virus

Six isolates of La Crosse (LAC) virus were obtained from sentinel gray squirrels and four from sentinel chipmunks placed in a known LAC endemic area. Viremia titers were measured by plaquing on Vero cells. Antibody responses of the animals were measured by a microneutralization test employing four California group viruses: LAC, snowshoe hare (SSH), trivittatus, and Jamestown Canyon. LAC antibody response titers in both species peaked at approximately twenty-one days and was still detectable at 300 days post viremia (Fig. 2). In chipmunks, homologous LAC antibody levels were consistantly higher than heterologous antibody responses throughout the period recorded. However, squirrels demonstrated approximately the same levels of antibody to LAC and the heterologous SSH initially; this heterologous SSH titer soon subsided while LAC antibody levels remained relatively high. Data indicate that antibody response persists from one summer season to the next. Also, viremia titers in both species indicate that these two species are capable of infecting Aedes triseriatus, the principle vector of LAC virus. This is the first reported field isolation of LAC virus from the squirrel.

Comparisons of La Crosse (LAC) virus strains, obtained from sentinel squirrels and chipmunks, were made using three viral markers: plaque size on Vero cells, virulence in eight day old laboratory mice, and antigenic characteristics as measured by the plaque reduction neutralization test. All strains were in their first suckling mouse brain passage. The mean plaque size of viruses isolated from squirrels was slightly larger than the mean plaque size of viruses isolated from chipmunks. There were no differences in virulence and antigenic characteristics between LAC strains isolated from chipmunks compared

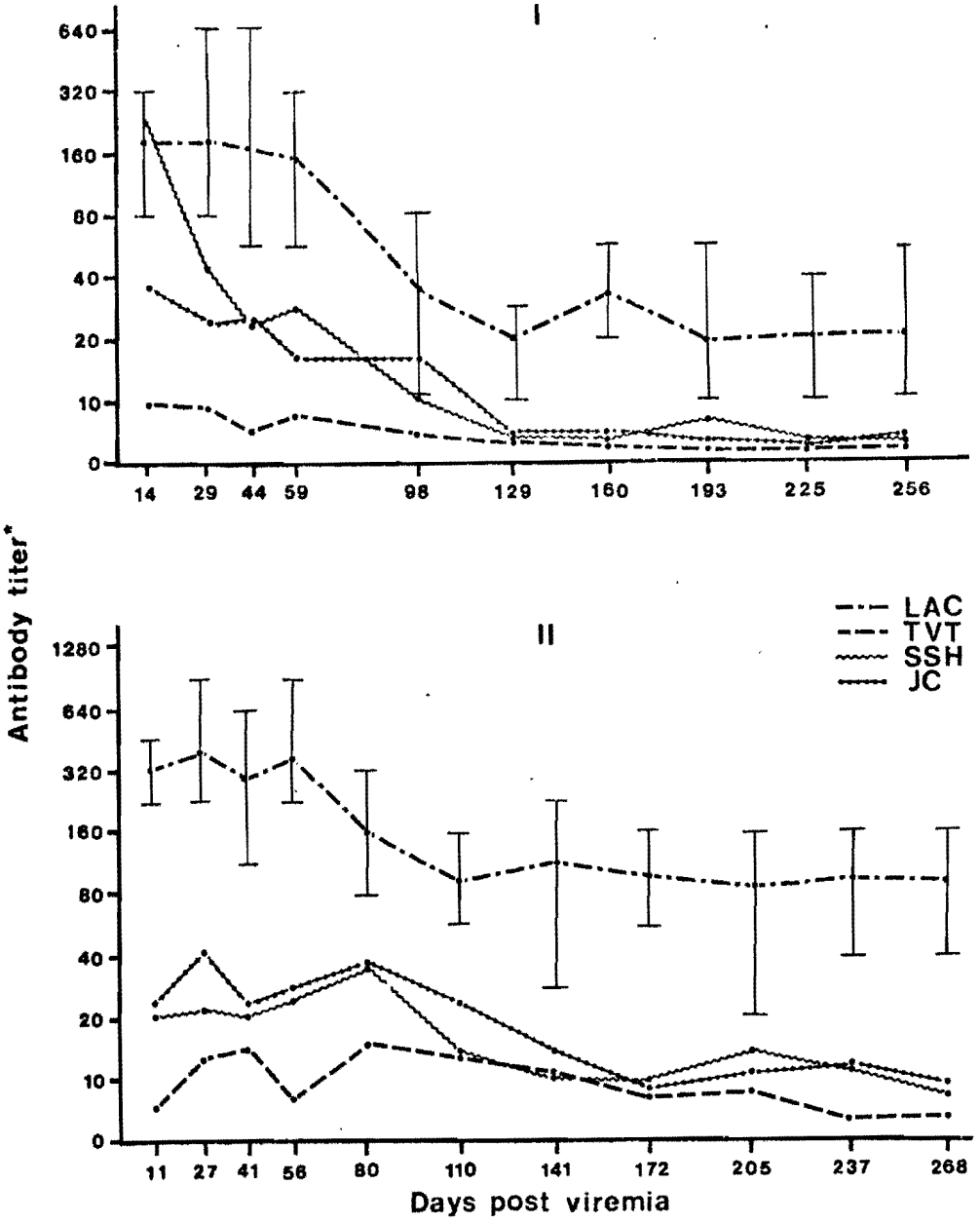

Figure 2. Geometric mean titers of six naturally infected squirrels (I) and four naturally infected chipmunks (II). Titers measured by microneutralization test using four California group arboviruses: La Crosse (LAC), snowshoe hare (SSH), trivitattus (TVT), and Jamestown Canyon (JC). The range of titers about the geometric mean of LAC virus antibody is represented by a bar.

* Antibody titer is expressed as the reciprocal of the highest dilution of serum neutralizing between 1.5 and 2.5 $\log_{10}$ of the test virus.

to those from squirrels (Table 3 and 4). However, significant differences in these characteristics between individual strains did occur. First suckling mouse brain passage of viremic blood apparently selected for smaller mean plaque size. These results indicate that the squirrel and chipmunk were not markedly and rapidly selecting for divergent subpopulations of the three measured markers in nature. There were some indications, however, that even one suckling mouse brain passage of field LAC virus apparently decreased mean plaque size.

(T. G. Ksiazek, T. M. Yuill)

AEDES TRISERIATUS - LACROSSE VIRUS MODELING

Preparatory to attempting to model the epidemiology of LAC virus several studies were initiated in 1974 with the aim of quantifying the vertical transmission component, especially as it exists in Iowa County, Wisconsin. One objective was to determine the virus prevalence in overwintering eggs of A. triseriatus at sites of known LAC virus endemicity. Isolations of virus from larvae from overwintering eggs revealed virus prevalence of between 0.28 and 0.59% at two sites during two consecutive winters. At other localities studied, virus prevalence did not exceed 0.14% in overwintering eggs.

Other objectives were to determine the transovarial and filial infection rates of LAC virus when passed transovarially through several successive generations of the mosquito vector and whether the virus retains its infectivity to warm-blooded hosts after such passage in the mosquito. The transovarial transmission rate observed was 98% with 44 of 45 A. triseriatus females used to perpetuate the line through 7 generations, transmitting virus to their offspring. The mean filial infection rate (percentage of transovarially infected offspring from an infected female) was 71% through the7th generation and showed no indication of declining in the later generations. Virus retained its infectivity to warm-blooded hosts through 6 generations of passage in the mosquito, as suckling mice and chipmunks fed upon by F_6 A. triseriatus females became infected. F_7 females was not fed on chipmunks but transmitted virus to suckling mice.

(G.R. DeFoliart, B. R. Miller, M. A. Lisitza, P. J. Scholl)

TABLE 3. Comparisons of virulence of virus isolates from sentinel squirrels and chipmunks in eight day old mice by IC and SC inoculation routes

Isolate Source	Titer (LD_{50}/ml) ± S. D.		
	IC/SC	IC	SC
LAC (Prototype)	1.68 ± 0.22	8.24 ± 0.10	6.56 ± 0.12
SQ-1-75	1.68 ± 0.23	8.24 ± 0.10	6.56 ± 0.12
SQ-14-75	1.94 ± 0.27	8.48 ± 0.14	6.54 ± 0.13
CM-1-75	2.01 ± 0.16	8.52 ± 0.09	6.51 ± 0.08
CM-10-75	2.02 ± 0.18	8.79 ± 0.09	6.77 ± 0.09
SQ-3-75	2.07 ± 0.18	7.23 ± 0.09	5.16 ± 0.09
SQ-7-75	2.10 ± 0.19	8.33 ± 0.09	6.23 ± 0.10
CM-3-75	2.14 ± 0.24	8.62 ± 0.11	6.48 ± 0.12
SQ-13-75	2.16 ± 0.18	8.34 ± 0.09	6.19 ± 0.09
SQ-6-75	2.18 ± 0.21	8.20 ± 0.12	6.02 ± 0.10
CM-9-75	2.32 ± 0.23	7.57 ± 0.08	5.25 ± 0.15

CM denotes isolate from a sentinel chipmunk
SQ denotes isolate from a sentinel squirrel
LAC prototype included for comparative purposes.

TABLE 4. Results of PRNT's comparing viruses isolated from sentinel squirrels and sera from those same sentinel animals

Virus	Serum (HMAF for LAC-5)										
	SQ-1	SQ-3	SQ-6	SQ-7	SQ-13	SQ-14	CM-1	CM-3	CM-9	CM-10	LAC-5
SQ-1-75	411*	2821	667	405	434	271	756	2422	1810	804	2153
SQ-3-75	422	3748	542	399	804	495	868	3130	2880	1736	3875
SQ-6-75	565	3774	523	378	553	534	735	2669	2860	850	3425
SQ-7-75	294	2123	453	297	459	267	553	1587	1940	745	2065
SQ-13-75	290	2763	428	350	565	284	523	1835	2323	821	1749
SQ-14-75	299	3521	431	299	614	341	472	2198	2490	772	2079
CM-1-75	391	1913	492	383	527	258	720	1887	2372	696	1654
CM-3-75	318	2405	459	336	585	311	636	2307	2706	899	2168
CM-9-75	360	3401	605	391	63[illegible]	416	833	3331	2560	1307	4614
CM-10-75	408	4551	691	449	997	422	886	2578	5681	1430	3595
LAC-5	440	5050	745	443	569	453	1316	2840	2801	1654	6803

* Expressed as the reciprocol of the dilution of serum calculated to have reduced by 50% an initial number of from 50-100 plaque forming units.

REPORT FROM THE VETERINARY FACULTY, UNIVERSITY OF ANTIOQUIA, COLOMBIA, AND THE VETERINARY SCIENCE DEPARTMENT, UNIVERSITY OF WISCONSIN (COLLABORATIVE PROGRAM)

Experiments are in progress to test the hypothesis that black flies can serve as vectors of Venezuelan equine encephalitis (VEE) virus. Sanmartin and colleagues at the University of Cali, Colombia, and we, working in Antioquia, have isolated VEE virus from Simulium spp. However, the vector capability of these black flies is unknown.

We located areas in Aburrá Valley where reasonable numbers of S. mexicanum could be captured by aspiration as they came in to feed on calves or horses. This species was selected because of the three black fly species from which VEE virus has been isolated, it was the largest and most robust, and appeared to be most easily adapted to laboratory conditions.

The past several months has been spent developing methods for laboratory maintenance, feeding and refeeding these blackflies. The black flies are held in individual 1-dram vials with screen tops, and have 5% honey in cotton continuously available. The flies are held at 4°C overnight following capture, lightly anesthetized for inspection to confirm the species as S. mexicanum and to discard partially engorged individuals, and then given access to a VEE viremic guinea pig. Guinea pigs 24 and 48 hrs postinoculation with 10^8 $SMLD_{50}$ of virus develop viremias sufficient for the flies to ingest 10^3 - 10^6 $SMLD_{50}$ of virus.

The flies experience continuous mortality. Half die within 5-8 days but a small percentage usually survives to day 12-15 post feeding. The flies refeed, albeit somewhat reluctantly, on guinea pigs, from postinfectious blood meal day 8 onward.

Virus has been detected in the flies in substantial titer ($10^{4.5}$) up to 14 days postinfectious blood meal. Mice dying following inoculation of the blood from guinea pigs upon which the black flies refed, and guinea pigs that died following refeeding by the flies, are being stored frozen, awaiting tests to determine if VEE virus is present. Thus, it is evident that VEE virus can persist for several days in S. mexicanum, but it is not yet clear whether this black fly is capable of transmission.

F. N. Zuluaga (Antioquia) and T. M. Yuill (Wisconsin)

REPORT FROM THE ARBOVIRUS RESEARCH UNIT, VECTOR BIOLOGY LABORATORY,

UNIVERSITY OF NOTRE DAME, NOTRE DAME, INDIANA 46556

Arbovirus research was initiated at the Vector Biology Laboratory in the fall of 1974. Emphasis is on genetics of vectorial capacity for arboviruses in species of Aedes. La Crosse virus (California group) and Aedes triseriatus (Say) are being studied as components in a model system. The role of another treehole mosquito, Aedes hendersoni Cockerell, in the cycle of La Crosse encephalitis is also being investigated.

Geographically diverse strains of A. triseriatus were infected with La Crosse virus and subsequently tested for ability to transmit the virus. Initially, mosquitoes were allowed to engorge on a blood-virus mixture offered in a membrane feeder. Titers of the blood-virus mixture averaged $10^{5.0}$ $SMLD_{50}$ per 0.03 ml inoculated intracerebrally (titer range of $10^{4.5}$ to $10^{5.4}$). After 20-24 days, individual female mosquitoes were refed on individual suckling mice. Among the 20 strains of A. triseriatus tested, 14 were recently collected from the field and 6 were established laboratory strains, including one marked with the mutant yellow larva. All strains were tested against a common control strain.

Transmission rates varied from 20% to 90% among the different strains tested. In 7 replicates with the control strain, transmission averaged 60%, with a range of 50-75%. Replicate trials with low- and high-transmitting strains never gave more than a difference of 12%. A distinct geographical pattern was noted. Field strains from the region where La Crosse virus occurs most frequently in humans (southeastern Minnesota, southwestern Wisconsin, Iowa, Illinois, Indiana, Ohio, upstate New York) had significantly lower transmission rates (mean 44%, range 27% to 65%) than strains obtained outside the above endemic region (North Carolina, Florida, northwestern Minnesota,

northern Wisconsin, northern and central Michigan, Texas) where there was a mean of 74% and a range of 67% to 90%.

Results also show that rapid changes in transmission rate can occur as strains become laboratory-adapted. This change can be noted as early as the 3rd or 4th generation of laboratory rearing. The size of the colony is also important; small colonies, under 200 pair, appear to exhibit more rapid changes in transmission rate than do large (500+ pair) freemating colonies.

(Paul R. Grimstad, George B. Craig, Jr.)

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

Arbovirus Surveillance

Our current surveillance program up to mid-August has failed to detect any evidence of reappearance of St. Louis encephalitis virus in New York State (in 1975 SLE virus infections were confirmed in 7 patients). A total of 224 patients with CNS infections were studied so far. No arboviruses were isolated and no antibody conversions to EE, WE, SLE, POW or CE were observed.

Specimens from 14 horses which had an onset of illness with signs of CNS infection between June 28 and August 24 were examined. Thirteen of these horses died, 1 was euthanized. An etiology of EE was confirmed in 5 of the horses by isolation of EE virus from the brain of 4 horses from Oswego County, and by demonstration of significant rises of HI antibody from 320 to 2560 against EE virus in a horse from Onondaga County. In both counties, EE virus activity was noted repeatedly in the past.

Isolation attempts on suspensions of 1, 648 pools of 76, 602 wild caught mosquitoes yielded 2 isolates of CE virus from Aedes communis mosquitoes collected in Saratoga County from June 3 - 8. CE virus has been shown to infect man in New York State and this year's isolates represent one of the earliest appearances of the virus during our summer surveillance season. In addition, one isolation of Flanders virus was made from Aedes vexans collected in Schenectady County on June 10.

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

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*Joined the staff in July, 1976.

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

Clinical Trial of Inactivated, TC-83 Venezuelan Equine Encephalomyelitis Vaccine.

Almost 15 years of experience with attenuated TC-83 vaccine in man has shown the vaccine to be effective, but fraught with several undesirable side effects. Adverse reactions have included febrile influenza-like illness in 15-30% of vaccinees together with circumstantial evidence for abortogenic and teratogenic effects. In addition, the vaccine has not proven very effective in boosting antibody titers in persons previously vaccinated and who have low or undetectable antibody titers at the time of attempted reimmunization. The use of an inactivated VEE vaccine prepared from the attenuated TC-83 strain should eliminate many of the adverse reactions and poor booster responses encountered with the live virus vaccine strain. By using an inactivated TC-83 vaccine the problem of incomplete inactivation, which was an undesirable feature of other inactivated VEE vaccines, becomes less critical. For even if the virus were not completely inactivated, vaccine reactions should be no more severe than those encountered routinely in the use of the attenuated vaccine. We have thus initiated studies to determine the reactogenicity and antigenicity of inactivated TC-83 virus vaccine in volunteers. Potency tests in animals indicated that this new vaccine induced antibodies and protected against lethal VEE virus challenge. The vaccine is the formalin-inactivated VEE virus harvested from the second passage of attenuated TC-82 virus grown in primary chick-embryo cell cultures. The vaccine was freeze-dried and reconstituted with sterile water before inoculation.

We studied 2 groups of volunteers. The first group of 6 persons was composed of healthy immune adults who were previously vaccinated with attenuated TC-83 vaccine. Each volunteer had VEE HI antibody titers $\geq$ 1:20 and plaque reduction neutralizing (NT) titers $\geq$ 1:80. Two subjects were administered 0.1 ml of the test vaccine intradermally and 4 subjects were inoculated subcutaneously with 0.5 ml. No adverse local or systemic reactions were encountered over a 14-day follow-up period which included repeated hemograms, platelet counts, liver and renal chemistries, chest x-rays, electrocardiograms, clinical reaction reporting forms, and twice-daily temperature checks. The VEE (TC-83) neutralizing antibody titers rose in all 6 volunteers to $\geq$ 1:320 by 7 days postvaccination and were sustained for at least 1 month. Four of the 6 volunteers had a $\geq$ 4-fold boost in HI antibody titers to VEE virus. Late postvaccination antibody titers are pending.

The second group of 6 volunteers was composed of healthy adults having no known immunity to VEE or other group A arboviruses. The prevaccination VEE, WEE, and EEE NT and HI antibody titers in this group were <1:10 and $\leq$ 1:20, respectively. They were inoculated subcutaneously with 0.5 ml of the test vaccine. Again, no local or systemic reactions were noted during the 14-day clinical follow-up period. VEE NT antibody titers were first detected 7 or 14 days after inoculation; maximim titers

of 1:160-1:1280 were achieved by days 14-28 in all vaccinees. VEE HI antibody titers in 4 of 6 vaccinees rose 4-fold by day 14 after the primary inoculation to maximums of only 1:20-1:40. A booster dose of 0.5 ml was safely administered 28 days after the primary dose. The postbooster antibody titers to TC-83 and other strains of the VEE virus complex are pending.

These preliminary clinical and serological results suggest that the inactivated TC-83 VEE vaccine is nonreactogenic and highly antigenic in healthy immune and nonimmune adult volunteers. The vaccine has the potential for use in human populations threatened by a VEE virus epidemic, because a single dose should induce serum neutralizing antibody within 7-14 days, and it can be used safely in adults of both sexes. Studies are needed to verify its safety and antigenicity in children.

ROBERT EDELMAN

REPORT FROM THE DEPARTMENTS OF ENTOMOLOGY AND VIRUS DISEASES, WALTER REED ARMY INSTITUTE OF RESEARCH, WASHINGTON, D.C. 20012

Serological surveillance studies conducted in 1972 and 1973 revealed evidence of Keystone (KEY) virus infection in gray squirrels of the coastal plains of Maryland and Virginia. In 1975 investigation was initiated to determine the extent and seasonal distribution of KEY virus infection in gray squirrels in relation to the seasonal occurrence of KEY virus infection in Aedes atlanticus.

A total of 90 gray squirrels consisting of 72 adults and 18 juveniles were captured between 24 March and 5 September, 1975 in the Pocomoke Cypress Swamp (PCS) of Maryland. Juvenile squirrels first appeared in traps during the middle of July. Twenty-six squirrels were single captures and 64 were recaptured 1 to 14 times. The mean recapture frequency was 4.2 times for males and 4.4 for females. Attempts to capture squirrels after 5 September were unsuccessful.

Of 88 squirrels captured, 56% possessed serum neutralizing antibody that caused 50% or more reduction of KEY virus plaque forming units. No significance difference in antibody prevalence rates was observed between adults and juveniles or between sexes of either age class. The seasonal distribution of KEY virus antibody in sera of squirrels in relation to the A. atlanticus activity period is presented in Figure 1. Antibody was detected in the squirrel population during the spring and early summer months, but antibody resulting from active KEY virus infection was not observed until 27 July (13th week of the study), or approximately 10 days after the emergence of A. atlanticus. Seroconversions continued to occur resulting in an increase in accumulative antibody prevalence rates from 10% on 27 July to 70% or greater for the 16th through the 19th week of the study.

No evidence of Jamestown Canyon (JC) virus infection was detected in the gray squirrel population; however, 86 sera of those that contained KEY virus antibody cross reacted with JC virus in PRNTS.

Only those gray squirrel blood specimens taken after 15 July, the beginning of the A. atlanticus activity period, were assayed for virus. Of 251 specimens tested, only a single virus isolation was made from an adult female captured on 3 September. This virus was apparently not JC or KEY. On 6 August (5th recapture), serum from the squirrel was positive for KEY virus antibody that was also detected on 3 and 5 September. Primary inoculation of 3 to 5 day old mice via the intracerebal (IC) route with a 1:5 dilution of the squirrel blood caused 1 of 5 mice to die on day 8 post inoculation (PI). Subsequent passages (IC) of a 20% mouse brain diluent suspension prepared from the mouse caused 100% mortality of mice on day 6 PI. Characterization and identification studies indicate that the virus was sensitive to chloroform and that it replicated in BHK-21, Clone 15 cell culture. Infectivity of the virus was not reduced in PRNTS employing KEY,

JC and St. Louis encephalitis (SLE) virus antisera. Similar studies employing antisera of other viruses enzootic in the PCS are in progress.

A total of 64,720 A. atlanticus were assayed for KEY virus during 1975. Preliminary results revealed approximately 200 virus isolates. Studies are being conducted to identify the virus isolates.

(D.M. Watts, D.E. Hayes, J.W. Taylor, C.L. Bailey, B.F. Eldridge, J.M. Dalrymple, F.H. Top and P.K. Russell)

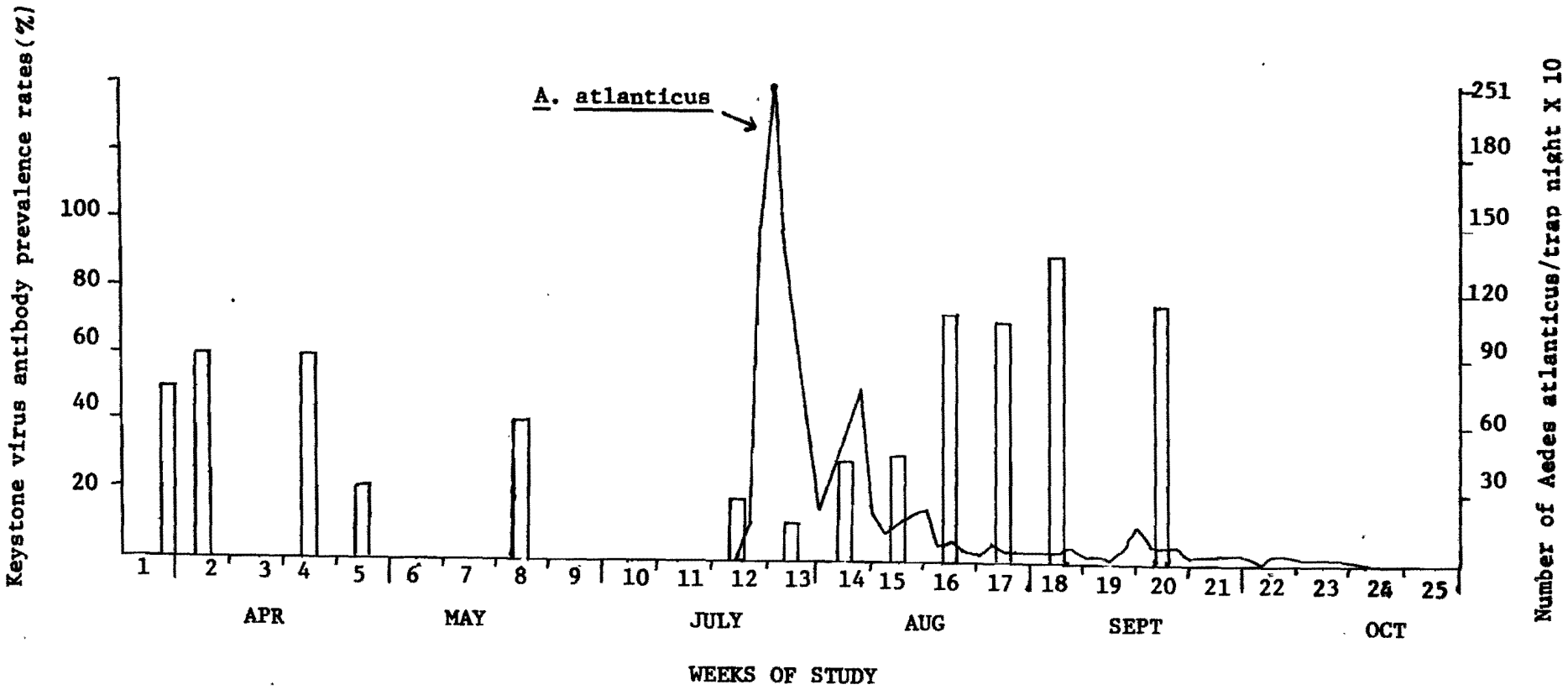

FIGURE 1. Accumulative Keystone virus antibody prevalence rates in gray squirrels in relation to Aedes atlanticus activity period.

REPORT FROM THE MEMPHIS-SHELBY COUNTY HEALTH DEPARTMENT

MEMPHIS, TENNESSEE

ST. LOUIS ENCEPHALITIS VIRUS SURVEILLANCE

Since April 5, 1976, the Insect Vector Control Division, Memphis-Shelby County Health Department, has maintained a continuous SLE Surveillance Program involving sparrows (including nestlings) and sentinel chicken flocks. Using HI determinations, 30-40 blood sera, from wild-caught sparrows and sentinel chicken flocks, are checked daily for SLE antibody activity. Tabulated below are data for the testing interval April 5, 1976 - July 31, 1976.

Collection Area	Species	Age	Test Performed	No. Tested	No. Positive*
Memphis	Sparrow	Adult	HI	467	18
Memphis	Sparrow	Juvenile	HI	1,017	41
Memphis	Sparrow	Nestling	HI	160	8
Memphis	Starling	Adult	HI	1	0
Memphis	Cowbird	Adult	HI	2	0
Memphis	Catbird	Adult	HI	2	0
Memphis	Chicken	-	HI	593	5
Memphis	Pidgeon	Adult	HI	2	0
Memphis	Mockingbird	Adult	HI	1	0

*Titers generally are $\geq$ 1:20; but some are $<$ 1:20

2,235 total sera were tested during the given interval. Of this number, 45 sera (2.00%) indicated SLE antibody activity.

Submitted by: James G. Hamm

REPORT . . . . ECTOR BIOLOGY AND CONTROL DIVISION (VBCD)

BUREAU OF TROPICAL DISEASES, CENTER FOR DISEASE CONTROL

ATLANTA, GEORGIA

At the VBCD, studies on arthropod vectors of disease include investigations on methods of surveillance and control of Aedes aegypti populations. Interest in this mosquito species is based on the history of dengue fever epidemics in the continental United States, the rather constant threat of dengue fever in Puerto Rico and other nearby Caribbean areas, and the more remote potential of urban yellow fever transmission in this hemisphere.

The Division is making a deliberate effort to determine: (1) the current status of the presence and distribution of Ae. aegypti in the in the United States; (2) the status of surveillance and control activities throughout the hemisphere; (3) the susceptibility of isolates of the species to existing insecticides and those under development; and (4) the status of resources and contingency plans for dealing with emergency control needs. With sufficient information of this type VBCD will be in a position to provide consultation and assistance during epidemic and interepidemic periods toward the establishment of adequate surveillance and control capabilities in affected areas.

Initially, efforts have been made to assess Ae. aegypti populations in areas of the United States potentially receptive to dengue and yellow fever viruses. Particular attention has been given to the presence and distribution of the species, its susceptibility to EPA-approved insecticides as space sprays, and existing surveillance and control activities. During the summer months of 1976, 20 urban areas (Harlingen, McAllen, Laredo, San Antonio, Houston, and Dallas, Texas; New Orleans, and Alexandria, Louisiana; Jackson and Meridian, Mississippi; Birmingham and Mobile, Alabama; Little Rock, El Dorado, and Texarkana, Arkansas; Memphis and Nashville, Tennessee; Spartanburg and Greenville, South Carolina; and Charlotte, North Carolina) have been selectively surveyed for presence of the species. All areas have been positive for Ae. aegypti with the exception of Memphis, Tennessee. The survey will be concluded during this summer and fall with studies in additional Atlantic and Gulf Coastal areas. The information will be used to establish baselines for future activities. Insectary colonies of the species from 15 of the locations have been established at Chamblee for insecticide susceptibility studies.

Plans are being developed to participate in collaborative field trials with the New Orleans Mosquito Control group to evaluate aerial Ultra Low Volume applications of EPA-labeled adulticides as an Aedes aegypti control measure.

(D. Eliason and S. Breeland)

REPORT FROM THE ARBOVIRUS DIAGNOSTIC LABORATORY

VIROLOGY DIVISION, BUREAU OF LABORATORIES, CENTER FOR DISEASE CONTROL

ATLANTA, GEORGIA

I. Serologic follow-up on St. Louis encephalitis cases 8-9 months post infection.

A cooperative St. Louis encephalitis surveillance project was set up in late spring of 1975 between the arbovirus diagnostic laboratory, CDC, Atlanta, and the Jefferson County Health Department, Birmingham, Alabama. Hospitals in the Birmingham area reported weekly or daily, as necessary, any newly admitted patients with suspect arboviral encephalitis. Serum samples were collected as early after onset as possible, and again approximately two weeks later. In some instances a third specimen was obtained. The acute specimens were tested immediately by hemagglutination-inhibition (HI) and plaque reduction neutralization (NT) in duck embryo cell culture. (CF tests were delayed until the convalescent specimens were available.) The NT titers were generally higher than the HI titers in these acute specimens and sometimes gave a more convincing indication of current infection than the HI titer.

From the latter part of July to late September, 1975, 45 cases were confirmed as SLE on the basis of 4-fold or greater rises by HI, CF and/or NT. An additional 9 cases were classed as presumptive, judged by high HI and NT titers, compatible with recent infection, plus a CF titer $\geq$ 16). Anticipation of possible carry-over of SLE activity into 1976 presented the problem of distinguishing, on the basis of antibody levels, old (1975) cases from any new ones which might occur. Therefore, arrangments were made to collect additional serum samples in May, 1976, from several of 1975's proven cases 8 to 9 months post infection in order to determine how much decline in HI, CF and NT titer to expect. The HI, CF and NT titers on 8 confirmed cases are shown in Table 1. HI titers dropped to low levels (10-20) in all cases except one, and the CF titers essentially disappeared. The NT titers also dropped but not as much as the HI; the lowest was 40 and the highest was 320.

These results are in concurrence with the accepted norm of antibody response. HI and NT titers rise early after infections to high levels, while the CF titer rises 2-3 weeks later. Several months post infection HI and NT titers still remain but at lower levels, and the CF titer has disappeared or become insignificantly low.

(Helen S. Lindsey (CDC) and Michael Maetz, Jefferson Co. Health Department, Alabama.)

Table 1. Serology for St. Louis encephalitis cases occuring in 1975 (Birmingham area)

ARL Serum #	(Age/Sex) Initials	Onset Date	Coll't Date	SLE Serology HI	CF	NT
2326	S.C. (46F)	8-16-75	8-20-75	10	<8	320
2430			8-29-75	160	<8	2560
4169			5-11-76	20	<8	160
2533	M.C. (_F)	9-01-75	9-05-75	20	<8	40
2708			9-15-75	80	32	640
4164			5-12-76	10	<8	40
2580	JEC (34M)	9-01-75	9-08-75	160	<8	1280
2901			9-18-75	40	<8	320
3313			10-13-75	40	8	320
4165			5-11-76	10	<8	160
2334	W.G. (51M)	8-01-75	8-20-75	40	<8	1280
2432			8-29-75	160	16	5120
4170			5-11-76	20	<8	80
2325	E.H. (34F)	8-05-75	8-20-75	10	QNS[a]	QNS
2434			8-28-75	40	8	160
4168			5-13-76	20	8	80
2206	C.M^{C}E (36F)	8-01-75	8-08-75	40	<8	80
2306			8-18-75	80	<8	320
2581			9-08-75	40	32	320
4167			5-11-76	10	<8	80
2528	V.M. (_F)	9-01-75	9-05-75	40	<8	160
2702			9-16-75	80	32	640
3295			10-08-75	80	32	160
4171			5-11-76	160	AC[b]	320
2416	D.T. (_F)	-	7-11-75	40	<8	640
2417			7-26-75	320	<8	10,240
2418			8-18-75	160	32	5,120
4166			5-12-76	20	<8	320

[a]Quantity not sufficient for testing
[b]Anti-complementary

II. La Crosse virus soluble cell culture antigen.

Supernatant fluids from La Crosse virus-infected BHK-21 cell cultures grown in roller bottles were clarified, virus pelleted, and treated with saturated ammonium sulfate to produce a soluble antigen. This antigen was applied in several test systems to evaluate its reactivity as compared with the standard suckling mouse brain preparations currently used by most arbovirologists.

A. Immunodiffusion (ID) and counterimmunoelectrophoresis (CEP) tests.

Evaluation of La Crosse cell culture antigen by ID tests and CEP tests showed its superiority over suckling mouse brain preparations. Whereas suckling mouse brain antigens in ID tests have been described as producing single arcs, double arcs, triple arcs and "multiple dense reaction" (1,2) the cell culture antigen did not show these confusing patterns but gave a precise, clear line. Equally by CEP, precipitin lines of the cell culture antigen were more precise, clear and reproducible. CEP was the preferable test due to its distinct precipitin reactions and rapidity (the CEP test is read in 1.5 hours).

Cross reactivity of the La Crosse cell culture antigen in CEP against hyperimmune mouse ascitic fluids of eight other California group viruses showed strong equivalent precipitation intensity against California encephalitis, snowshoe hare, and Jamestown Canyon; a much lesser reaction for San Angelo and Trivittatus; and little or no reaction to Melao, Keystone and Tahyna. Specific reactions probably would have been obtained had single shot antisera been available for testing rather than hyperimmune ascitic fluids.

B. CF test.

Ammonium sulfate precipitated cell culture antigen could not be used for complement-fixation (CF) due to the considerable anticomplementary (AC) reactions. Extensive dialysis against veronal-buffered diluent was unsuccessful in removal of the AC activity. Soluble cell culture CF antigen for La Crosse (ccAg) was prepared from virus-free supernatant fluids of infected BHK-21 monolayers by dialysis, then concentration by evaporation. The antigen titer increased from 1:8 before concentration to 1:256 after concentration. Cross reactivity occurred with heterologous California virus hyperimmune ascitic fluids but to a lesser degree than with the standard sucrose-acetone extracted suckling mouse brain antigens (ISMB) (Table 2).

C. IHA test.

The need for a less complicated yet sensitive test for early diagnosis of California virus infections is evident. Preliminary results for an indirect hemagglutination-inhibition (IHA) test for La Crosse is encouraging. The cell culture ammonium sulfate antigen for La Crosse was coupled to sheep RBC; hemagglutination occurred with homologous hyperimmune ascitic fluid, rabbit antisera, and an antibody-positive human serum. Continued investigation is planned to standardize the procedure and to establish optimal reaction conditions. Hopefully the IHA will be the needed diagnostic tool necessary for early diagnosis of California infections.

D. Protein analyses.

La Crosse virus has been found to possess three major virion proteins, G1 and G2 (glycosylated and found on the surface of the virus particle), plus N, the virion nucleocapsid (3). To identify the soluble antigen protein responsible for precipitation by ID and CEP, immune complexes were cut from ID and CEP agar gels and examined by polyacrylamide gel electrophoresis. Coomassie blue-stained and scanned gels of La Crosse virus proteins versus the soluble immune precipitin complex indicated that the soluble antigen is probably the N viral protein species. Further evidence was obtained by co-electrophoresis using 14C-labeled La Crosse virus and 3H-amino acid-labeled soluble antigen (Fig. 1).

(Helen S. Lindsey and J. F. Obijeski)

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2. Wellings, F. M., G. E. Sather, and W. McD. Hammon. 1970. A type-specific immunodiffusion technique for the California encephalitis group. J. Immunol. 105:1194-1200.

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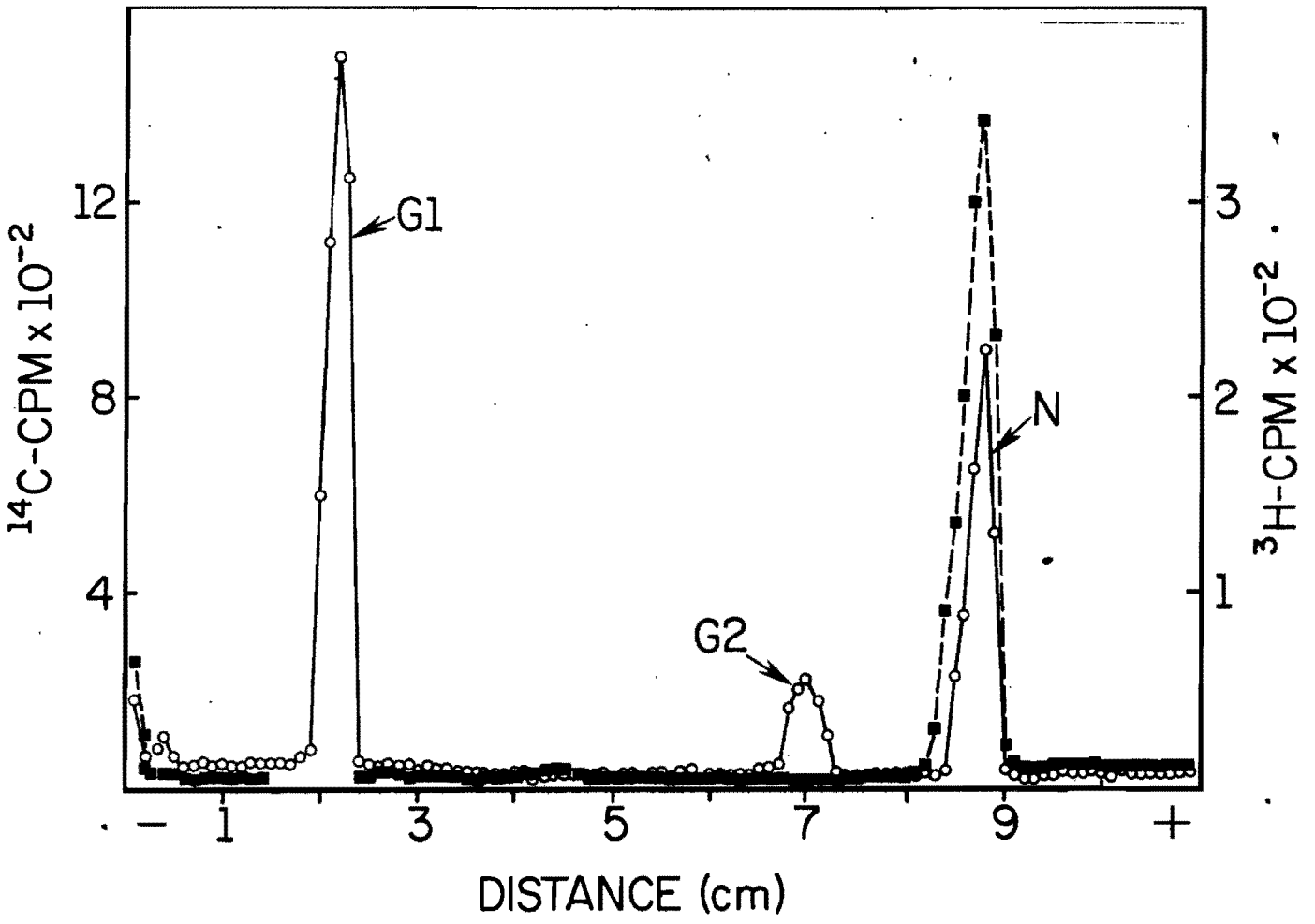
Table 2. Complement-fixation reactivity of La Crosse cell culture soluble antigen versus La Crosse suckling mouse brain antigen.

	Antiserum titers[1]									
ANTIGEN	LAC	CEV	SSH	JC	TVT	SA	MEL	KEY	TAH	EEE/SLE
LAC (ccAG)	256	16	32	16	16	16	8	<8	<8	<8/<8
LAC (ISMB)	256	64	128	64	32	64	16	16	16	<8/<8
Homologous (ISMB)		64	256	128	256	64	32	64	16	128/64

[1]Hyperimmune ascites fluids

Figure 1. Electropherogram of La Crosse virus (14C. labeled) and La Crosse virus soluble cell culture antigen (3H-amino acid labeled).



REPORT FROM THE VIRAL AND RICKETTSIAL PRODUCTS BRANCH,
BIOLOGICAL PRODUCTS DIVISION, BUREAU OF LABORATORIES, CDC,
ATLANTA, GEORGIA

Antisera for Identifying Insect Blood Meals by Double Immunodiffusion

In response to a number of requests during the past few years, we have produced a variety of rabbit antisera for identifying insect blood meals. These antisera were produced for the agar gel double diffusion test using microscope slides as described by Chamberlain and Sudia in 1967 (1).

New Zealand white rabbits ranging from 3.8 to 5.0 kg were injected with 1.25 ml of emulsified serum into each front footpad by the subcutaneous route. Serum emulsions contained equal parts of undiluted animal serum and Freund's incomplete adjuvant.

Five rabbits were immunized with serum from each of the following animals: human, dog, swine, rat, chicken, opossum, cat, horse, bovine, Rhesus, and armadillo. Antiserum samples were collected from each rabbit on days 11, 18, 25, and 61 after serum injection. Miniature proportionate pools of serum were made from each group of 5 rabbits and for each bleeding. They were stored at -20 C.

To determine homologous titers of antisera, undiluted antisera were tested with twofold dilutions of the same serum as used for immunization. The greatest dilution of serum that produced a distinct precipitate line with undiluted antiserum was considered to be the homologous titer of antiserum. Undiluted antisera were tested with 1:200 dilutions of 17 sera, as shown in Table 1, to determine heterologous reactions.

Undiluted antisera were tested with triatome and mosquito blood meals, as shown in Table 1. All of the engorged triatomes and mosquitoes were supplied by Dr. Donald A. Eliason of the Bureau of Tropical Diseases, Center for Disease Control. Insects were fed on known blood by membrane feeding and then were frozen within an hour after engorgement. Blood meal antigens consisted of 1 engorged mosquito or triatome ground in 1.0 ml of frozen saline. Twofold dilutions of blood meal suspensions were tested with undiluted antisera to determine homologous reactions. Undiluted blood meal suspensions were tested with undiluted antisera to determine heterologous reactions. The results of these tests are presented in Table 1.

The range in titers of antiserum collected on day 11 was less than 1:200 to greater than 1:25,600.

Some antisera were at peak titer by day 11 followed by a decline at day 25 and 61. Some were at maximum titer by day 11 and did not decline by day 61. Some antisera rose from 1:3,200 at day 11 to 1:12,800 by day 61. Antiserum for Rhesus rose from less than 1:200 at day 11 to greater than 1:25,600 by day 18.

The range in antiserum titers using triatoma blood meal antigen was 1:1 to greater than 1:32. Titers ranging from 1:1 to 1:8 were observed using mosquito blood meal antigen.

The only heterologous reactions observed with antisera collected on day 11 were anti-human and anti-bovine sera. Anti-human serum reacted with 1:200 dilutions of Rhesus and Vervet sera. Anti-bovine serum reacted with 1:200 dilution of sheep serum. There were no heterologous reactions between antisera collected on day 11 and undiluted blood meal antigens except with anti-human serum. It reacted with triatome Rhesus blood meal antigen.

The day 18 antisera that reacted with heterologous sera consisted of anti-human, anti-bovine, anti-rat, anti-cat, and anti-Rhesus sera. Only anti-bovine, anti-human and anti-Rhesus sera reacted with heterologous blood meal antigens.

The day 25 antisera that reacted with heterologous sera included anti-dog serum in addition to those listed in the previous paragraph. Only anti-bovine, anti-human, anti-Rhesus, and anti-cat sera reacted with heterologous blood meal antigens.

The day 61 antisera that reacted with heterologous sera consisted of anti-human, anti-bovine, anti-rat, anti-cat, anti-Rhesus, anti-dog, anti-horse, and anti-armadillo sera. Only anti-human, anti-Rhesus, anti-dog, anti-cat, anti-bovine, and anti-armadillo sera reacted with heterologous blood meal antigens.

These results indicate that following a single injection of whole serum by the footpad-subcutaneous route, rabbits respond in a variety of ways with respect to precipitating antibody formation. In general, antisera collected by the 11th day are more specific than antisera collected later. The least specificity was observed in testing day 61 antisera; however, there were many exceptions to these generalities. All antisera for chicken, opossum, and swine were free of heterologous reactions regardless of when they were collected. Anti-human serum crossed only with Rhesus and Vervet antigens but these crosses were observed in antisera collected as early as day 11. Anti-Rhesus serum reacted with human and Vervet antigens after the titer rose to 1:12,800. The antiserum reacting with the greatest number of heterologous antigens was day 61 anti-cat serum. It reacted with 13 heterologous antigens.

Specificity of antiserum appears to be more related to time after immunization than to homologous titer. For example, day 25 anti-armadillo serum had a homologous titer of 1:12,800 and had no heterologous reactions; whereas, day 61 anti-armadillo serum had the same homologous titer but reacted with bovine antigen. Another illustration of this was observed with day 11 and day 18 anti-bovine sera.

The most specific antisera collected on day 11 and day 18 have been placed on the Biological Products Division inventory for distribution in small volumes. These reagents are packaged in 1.0 ml volumes as undiluted antisera. They are intended to be used undiluted in agar gel slide double

immunodiffusion tests to identify blood meals from insects. Requests for these reagents should be addressed to Center for Disease Control, Attention: Biological Products Division, Atlanta, Georgia 30333.

1. Chamberlain, Roy W. and W. Daniel Sudia. 1967. Methods for the study of mosquitoes as virus hosts and vectors. Methods in Virology 1:63-103.

(W. Adrian Chappell, William C. Gamble, Helen S. Lindsey, and Edwin H. George)

Table 1. Precipitin Reactions in Double Immunodiffusion Tests Between Rabbit Antisera and Insect Blood Meals and Animal Sera

Titer of Antisera in Reaction with Antigens Below*

Rabbit Antiserum	Day Serum Collected After Immunization	Human Serum	Triatome Blood Meal (Human)	Mosquito Blood Meal (Human)	Dog Serum	Triatoma Blood Meal (Dog)	Mosquito Blood Meal (Dog)	Swine Serum	Triatome Blood Meal (Swine)	Mosquito Blood Meal (Swine)	Rat Serum	Triatoma Blood Meal (Rat)	Mosquito Blood Meal (Rat)	Chicken Serum	Triatoma Blood Meal (Chicken)	Mosquito Blood Meal (Chicken)
Human	11	12,800	≥32	4	-	-	-	-	-	-	-	-	-	-	-	-
	18	12,800	≥32	4	-	-	-	-	-	-	-	-	-	-	-	-
	25	6,400	≥32	4	-	-	-	-	-	-	-	-	-	-	-	-
	61	6,400	≥32	8	-	-	-	-	-	-	-	-	-	-	-	-
Dog	11	-	-	-	3,200	16	Undil	-	-	-	-	-	-	-	-	-
	18	-	-	-	3,200	≥32	Undil	-	-	-	-	-	-	-	-	-
	25	-	-	-	3,200	≥32	Undil	-	-	-	-	-	-	-	-	-
	61	Pos.	-	Pos	12,800	≥32	Undil	-	-	-	-	-	-	-	-	-
Swine	11	-	-	-	-	-	-	12,800	≥32	4	-	-	-	-	-	-
	18	-	-	-	-	-	-	12,800	≥32	4	-	-	-	-	-	-
	25	-	-	-	-	-	-	12,800	≥32	4	-	-	-	-	-	-
	61	-	-	-	-	-	-	12,800	≥32	4	-	-	-	-	-	-
Rat	11	-	-	-	-	-	-	-	-	-	≥25,600	4	Undil	-	-	-
	18	-	-	-	-	-	-	-	-	-	≥25,600	4	4	-	-	-
	25	-	-	-	-	-	-	-	-	-	≥25,600	4	4	-	-	-
	61	-	-	-	-	-	-	-	-	-	≥25,600	4	4	-	-	-
Chicken	11	-	-	-	-	-	-	-	-	-	-	-	-	3,200	≥32	4
	18	-	-	-	-	-	-	-	-	-	-	-	-	12,800	≥32	2
	25	-	-	-	-	-	-	-	-	-	-	-	-	3,200	≥32	Undil
	61	-	-	-	-	-	-	-	-	-	-	-	-	3,200	≥32	4
Opossum	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cat	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	Pos.	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	Pos.	Pos	-	-	-	-	-	-	-	-	-	-
	61	Pos.	-	Pos	Pos.	Pos	Pos.	Pos.	Pos	Pos	-	Pos	Pos	-	-	-
Horse	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bovine	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	Pos	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	Pos	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	Pos	Pos	-	-	-	-	-	-
Rhesus	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	Pos.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	Pos.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	Pos.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Armadillo	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Rabbit Antiserum	Day Serum Collected After Immunization	Opossum Serum	Triatome Blood Meal (Opossum)	Mosquito Blood Meal (Opossum)	Cat Serum	Triatome Blood Meal (Cat)	Mosquito Blood Meal (Cat)	Horse Serum	Triatome Blood Meal (Horse)	Mosquito Blood Meal (Horse)	Bovine Serum	Triatome Blood Meal (Bovine)	Mosquito Blood Meal (Bovine)	Rhesus Serum	Triatome Blood Meal (Rhesus)	Mosquito Blood Meal (Rhesus)
Human	11	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	-
	61	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Pos
Dog	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	Pos	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	Pos	Pos	Pos	-	-	-	-	-	-	Pos	-	Pos
Swine	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rat	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Opossum	11	3,200	Undil	Undil	-	-	-	-	-	-	-	-	-	-	-	-
	18	6,400	Undil	Undil	-	-	-	-	-	-	-	-	-	-	-	-
	25	6,400	Undil	Undil	-	-	-	-	-	-	-	-	-	-	-	-
	61	12,800	Undil	Undil	-	-	-	-	-	-	-	-	-	-	-	-
Cat	11	-	-	-	12,800	16	4	-	-	-	-	-	-	-	-	-
	18	-	-	-	≥25,600	4	4	-	-	-	-	-	-	-	-	-
	25	-	-	-	≥25,600	8	4	-	-	-	-	-	-	-	-	-
	61	-	Pos	-	>25,600	≥32	4	Pos.	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Horse	11	-	-	-	-	-	-	≥25,600	≥32	4	-	-	-	-	-	-
	18	-	-	-	-	-	-	≥25,600	≥32	4	-	-	-	-	-	-
	25	-	-	-	-	-	-	≥25,600	≥32	4	-	-	-	-	-	-
	61	-	-	-	-	-	-	≥25,600	≥32	4	-	-	-	-	-	-
Bovine	11	-	-	-	-	-	-	-	-	-	12,800	≥32	4	-	-	-
	18	-	-	-	-	-	-	-	-	-	12,800	≥32	4	-	-	-
	25	-	-	-	-	-	-	-	-	-	12,800	≥32	4	-	-	-
	61	-	-	-	-	-	-	-	-	-	12,800	>32	4	-	-	-
Rhesus	11	-	-	-	-	-	-	-	-	-	-	-	-	<200	2	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	≥25,600	≥32	2
	25	-	-	-	-	-	-	-	-	-	-	-	-	12,800	≥32	4
	61	-	-	-	-	-	-	-	-	-	-	-	-	12,800	>32	4
Armadillo	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	Pos	-	Pos	-	-	-

Rabbit Antiserum	Day Serum Collected After Immunization	Armadillo Serum	Tritome Blood Meal (Armadillo)	Mosquito Blood Meal (Armadillo)	Goat Serum	Guinea Pig Serum	Vervet Serum	Rabbit Serum	Goose Serum	Sheep Serum	Mouse Serum
Human	11	-	-	-	-	-	Pos	-	-	-	-
	18	-	-	-	-	-	Pos	-	-	-	-
	25	-	-	-	-	-	Pos	-	-	-	-
	61	-	-	-	-	-	Pos	-	-	-	-
Dog	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-
Swine	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-
Rat	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	Pos
	25	-	-	-	-	-	-	-	-	-	Pos
	61	-	-	-	-	-	-	-	-	-	Pos
Chicken	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-
Opossum	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	-	-	-	-	-	-	-
Cat	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	Pos	-	-	-	-	-
	25	-	-	-	-	Pos	-	-	-	-	-
	61	-	Pos	Pos	-	Pos	Pos	-	-	Pos	Pos
Horse	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	61	-	-	-	Pos	-	Pos	-	-	-	-
Bovine	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	Pos	-
	25	-	-	-	Pos	-	-	-	-	Pos	-
	61	-	-	-	Pos	-	-	-	-	Pos	-
Rhesus	11	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	Pos	-	-	-	-
	25	-	-	-	-	-	Pos	-	-	-	-
	61	-	-	-	-	-	Pos	-	-	-	-
Armadillo	11	3,200	≥32	4	-	-	-	-	-	-	-
	18	12,800	≥32	16	-	-	-	-	-	-	-
	25	12,800	≥32	16	-	-	-	-	-	-	-
	61	12,800	≥32	16	-	-	-	-	-	-	-

*Titers are expressed as highest dilution of serum antigen or blood meal antigen that produced a distinct precipitate line with undiluted antiserum (3,200 = 1:3,200).
Heterologous serum antigens consisted of 1:200 dilutions of serum in saline.
Heterologous triatome blood meal antigen consisted of one engorged triatoma ground in 1.0 ml of saline.
Heterologous mosquito blood meal antigen consisted of one engorged mosquito ground in 1.0 ml of saline.
Undil. indicates reaction with undiluted antigen.
Pos. indicates distinct precipitate line showing positive reaction.
- indicates negative, no precipitate line.

REPORT FROM THE OFFICE OF LABORATORY SERVICES

FLORIDA DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES

JACKSONVILLE, FLORIDA

With the increased number of human SLE cases reported in neighboring states in 1975, overall surveillance is being conducted throughout the state of Florida. As in the past, as part of the general virus diagnostic services provided to the medical community, we have tested the sera of 473 patients against a battery of antigens associated with central nervous system diseases during the period from January, 1976 - June, 1976. Where histories could be obtained, the patients with constant Group B titers had previously resided in known endemic areas in the Caribbean. No human case of SLE has been detected in Florida thus far in 1976 (August 26, 1976).

Human and Animal Sera Screened
by the HI Technique with Arbovirus Antigens*

January 1976 - June 1976

Species	Number of Sera	Reactors
Human	473	6 Group B**
Horses	16	5 EEE 1 WEE
FIELD SPECIMENS		
a. Small Mammals	126	2 EEE
b. Avian Sera	434	2 SLE***
TOTAL	1049	16

* Arbovirus Antigens:
EEE - Eastern Equine Encephalitis
WEE - Western Equine Encephalitis
SLE - St. Louis Encephalitis TBH-28
VEE - Venezuelan Equine Encephalitis TC-83 and/or Fe3-7c

** Patients paired sera had constant low level HI antibodies to SLE and Dengue antigens.

*** North Florida Counties.

(Nathan J. Schneider and Elsie E. Buff)

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

Dengue in Puerto Rico in 1976

The dengue epidemic which began in September 1975 terminated in early 1976. Cases of clinical dengue from which blood specimens were received in 1976 totalled 273, with onset as follows:

January	128
February	78
March	34
April	16
May	3
June	4
July	8
August	2

The last confirmed case was from the San Juan metropolitan area, had onset on May 1, and was confirmed by virus isolation. Ten isolates from the epidemic have been identified; all were dengue-2.

A post-epidemic serum survey of more than 1200 people from the San Juan metropolitan area and the eastern part of the island has been completed and the sera are being tested.

On September 1, 1976, Dr. Barnett L. Cline left the San Juan Laboratories to assume the position of Chairman, Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University, New Orleans. He has been replaced by Dr. John P. Woodall as Director, San Juan Laboratories.

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

REPORT FROM THE BUREAU OF LABORATORIES

TEXAS DEPARTMENT OF HEALTH RESOURCES

AUSTIN, TEXAS

January 1, 1976 through July 31, 1976

Mosquitoes

557 pools of mosquitoes were tested, numbering 6,013 mosquitoes. There were 14 isolates, all of them Hart Park. Following is a list of isolates:

State #	Location	Species of Mosquitoes	Collection Date
10830	Cameron County	Culex quinquefaciatus	6-1-76
10859	Dallas City	C. quinquefaciatus	6-1/7-76
10861	Tarrant County	C. restuans	6-8-76
10872	Tarrant County	C. restuans	6-8-76
10878	Hidalgo County	C. quinquefaciatus	6-7-76
10922	Dallas City	C. quinquefaciatus	6-7/11-76
10923	Dallas City	C. quinquefaciatus	6-7/11-76
10937	Hidalgo County	C. quinquefaciatus	6-14-76
10978	Dallas City	C. quinquefaciatus	6-17/21-76
11006	Wichita Falls	C. quinquefaciatus	6-22-76
11040	Dallas City	C. quinquefaciatus	6-22-76
11069	Dallas City	C. quinquefaciatus	6-25-76
11079	Hidalgo County	C. quinquefaciatus	6-29-76
11123	Cameron City	C. quinquefaciatus	7-13-76

Sentinel Flocks

There have been 741 chicken sera tested by hemagglutination-inhibition for encephalitis. Of these sera, one was positive for VEE with a titer of 1:20. This serum was collected in Dallas, Texas.

Wild Birds

69 wild birds have been tested over the six-month period. One was positive for SLE with a titer of 1:20. This bird also came from Dallas.

(Charles E. Sweet)

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

Experimental infection of Anopheles albimanus and Culex thriambus mosquitoes with Venezuelan Equine Encephalomyelitis virus (VEE), TC-83 strain.

Experimental infection of Anopheles albimanus and Culex thriambus mosquitoes was achieved in the following experiments.

In the first experiment Anopheles albimanus was allowed to feed on suckling mice infected with TC-83 virus. The viremia titer was $10^{7.3}$ LD_{50}/sm/ic/ml. After 30 minutes of feeding the mosquitoes were separated in cardboard cups and maintained at 28°C and relative humidity of 70-80%. Immediatly after feeding and at 3,7,8,9,10,11,12 and 13 days after feeding, the mosquitoes were killed, suspensions were made and inoculated IC into suckling mice. The inocula with virus were titrated in suckling mice. The average viral titers per mosquito as LD_{50}/sm/ic/ml were: Negative at 3 days, $10^{3.3}$ at 7 days, $10^{4.3}$ at 8 days, $10^{4.3}$ at 9 days, $10^{7.3}$ at 10 days, $10^{5.2}$ at 11 days, $10^{3.9}$ at 12 days and $10^{4.2}$ at 13 days.

In the second experiment, mosquitoes that had been infected 10,11,12 and 13 days earlier were allowed to feed on normal suckling mice. Only one mouse of six exposed died of VEE. This group corresponded to the mice exposed to

mosquitoes after 10 days of incubation with an average viral titer per mosquito of $10^{7.3}$ LD_{50}. In the animal that died the virus could be recovered and was neutralized with an anti-VEE specific serum.

In the third esperiment, Culex thriambus mosquitoes were inoculated by intrathoracic route with approximately 0.001 ml of TC-83 virus of a titer of $10^{5.0}$ LD_{50}/sm/ic/ml. The mosquitoes were separated in cardboard cups and maintained at a temperature of 28°C with a relative humidity of 70-80% . At 16, 23, 26, 36 and 43 days post-inoculation, the mosquitoes were killed and suspensions were made. The average viral titers per mosquitoe were: $10^{5.9}$ at 16 days, $10^{8.0}$ at 23 days, $10^{6.3}$ at 26 days, $10^{7.7}$ at 36 days and $10^{4.9}$ at 43 days.

According to the results, TC-83 strain of VEE virus is able to infect Anopheles albimanus and Culex thriambus mosquitoes. In addition An. albimanus was able to transmit the virus to one suckling mouse.

(C.R. Bautista-Garfias, S. Mercado-Sánchez, A. Morilla-González)

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

In July 1968, sera from pigs and chickens were collected in the slaughterhouse of Tampico, Mexico. The pigs came from nearby places: Altamira, Tamaulipas; Ebano, San Luis Potosí and Pánuco, Veracruz, where the first cases of the 1971 Venezuelan equine encephalitis epidemic occurred. The chickens were from a poultry farm outside Tampico, very near where a small epizootic of VEE occurred in 1966.

The 68 sera from pigs and the 61 from chickens were tested for arbovirus activity by the hemagglutination-inhibition test. The results are presented in the following table:

SERA FROM PIGS* AND CHICKENS** COLLECTED ON JULY 1968 INVESTIGATED FOR EVIDENCE OF ARBOVIRUS INFECTION BY THE HEMAGGLUTINATION-INHIBITION TEST***

ANTIGEN	PIGS POSITIVE/TESTED	CHICKENS POSITIVE/TESTED
VEE	12 / 68	4 / 61
WEE	7 / 68	1 / 61
EEE	1 / 68	0 / 61
YF	11 / 68	0 / 61
SLE	11 / 68	1 / 61
IIH	11 / 68	2 / 61

* Pigs came from Tampico, Tamaulipas; Ebano, San Luis Potosí and Pánuco, Veracruz, Mexico.

** Chickens were from a poultry farm outside Tampico.

*** HI positive titers ranged from 10 to ≥ 80 against 4 HA Units of each antigen.

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REPORT OF THE NATIONAL INSTITUTE OF HEALTH OF COLOMBIA

BOGOTA, COLOMBIA

VIRAL ANTIGEN, PRESUMABLY DENGUE, DEMONSTRATED BY IMMUNOFLUORESCENT TECHNIQUES IN AEDES AEGYPTI

During the course of a dengue 3 outbreak in Armero, Colombia, a survey carried out in 352 houses demonstrated that 24 per cent of the 2,245 persons living there probably had experienced dengue infections during the ninety days prior to the survey, as indicated by clinical histories and serological studies. The same survey showed that the house larval index for A. aegypti was 44 per cent and that the attack rate of females was never greater than two mosquitoes per man per house. In the course of the survey 360 females of A. aegypti were captured, 20 of which (5.6 per cent) exhibited in their brains antigen reacting with anti-dengue serum, when the immunofluorescent techniques developed by Kuberski and Rosen were used. Other surveys in the same area during the outbreak showed 11 positive females among 268 studied. Although such an antigen could correspond to diverse viruses of the Casal's group B, we are inclined to believe that it corresponds to dengue 3, since this was the only group B agent active in the area at that time. Studies to make a final identification of the antigen in these mosquitoes are in process.

(Hernando Groot, Alberto Morales, Margarita Romero and Hernando Vidales)

Bogota, August 26, 1976

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUTO DE INVESTIGACIONES VETERINARIAS
MARACAY, ESTADO ARAGUA, VENEZUELA

EEE IN VENEZUELA:

Since 1938, the year in which the first isolation of VEE virus was made, to date this was the only group "A" virus which had been recognized in Venezuela as causing equine encephalomyelitis.

On February 26 a brain specimen collected at "El Delirio", a farm located in the Municipium of Santa Cruz, District of Colon, State of Zulia (south end of Lake Maracaibo), was forwarded to this laboratory for diagnosis. From seven susceptible animals on this farm, only one horse became sick and died. A virus was recovered in newborn mice. Crude antigen with infected mouse brain by CF showed titers of 32/32 against EEE, 16/8 against WEE and no reaction with VEE and Maguarí; all sera were reference from WHO, except the last one. A presuntive diagnosis of EEE was made on the base of CF tests and confirmed by neutralization test with the same sera showing a clear protection with EEE and none with WEE, VEE or Maguari.

Validity of the isolation is enhanced by the absence of EEE in this laboratory at the time of isolation.

A hemagglutinating antigen was obtained from infected mouse brain with this strain called "El Delirio" and tested against horse sera from the Institute's serum bank collected in previous years. Up to the present, 1549 sera from different species have been tested (see tables 1 and 2). Results showed that EEE is not new in the area and the virus is present also in other regions of Venezuela (see table 2, Delta Amacuro for instance). Many of them have high titers for EEE with no HI or NT antibodies against VEE.

Later, June 3, 1976 another strain was recovered from a one year old male horse that died in "La Trinidad", another farm close to "El Delirio" located in the same district.

A trip was made on March to Zulia where the virus was present in order to study the whole situation. It was found that a small epizootic was taking place. Bloods from 176 animals in the area were examined for HI and in some cases CF antibodies against EEE, VEE and WEE.

Recent activity for both EEE and VEE virus was demonstrated through serology. However, none of these viruses were recovered so far from any of the serum samples also used for isolation attempts.

On July 30, 1976 another horse died, this time in "La Cumaca", San Felipe in Yaracuy State, about 400 Km. northeast of the region of the first isolations. A virus was isolated from the brain of this horse which seems to be EEE by CF; neutralization test is presently under way.

Efforts are in progress to elucidate the posibility of the endemicity of VEE and EEE in the area of the south end of Lake Maracaibo in the State of Zulia.

The isolation of EEE in Venezuela is new for horses; however, in April 1975 in studies done by another Institute (IVIC) three strains of EEE were isolated from sentinel hamsters exposed in Catatumbo, close to the area where the first isolation from a horse was made.

(Norma E. mettler and Guillermo Dumuth Arteaga)

TABLE 1

RESULTS OF HI (VEE) AGAINST SERA FROM THE STATE OF ZULIA

SPECIES	DISTRICTS AND YEARS											TOTALS 1973-74	%
	COLON 1973	PERIJA 1973	SUCRE 1973	URDANETA 1973	BARALT 1973	MARACAIBO 1973	BOLIVAR 1973	MARA 1973	MIRANDA 1973	PAEZ 1973 - 1974			
Horses	29/56*	11/33	3/26	14/27	3/11	1/3	3/12	4/12	1/10	4/11	1/6	74/207	35.75
Donkeys	4/97	-	1/1	0/2	4/20	0/10	0/7	8/32	1/21	11/99	4/111	33/400	8.25
Mules	1/8	2/6	0/6	2/6	1/2	1/1	0/5	0/2	-	0/2	0/1	7/39	17.95
Bovines	0/4	1/54	1/8	0/25	2/14	0/44	0/16	0/7	0/10	-	2/14	6/196	3.06
Ovines	0/2	0/14	-	0/11	-	0/4	-	0/25	-	1/10	0/30	1/96	1.04
Caprines	-	0/1	-	0/19	0/7	0/8	-	1/16	0/20	0/18	1/20	2/109	1.33
Canines	0/4	-	0/4	0/2	-	0/4	0/1	0/7	0/3	0/7	0/8	0/40	0

* Number of Sera with HI titers $\geq$ 1:20/Number of sera tested

TABLE 2

RESULTS OF HI WITH EEE AGAINST SERA OF SEVERAL STATES

SPECIES	FALCON 1972	MONAGAS 1972	SUCRE 1972	CARABOBO 1972 (de haras)	DELTA AMACURO 1973
Horses	0/3	7/30	0/2	0/56	43/61
Donkeys	3/56	9/28	0/53	-	7/8
Mules	-	0/1	-	-	-
Bovines	-	-	-	0/12	12/62
Ovines	-	-	-	0/5	-
Caprines	-	-	-	-	1/21
Canines	-	-	-	-	0/8
Humans	-	-	-	-	3/56

REPORT FROM THE EVANDRO CHAGAS INSTITUTE, FSESP

BRAZILIAN MINISTRY OF HEALTH

BELEM, BRAZIL

Possible new arenavirus isolated from wild rodents

A possible new arenavirus has been isolated from wild rodents captured at the Flexal area in November 1975.This area is located in the Itaituba - Jacareacanga section of the Transamazon highway, some 212 Km southwest of Itaituba, in Pará State. This road section is more or less paralel to the Tapajós river.

Three rodents were found infected with the virus. Two of them were Oryzomys oecomys and one was a Oryzomys macconnelli. 8 and 3 animals from these species respectively were captured during the study period (October-December 1975). The virus was obtained from the blood of all three animals and from the viscera of one Oryzomys oecomys only. From one of these rodents the virus was isolated twice, from blood samples taken from him one month apart.

The viruses were considered as arenaviruses on the basis that they showed a cross reaction in the CF test with a Tacaribe group hyperimmune ascitic fluid. CF tests performed at the YARU with the strain Be An 293022 of the virus showed that it was different from Amapari, Junin, LCM, Machupo, Tacaribe, Pichinde and Tamiami viruses (Table 1). A strong CF cross reaction was noted with Parana antisera in tests carried out in Belém. It also cross reacts with Latino antiserum, although to a less extent, but no reaction was observed with Lassa antiserum. (Table 2).

The A.S.T. in infant mice at 1st and 2nd passage levels varied from 11.5 to 13.7 days in the i.c. route and reached 14.6 days when the i.p. route was used. The LD_{50} reached 5.5 and 4.0 log/0.02 ml by the i.c. and i.p. routes

respectively. A few adult mice died after ic inoculation but they usually survived after i.p. inoculation.

Interestingly the adult mice show partial alopecia which becomes evident about 3 weeks after the inoculation. One or two weeks afterwards the animals regain their hair.

No CPE was observed in Vero cells during a 21 days of observation after infection. Plaque formation, however, was observed in that cell line.

The following animals captured at the Flexal area were examined for the presence of this arenavirus, with negative results: Oryzomys sp (47), Neacomys (19), Proechimys (59), Nectomys (29), other rodents (11), marsupials (63), primates (13, birds (530), miscellaneous (7).

One of the virus strains was sent to Dr. P. Webb, NCDC, Atlanta, for further characterization of the agent.

Fatal case of yellow fever

A 23 y.o, male died of yellow fever in Alenquer County, Pará State, on the April 30. 1976. He had arrived recently in Alenquer and had been working since April 17 in a forested area located about 40 km from the seat county. His illness begun on the April 22.

The diagnosis was confirmed by histopathology of the liver. Attempted virus isolation from a liver sample resulted negative. The sample was collected a few hours after the patient's death and was preserved in wet ice for a few days until it was inoculated.

Field investigation in the area were undertaken from May 24 until June 5. No other deaths or clinical cases compatible with yellow fever were found in the area where the patient had been working until he sickened.

207 persons were bled for serological studies; 14 of these persons had a fever at the time of bleeding, therefore virus isolation was attempted from their blood, Serological results are awaited. But two virus strains were isolated in

baby mice from the blood of two persons. One of the strains was yellow fever virus obtained from a 3 y.o. girl. This isolate probably represents a 17 D strain of yellow fever virus since this strain was given to the child 2 days before she was bled. She had an uneventful recover from her febrile episode.

The other virus strain was isolated from the blood of a 27 y.o. male who was in the first day of a febrile illness.

Preliminary serological tests indicate that this agent belongs to the Phlebotomus group.

1084 mosquitoes were collected on the ground and at tree level. 252 of the mosquitoes belonged to the genus Haemagogus . These were inoculated into baby mice as 15 pools, 3 of which yield YF virus; from another pool Mayaro virus was isolated. The four strains were reisolated in Vero cells.

Five monkeys (3 Cebus, 1 Saimiri, 1 Ateles) one marmoset (Saguinus) and one rodent (Cuniculus paca) where shot and speciemens collected for serology and virus isolation attempts. These results are awaited.

Serological studies with an agent isolated from fatal case of encephalitis ocurred in S.Paulo

At the request of Dr. Oscar S. Lopes, I.A.L., S.Paulo, serological studies were performed with the SPH 34675 virus that he had isolated from nervous tissue of a fatal human case of encephalitis.

The results summarized in Tables 3 and 4 show clearly that SP H34675 is a group B arbovirus although it is distinct from all flaviviruses present in Brasil, as well as from others found abroad included in our tests. It seems though that the agent is more closely related to Ilhéus virus.

These results confirm and extend Dr. Souza Lopes findings.

(Francisco P. Pinheiro, Amélia A. T. Rosa and Jorge F. T. Rosa)

Table 1. Cross CF test with arena group viruses including the new isolate Be An 293022.

Antigens	Antisera								
	AMA	JUN	LCM	MAC	PIC	TCR	TAM	758	022
Amapari	64/≥40	≥64/≥40	0	32/≥40	0	16/≥40	0	0	0
Junin	32/≥40	≥64/≥40	8/4	≥64/≥40	0	16/≥40	0	0	0
LCM	0	0	≥64/≥40	0	0	0	0	0	0
Machupo	8/4	≥64/≥40	0	≥64/≥40	0	16/4	0	0	0
Pichinde	0	0	0	0	8/4	0	0	0	0
Tacaribe	8/≥40	≥64/≥40	0	≥64/≥40	0	≥64/≥40	0	0	0
Tamiami	0	0	0	0	0	0	≥64/≥40	0	0
Be 238758	0	0	0	0	0	0	0	32/8	0
Be 293022 br.	0	16/4	16/4	16/4	0	0	8/4	8/16	16/16
" lv.	0	8/4	0	0	0	0	0	0	16/16
Normal	0	0	0	0	0	0	0	0	0

Table 2. CF test with Be An 293022 virus and certain arenavirus antisera.

Antisera	Titer to Antigen	
	An 293022	Homologous
An 293022	64-128/16	
Paraná	512/16	4096
Latino	32/8	2048
Machupo	<8/<4	
Lassa	<20/<4	160-320

Table 3. CF test with SP H34675 and group B viruses present in Brazil *

ANTIGEN	SERA				
	SP H 34675	YF	Bus	ILH	SLE
SP H 34675	64/≥8	0	0	32/≥8	32/≥8
YF (H 111)	0	≥32/≥16			
Bus, (An 4116)	0		16/≥16		
Ilhēus (H 7445)	0			≥32/≥16	
SLE (Ar 23379)	0				≥32/≥16

* No reactions found with antisera for Dengue, RSSE, JBE, B.S. Gland Viruses.

Table 4. N test with SP H 34675 *

SERA	ANTIGENS			
	SP H34675	SLE(Ar23379)	SLE(H203235)	ILH(H7445)
SP 34675	4.5**	1.7		2.1
ILH (H7445)	2.5			5.3
SLE (Ar 23379)	1.3	≥ 4.7	5.0	1.8
SLE (H 203235)	0	≥4.7		2.1
SLE(Parton)	1.0	3.5	4.7	1.0
Dengue Hawaii	0,8			
Dengue TR H51	0.7			
RSSE	0			
JBE	0.8			
B S Galnd	0			

* N test in baby mice, i.p. route.
** Results expressed in log. 10 of N. index.

REPORT FROM THE VIRUS DEPARTMENT, NATIONAL

INSTITUTE OF MICROBIOLOGY "DR. CARLOS G. MALBRAN"

MINISTRY OF SOCIAL WELFARE, BUENOS AIRES, ARGENTINA

In the first 6 months of 1976, 212 suspect cases of AHF were studied by CF tests with Junin and LCM viruses at the Instituto Nacional de Microbiologia. A breakdown of the results by month, location, age, and sex is shown in the following tables.

Comparative studies by CF, IF, and NT against Junin and LCM viruses, as well as by virus isolation, are being carried out to further clarify the meaning of the concomitant presence of antibodies against both viruses in some cases of AHF.

It has been already demonstrated that some AHF suspects suffered a recent infection by Junin virus and a previous infection by LCM virus. These cases show an apparent simultaneous serologic conversion by CF against both viruses.

It is hopped that more data will be available for the next issue of the Arthropod-borne Virus Information Exchange.

(Julio G. Berrera Oro)

Table 1. MONTHLY DISTRIBUTION OF AHF SUSPECT CASES OF 1976 (first 6 months) BY CF TESTS.

Onset of illness	Positive cases with Jun virus antigen / number of suspects With paired sera (4-fold rise)	With single sera (1/4)
Jan.	3/3	0/0
Feb.	2/4	0/0
Mar.	12/15	0/0
Apr.	27/35 (4)[a]	1/1
May	60/78 (6)[a]	0/3
Jun.	38/71 (4)[a]	0/2
Total	142/206 (14)[a]	1/6

Table 2. GEOGRAPHIC DISTRIBUTION

Province	Positive cases with Jun virus antigen / number of supects With paired sera	With single sera
Buenos Aires	97/150 (10)[a]	1/6
Córdoba	3/4	0/0
Santa Fe	42/52 (4)[a]	0/0
Total	142/206 (14)[a]	1/6

Table 3. AGE DISTRIBUTION

Age (years)	Positive cases with Jun virus antigen / number od suspects With paired sera	With single sera
0-5	1/1	0/1
10-14	6/10	0/1
15-24	30/45 (2)[a]	0/0
25-40	38/63	0/0
41-59	45/56 (8)[a]	0/0
59	14/18 (4)[a]	0/0
Unknown	8/11	1/4

a: Number of suspect cases that gave a concomitant rise with both, Jun and LCM virus antigens.

Table 4. AGE DISTRIBUTION

Sex	Positive cases with Jun virus antigen / number of suspects With paired sera	With single sera
Male	110/158 (13)[a]	1/5
Female	32/48 (1)[a]	0/1
Total	142/206 (14)[a]	1/6

a: Number of suspect cases that gave a concomitant rise with both, Jun and LCM virus antigens.

REPORT FROM THE PACIFIC RESEARCH SECTION, NIAID, NIH

HONOLULU, HAWAII

Isolation of a new vesicular stomatitis virus serotype from sandflies in Iran

During field studies on the epidemiology of sandfly fever in Iran in the summer of 1975, two strains of an apparently new vesicular stomatitis virus (VSV) serotype, designated Isfahan virus, were isolated from pools of female Phlebotomus papatasi. The animal pathogenicity, growth rate, cytopathic effect and plaque morphology of Isfahan virus are similar to the other VSV serotypes. Electron microscopic examination of the virus demonstrated a bullet shape, the presence of numerous truncated (T) particles and maturation at cell surfaces. In serologic tests (Tables 1 and 2), Isfahan virus was inhibited by a VSV group antiserum but was antigenically distinct from the 6 known VSV serotypes.

A high prevalence of Isfahan neutralizing antibodies was found in human sera from several regions of Iran (Table 3), suggesting that the virus may be of some public health importance. All of the residents over 5 years of age in Dormian village where the virus was isolated had been infected. Similar infection rates have been reported among other human populations with VSV-Indiana and New Jersey in Panama and with Chandipura virus in India. Neutralizing antibodies to Isfahan virus were also found in sera of Iranian gerbils (Table 4), but interestingly not in domestic animals. Results of our studies suggest that the ecology of Isfahan virus is distinct from the other VSV serotypes and involves chiefly humans, gerbils and sandflies, a pattern also observed with cutaneous leishmaniasis and sandfly fever in Iran. Isfahan virus is the third VSV serotype to be associated with phlebotomus sandflies.

These studies were a collaborative effort between the Pacific Research Section, NIAID, NIH and the School of Public Health and Institute of Public Health Research, University of Tehran.

R. B. Tesh

Table 1

Results of complement fixation tests with Isfahan virus and 6 VSV serotypes

Immune serum	ANTIGEN** New Jersey	Indiana	Cocal	Piry	Chandipura	Isfahan
New Jersey	128‡	<4	<4	<4	<4	<4
Indiana	<4	2,048	64	<4	<4	<4
Cocal	<4	32	128	<4	<4	<4
Alagoas*	<4	4	8	<4	<4	<4
Piry	<4	<4	<4	128	<4	<4
Chandipura	<4	<4	<4	<4	128	<4
Isfahan	<4	<4	<4	<4	<4	128

* Homologous titer of the Alagoas antiserum was 1:200

** 8 units of antigen used

‡ Reciprocal of highest positive serum dilution

TABLE 2

RESULTS OF PLAQUE REDUCTION NEUTRALIZATION TESTS WITH ISFAHAN VIRUS AND 5 VSV SEROTYPES

IMMUNE SERUM	VIRUS					
	NEW JERSEY	INDIANA	COCAL	PIRY	CHANDIPURA	ISFAHAN
NEW JERSEY	10,240*	<10	<10	<10	<10	<10
INDIANA	<10	327,680	320	<10	<10	<10
COCAL	<10	160	5,120	<10	<10	<10
ALAGOAS	<10	20	20	<10	<10	<10
PIRY	<10	<10	<10	10,240	<10	<10
CHANDIPURA	<10	<10	<10	<10	10,240	<10
ISFAHAN	<10	<10	<10	<10	<10	2,560
VSV - GROUP**	640	2,560	160	5,120	1,280	40

* RECIPROCAL OF HIGHEST SERUM DILUTION PRODUCING $\geq$ 95% PLAQUE INHIBITION

** PREPARED IN A GUINEA PIG SEQUENTIALLY INOCULATED WITH PIRY, CHANDIPURA, INDIANA, NEW JERSEY AND COCAL VIRUSES

Table 3

Prevalence of Isfahan virus neutralizing antibodies among residents of 15 selected Iranian communities

Community - Province	Number positive / Total tested	Percentage positive
Tabriz, East Azerbaijan	0/30	0.0
Rasht, Gilan	0/30	0.0
Ali-Abad, Khorassan	51/73	69.9
Esmaill-Abad, Khorassan	6/29	20.7
Neishabor, Khorassan	14/30	46.7
Mashad, Khorassan	8/32	25.0
Tehran, Tehran	2/30	6.7
Varamin, Tehran	0/30	0.0
Dormian, Isfahan	49/57	86.0
Komshecheh, Isfahan	38/67	56.7
Gholounabad, Isfahan	16/30	53.3
Kermanshah, Kermanshah	0/32	0.0
Dezful, Khuzestan	4/31	12.9
Khorousi, Khuzestan	0/30	0.0
Abadan, Khuzestan	1/31	3.2

Table 4

Prevalence of Isfahan virus neutralizing antibodies among humans and animals in Iran

Species	Locality (Province)		
	Isfahan	Tehran	Khuzestan
Humans	103/154 (66.9)*	2/60 (3.3)	5/92 (5.4)
Sheep	0/78	0/72	0/25
Goats	0/36		0/ 5
Cattle	0/24	0/56	0/ 6
Gerbils	26/33 (78.8)		3/44 (7.0)
Chickens	0/ 5		
Pigeons	0/23		

* Number positive/Total tested (Percentage positive)

REPORT FROM THE NATIONAL INSTITUTE OF HEALTH

TOKYO, JAPAN

Establishment of a bat kidney cell (BKC) line and its susceptibility to some arboviruses

Bats are known to become persistently infected after experimental infection with Japanese encephalitis (JE) virus during the active period, but to have almost zero response if inoculated during hibernation in the winter season (Ito and Saito, 1952). A similar phenomenon was observed by La Motte (1958) and Sulkin et al. (1963) in experimental infection of bats with JE and St. Louis encephalitis (SLE) viruses. Miura(1968) showed that by mincing antibody-negative bat embryo tissue and trypsinizing in a bottle, the cells were easily obtained and grew out in a monolayer sheet. When JEV was inoculated into such monolayer culture, the virus was released persistently from 5 days to 237 days, with only an occasional medium change required.

Eighty bats (Rhinolophus cornatus) were all collected at the same cave in Oita Prefecture in 1968. They were divided into 8 pools. They were bled individually with surgical precautions by heart puncture after ether anaesthesia. All were negative by hemagglutination-inhibition (HI) test to JEV, and no virus was isolated. Minced and trypsinized kidney cells from these proven non-infected bats were adjusted to 10^4 - 10^5 per ml in stationary bottle culture (4.5 x 4.5 x 10.0 cm). The culture medium used was composed of Hanks balanced salt solution, yeast extract 0.1%, lactalbumin hydrolysate 0.5%, calf serum 10%, in distilled water pH 7.4 containing 100 units of penicillin and 1 mg of streptomycin per ml. Phenol red indicator was present in a concentration of 0.0002% at 37°C. No growth medium was needed. The first medium change was in 4-7 days, and later changes were at much longer intervals. Cell passage was made with a mixture of 1.0% trypsin and 0.02% EDTA solution at room temperature, once each 2 weeks or longer. Thus the established bat

kidney cell (BKC) line was obtained.

Infected suckling mouse brain suspension of each virus was inoculated to the cell culture with m.o.i. = 0.3 - 0.7. After adsorbtion at 37°C for 60 min, the residual virus suspension was thrown away and 2% calf serum-minimum essential medium was added. The cultures were then incubated at 37°C or 4°C. The virus titration was mostly carried out by plaque assay (PFU/ml) on primary chick embryo fibroblast monolayer culture, but also by i.c. inoculation of suckling mice (LD_{50}/ml) (Reed and Muench, 1938). In a part of the experiment, virus titer was estimated by cytopathic effect (CPE) in the tube method.

The terminal dilution of cell suspension was distributed in petri dishes and cultivated at 37°C in a 5% CO_2 incubator. When any cell colony was recognized, it was picked up by pressing it with a sterilized slip of filter paper which had been immersed in trypsin-EDTA mixture (Okumura, 1964).

1. Susceptibility of BKC to some arboviruses.

The BKC was found susceptible, with CPE, to Flaviviruses (2 JE, Nakayama-NIH and JaGAr-01; St. Louis encephalitis (SLE); Murray Valley encephalitis (MVE); and Apoi viruses) and to an Alphavirus (Sindbis); but was not susceptible to dengue 1 and AMM 2021 (Getah) viruses. The titer of JEV and MVE virus in fluid was a little higher than in cells; and for Apoi and Sindbis the fluid and cell-associated titers were almost equal. Dengue 1 and Getah viruses were not found in either fluid or cells. Both JE (JaGAr-01 strain) and SLE viruses titered about one log more in chick embryo cells (PFU/ml) than in BKC by the tube method ($TCID_{50}$/ml) (Table 1). CPE in BKC was recognized with MVE, Apoi and Sindbis viruses but was not clear, and no growth of dengue 1 and Getah viruses was seen. CPE was clearer and the virus yield

was generally greater with the JaGAr-01 strain of JEV than with the Nakayama-NIH strain of longer mouse brain passage history.

2. Susceptibility of 8 cloned BKC lines to JE virus.

It is possibble that the primary BKC culture is composed of a mixture of various cells with different biological characteristics. Accordingly, cloned cell lines should first be tested to suitably evaluate the susceptibility of BKC to various arboviruses before use. Our studies on the susceptibility of 8 clones to two strains of JEV (Fig. 1) failed to show marked variations in virus yield. However, the patterns of clones 2, 3 and 6 appeared to be somewhat different from the others.

3. Long-term persistence of JE virus in BKC culture.

When the BKC was infected with the Nakayama-NIH strain of JE virus and incubated at 37°C, CPE occurred as mentioned above. Reparative multiplication of some BKC remains on the surface of the bottle culture, adequate to pass to the next cell subculture. Table 2 indicates that such cell culture showed virus yield as high as 2.8 log PFU/ml at the 16th passage (215 days at 37°C) after initial inoculation, even though the infectious center was only 0.3%. When the already infected BKC culture was incubated at 4°C, the JE virus yield was poor.

The other experiment, at 4°C (Table 3), indicates that the virus yield was zero after 118 days of incubation. However, it is evident that the virus was in a latent state, because when the cell culture temperature was elevated to 37°C (after the 118 days at 4°C), virus release could be detected (Table 3). When the direct fluorescence antibody technique was applied to the cells, the precursor or early stage of the virus could be recognized as

reported by Aizawa et al. (1975) with the mouse embryo cells.

(Masami Kitaoka, Akira Shimizu and Takayuki Ogata)

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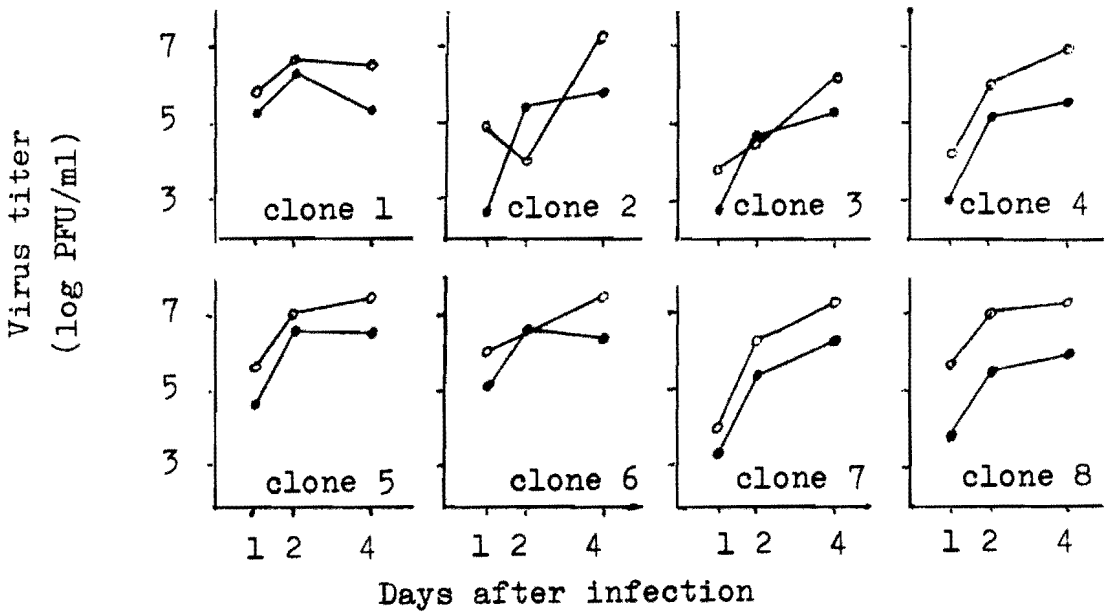
Fig. 1

Susceptibility of the clonized bat kidney cells to Japanese encephalitis virus



o——o, JaGAr-01 strain; •——•, Nakayama-NIH strain

Table 1 Virus titration on bat kidney cells

Virus	Strain	Chick embryo cell (PFU/ml)*	Bat kidney cell (TCID50/ml)**
Japanese encephalitis	JaGAr-01	9.5	8.8
St. Louis encephalitis		9.3	7.7

*PFU, plaque forming units on primary chick embryo cells.
**TCID50, 50% tissue culture infective doses.

Table 2 Persistent infection of Japanese encephalitis virus in bat kidney cells cultivated at 37°C

Days after infection	No. of passage of the cells	Existence of virus in culture fluids	Infectious center (%)
22	3	+ (4.6*)	N.D.**
75	8	+ (3.9)	2.0
108	9	+ (3.5)	N.D.
215	16	+ (2.8)	0.3

*Virus titer (log PFU/ml)
**N.D.: not done

Table 3 Long-term existence of Japanese encephalitis virus in bat kidney cells incubated at 4°C

	Days after infection	Virus yield in culture fluids	Recovery of virus after cell culture at 37°C
Exp. 1	1	+ (1.5*)	+
	12	+ (1.1)	+
	23	+ (0.9)	+
Exp. 2	118	- (0)	+

*Virus titer in culture fluids (log PFU/ml).

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY,

KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Antibody response of mini-pigs to dengue virus

Mini-pigs (Sus scrofa), 1-month-old, were procured from a Japanese farm. Two strains of DEN-1 virus were used: One was the human-attenuated Mochizuki strain, in the form of infected fluid from cultures of a human diploid cell line, WI-38; and the other was a newly isolated virus, in the form of an emulsion of infected mosquitoes which were kindly provided us by Dr. L. Rosen, of the Pacific Research Section, NIAID, Hawaii.

The viruses of the live state were subcutaneously injected into the animals, 1 ml each (approximately 2×10^3-2×10^4 PFU). No abnormal clinical signs were noted in all the test animals. Two weeks after the virus inoculation, $PRNT_{50}$ titers (50% plaque reduction NT titers per 0.025 ml, determined on BHK-21 cell monolayers in microplate cultures) were 6-8; no HI antibody was detected. After a booster injection given 7 weeks following the first, the $PRNT_{50}$ titers were 37-64, and the HI antibodies (20-40 HIU per 0.025 ml) were demonstrated.

It was regarded that the responses of mini-pigs to the mouse-passaged virus (human-attenuated) and the mosquito-grown virus (probably human-virulent) were almost the same, so far as concerning the titers of anti-DEN antibodies produced. Detection of viremia is being done using the heparinized blood samples.

(N. Fujita and M. Yamamoto)

REPORT FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY

INSTITUTE OF PUBLIC HEALTH, UNIVERSITY OF THE PHILIPPINES, MANILA

Studies on human dengue virus infections; experimental model of man's immunity against dengue virus infections*

Adult swiss white mice-adapted (ASWM) dengue 1 (now in its 60th serial intracerebral passage) and dengue 2 (now in its 57th passage), but not dengue 4 (discontinued after the 20th passage), produce active infection manifested by paralysis of the extremities and concomittant involvement of the eyes with profuse secretions leading to death.

It took 34 passages for dengue 1 and 26 passages for dengue 2 before a definite incubation period of 7 days was established.

These ASWM-adapted dengue strains have been utilized to set up an experimental model for direct assessment of the immunogenicity of the dengue viruses. The experimental model consists of adult swiss white mice immunized with one intradermal dose of 100 LD_{50} of the wild strain of dengue virus. Solid immunity among the immunized mice is measured by intracerebral challenge with the ASWM-adapted strain of dengue at definite intervals of time.

This model system offers a big advantage over previous systems so far reported. While the previous systems monitored solid protection indirectly by detection of absence of viremia in conjunction with a negative or transient HI antibody response or absence of histologic brain lesions in the animal after challenge, this experimental model directly and accurately quantitates the solid protection or immunity of preimmunized animals against a lethal dose of challenge virus.

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* This project was supported by the National Research Council of the Philippines, Grant I.C. - 32.

Using the experimental model described, we observed that:

1. A single intradermal dose of 100 LD_{50} of the wild dengue strain is the least effective immunizing dose.
2. Dengue 2 produces a solid and lasting immunity while dengue 1 produces a partial and transient immunity.
3. Dengue 2 is a much better immunogen than dengue 1.
4. Dengue 4 stimulates intra-heterotypic solid and lasting immunity against dengue 2, intra-heterotypic partial and transient immunity against dengue 1 and no extra-heterotypic immunity against Japanese encephalitis virus.

The full text of the work is under preparation for publication.

(Veronica F. Chan, Marieta R. Maaba & Cynthia C. Lusica)

REPORT FROM THE SEATO MEDICAL RESEARCH LABORATORY
VIROLOGY DEPARTMENT, BANGKOK, THAILAND

Primary Dengue Infections with Shock

Dengue infection presents a wide spectrum of clinical severity. Dengue hemorrhagic fever (DHF), a syndrome that affects Asian children, may be differentiated from "classical" dengue infections by fever which subsides at the time of increased severity of disease, hemorrhagic phenomena associated with thrombocytopenia and hepatomegally with moderate to marked hemoconcentration. The most severe and sometimes fatal manifestation of this syndrome, a decrease plasma volume with vascular collapse, is refered to as dengue shock syndrome (DSS). Halstead (Yale J. Biol. Med., 42:350-360) has hypothesized that the severe aspects of dengue disease occur as a consequence of dengue infection in the presence of pre-existing antibody. This is been refered to as the "secondary infection hypothesis." Studies in Bangkok, Thailand in 1974 have demonstrated that the shock syndrome and even death may occur with the first as well as with a second dengue infection.

Three patients with shock (see Fig. 1 for clinical data from one of these), all over one year of age, were initially classified as primary dengue infection using the criteria of Winter et al (Am. Soc. Trop. Med. Hyg. 17:590-599). Sera from these three patients were submitted for immunoglobulin separation by sucrose gradient ultracentrifugation and plaque reduction neutralization. Plaque

reduction neutralization showed higher titers to one dengue type than to others. Immunoglobulin separation followed by hemagglutination inhibition testing of the sucrose gradient fraction documented sequential formation of IgM followed by IgG in all three patients. IgM against the putative homotypic virus appears to be higher titered and to last longer.

Two fatal cases in patients also over one year of age appeared to be primary infections. Although there was no convalescent sera for confirmation of primary infection by specific immunoglobulin determination, these two patients did have low level neutralizing antibody in the premortal serum against the isolated dengue types.

(Accepted for publication in the American Journal of Tropical Medicine and Hygiene, Robert McNair Scott, Suchitra Nimmannitya, William H. Bancroft, and Pethai Mansuwan)

PATIENT 77

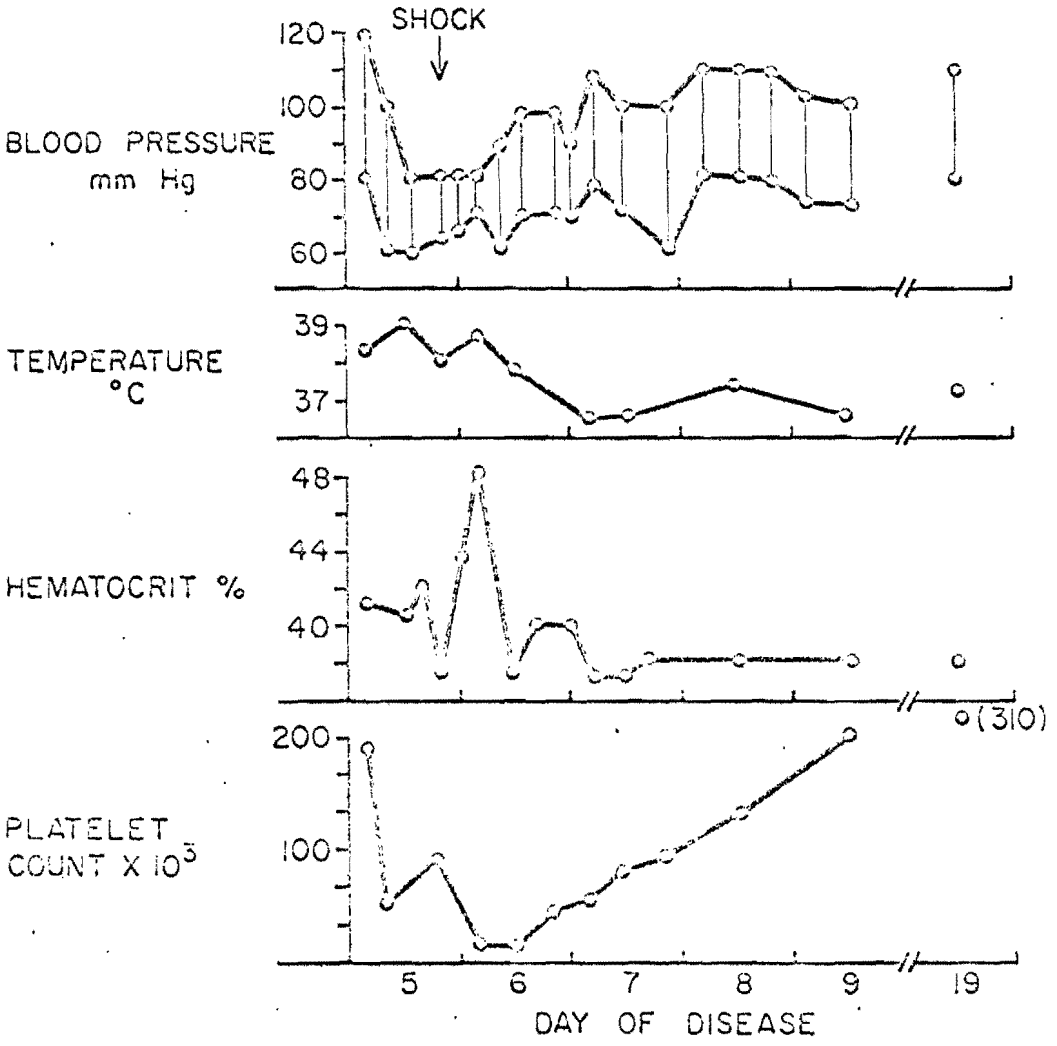

Figure 1. Diagram of the clinical course of Patient 77, showing the relationship of several clinical and laboratory variables to the onset of shock. This patient had a primary dengue infection.

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

STUDIES OF TEMBUSU AND UMBRE VIRUSES

By studying antibody prevalence, current infection rates and clinical or inapparent illness in a population of vocational trainees in the Dusun Tua Youth Training Centre in Ulu Langat, Malaysia, we hoped to determine the extent and significance of tembusu and Umbre infections in man. Investigations or virus transmission in mosquitoes and non-human hosts were conducted simultaneously to determine what species serve as principal vectors and what vertebrates may be involved. Vector mosquito populations, mainly *Culex (Culex)* species, were monitored for one year for virus infection rates. A sentinel chicken flock was maintained to determine transmission indices by virus isolation and antibody changes and suspect avian hosts were captured by mist-netting for antibody studies.

Human Studies:

To date, laboratory studies have demonstrated specific neutralizing antibody and serologic conversions to Umbre virus, indicating current infections. This is the first evidence of Umbre infection in man in Malaysia. Serologic conversions to group B and Sindbis viruses as shown by PRNT tests indicated low-level infection by other arboviruses in the Centre.

Of 909 sera tested by PRNT's, 406 (44.7%) were positive to group B viruses; tembusu antibody was shown in 55 sera (60%) with concurrent group B antibodies. The PRNT test could not identify specific infection with tembusu virus in those sera with broad group B antibody patterns.

Testing of 915 sera for Umbre neutralizing antibody demonstrated a prevalence rate of 14.6%. Nine serologic conversions (3.0%) occurred, indicating current infections in Dusun Tua.

This is the first evidence of Umbre antibody in man in Malaysia. (Banerjee reported low levels (3.7%) of Umbre antibody in man in South India: Ind. J. Med. Res., 1973, 61:334-351). Current infection rates in man have not been reported anywhere.

Whether or not clinical or inapparent illness is associated with Umbre virus is, at this time, not known and is yet to be determined. Studies of clinical histories of illnesses reported in the trainees with serological conversions to group B, Umbre and Sindbis viruses are still in progress.

Vector Studies:

Twelve strains of tembusu and six strains of Umbre viruses were recovered from 406 pools of *Culex (Culex)* mosquitoes. All but one virus strain were isolated from *C. vishnui* (syn. *C. annulus)* and *C. pseudovishnui* of the *vishnui* complex and *C. tritaeniorynchus* of the *tritaeniorynchus* complex, both in the *vishnui* subgroup.* *C. pseudosinensis* of the *bitaeniorynchus* subgroup yielded a single strain of Umbre.

Of all the mosquitoes in the *C. vishnui* subgroup collected by various methods, *C. vishnui* was by far the most numerous, caught principally in modified CDC light traps enhanced with CO_2 blocks. Further, four other methods yielded more *C. vishnui* than all other species: chicken-baited traps with CO_2, malaise traps, a sentinel chicken coop trap and sweeping collections of vegetation. *C. vishnui* populations appeared to be low in the first four months of the study, but malfunctional light traps at the onset may have been a contributory factor. However, numbers collected increased in February and March, almost tripled in April and remained at high levels until the end of the study in August. The peak month for *C. vishnui* was in June, as was the greatest number of virus isolations. Tembusu activity was shown to be in April through August, with a peak in June, and Umbre activity occurred only in June and July.

Sentinel Chicken Studies:

Of a total of 76 chickens exposed over the year, 48 (63.2%) were infected with one or more of four viruses: tembusu, Umbre, Sindbis and Japanese encephalitis.

Very high infection rates occurred with tembusu and Umbre viruses. Of the 70 chickens susceptible to tembusu, 35 (50%) were infected; of the 66 susceptible to Umbre, 37 (56%) were infected. As mosquitoes of the same *Culex vishnui* subgroup are vectors for Sindbis and Japanese encephalitis viruses in Malaysia and other southeast Asian countries, it was not surprising to find low-level infections to these viruses also. Of the 76 chickens susceptible, five (6.6%) were infected with Sindbis and two (2.6%) with Japanese encephalitis viruses. A total of 79 infections with four viruses occurred in 48 chickens, allowing for a ratio of 1.7 infections per chicken. Of the 48 chickens infected, 28 (58.3%) had multiple infections: TMU/UMB/SIN:3; TMU/UMB:21; TMU/JE:2; TMU/SIN:1; and UMB/SIN:1.

So far, ten virus strains have been recovered from chicken sera drawn just prior to the serologic conversions, showing an overall virus recovery rate for all 79 infections of 12.7%; eight strains of tembusu and one strain each of Sindbis and Umbre were isolated.

* Most recent classification of the *vishnui* subgroup according to Sunthorn Sirivanakarn in Mosquito Systematics, 1975, 7:1.

Wild Bird Studies:

Sera taken from wild-caught birds by mist-netting from sites around the chicken farm and in the adjacent secondary forest were tested for tembusu and Umbre antibodies by PRNT tests. All sera were negative to tembusu. Of sera from 375 birds tested so far, 12 (3.2%) had Umbre antibody.

Included among the species with antibody were: house sparrows *(Passer flaveolus)*, little spiderhunters *(Arachnothera longirostris)*, a reddish scops owl *(Otus rufescens)*, a mangrove whistler *(Pachycephala cinerea)*, and an *Abbott's jungle babbler (Trichastoma abbotti)*. Testing of other forest-dwelling species has not been completed yet. (Carey, D.E., *et al.*, reported on an isolation of Umbre virus from a grey partridge in south India: 1968, Ind. J. Med. Res., *56*:1340-1352).

JUNGLE DENGUE STUDIES

In the last issue of the Information Exchange, we reported the isolation of a group B virus from a pool of *Aedes (Finlaya) 'niveus'* mosquitoes collected in the sentinel monkey bait trap in the high canopy of the Gunong Besout Forest Reserve.

The virus has been successfully reisolated from the original mosquito suspension as well as from the blood of an experimental monkey inoculated with the virus. Mice surviving inoculation with the virus resisted lethal challenge with a mouse-adapted strain of dengue, indicating that the isolate is a strain of dengue virus. Final identification of the original isolate, the re-isolate, and the isolate from the experimentally-inoculated monkey have been made by plaque-reduction neutralization test and all are strains of dengue type 4. This represents the first isolation of dengue virus in nature from a jungle mosquito. Dengue virus had been isolated previously only from two other species of mosquitoes, *A. aegypti* and *A. albopictus*, both associated with man. The first isolations in nature from both species were made by Dr. Rudnick in earlier studies.

When these studies were initiated, there was no conclusive evidence available that a jungle dengue cycle existed. The principal objectives originally outlined for this study have now been satisfied to a large extent. It has been established by serological study and by virus isolation that monkeys in the high canopy of isolated primary forest are infected with at least three of the four known types of dengue; that the canopy-dwelling *Aedes 'niveus'* is the important vector in the canopy; that the canopy vector will descend to the ground to feed on man, thus allowing transfer of the virus from the forest to the human environment. *Aedes albopictus*, a known vector of dengue to man, also occurs in the forest. However, it is found in low numbers and principally at ground level. Since monkeys held at ground level failed to become infected at the same time that monkeys in the canopy were infected, it appears that *A. albopictus* does not play an important role in the transmission of dengue among monkeys in this type of forest.

Further work is required to confirm the vectorial ability of *A. 'niveus'*, to assess its role in other forest habitats, and to gather additional evidence in an attempt to correlate the jungle cycle with the cycle in man. Future plans include establishment of a laboratory colony of *A. 'niveus'* for use in dengue transmission experiments, assessment of *A. 'niveus'* in a variety of habitats, and study of dengue infection in man in rural areas.

(Albert Rudnick and Hazel Gim Wallace)

REPORT FROM THE DEPARTMENT OF VIROLOGY, NAMRU-2

JAKARTA DETACHMENT, JAKARTA, INDONESIA

Dengue Hemorrhagic Fever in Jakarta, Indonesia

A study has been initiated in Jakarta, Indonesia to investigate whether there is a relationship between the magnitude and duration of viremia in dengue hemorrhagic fever and the severity of illness, virus strain and serotype. Patients admitted to 3 hospitals in the city are studied clinically and daily specimens are taken for virological and serological study.

Table 1 shows the number of admissions in the 3 study hospitals and the number of confirmed and presumptive dengue hemorrhagic fever patients by month. Through May 1976, 279 patients had been admitted for dengue hemorrhagic fever and of these, 134 were confirmed or presumptive. The increased number of admissions in April 1976, reflects the addition of a third study hospital rather than an actual increase in admissions. It will be noted that the total number of confirmed and presumptive cases was fairly constant each month, except for September 1975 when the study was just started and February 1976. At this time we have no good explanation for the drop in cases during this latter month.

Table 2 shows the virus isolation rate from serologically confirmed patients by month. Through May 1976, 33 dengue

viruses had been isolated from dengue hemorrhagic fever patients. To date, 29 of these isolates have been typed with all 4 dengue serotypes occurring in the city of Jakarta. Dengue types 1, 2 and 3 have been isolated in about equal numbers with 9, 8 and 9 isolates, respectively. Dengue type 4 has been isolated from only 3 patients.

Table 3 shows the duration and magnitude of viremia in patients by dengue serotype. These studies have not been completed on all patients and therefore the numbers are small. There did not appear to be any significant difference in either duration or magnitude of viremia among the 3 serotypes studied to date. The longest viremia was 10 days, but most patients circulated detectable virus for only 4 to 5 days. It should be pointed out, however, that the duration of viremia was estimated from the day of onset given at the time of admission to the hospital. Furthermore, the assumption is being made that viremia was present at the time of onset. Thus, the estimates of duration of viremia may be subject to some error.

The highest viremia was over 10^8 MID_{50} per ml and occurred on the fifth day of illness with a dengue type 2 infection. It is difficult to obtain accurate data on the maximum virus titers because the patients are usually brought to the hospital late in the infection when viremia is probably

decreasing. Again, it will be noted that there does not appear to be any marked differences in viremia level between the 3 serotypes.

These data are preliminary and therefore, the observations made at this time are tentative. Further studies are in progress. We also have a program in Jakarta studying the vector competence of Aedes aegypti for dengue viruses. Report on the progress of this work will be made at a later date.

(Duane J. Gubler)

Table 1

Hospital Admissions for Dengue Hemorrhagic Fever and Number of Confirmed or Presumptive Cases in Jakarta by Month

Month	No. of Admissions	No. with* confirmed DHF	No. with** Presumptive DHF	Total Presumptive or confirmed DHF
September 1975	5	1	2	3
October 1975	20	7	11	18
November 1975	30	9	5	14
December 1975	33	7	8	15
January 1976	34	12	5	17
February 1976	21	3	1	4
March 1976	36	7	12	19
April 1976	50	13	9	22
May 1976	50	14	8	22
Total	279	73	61	134

* Includes those patients who had a 4-fold or greater rise in antibody titer between acute and convalescent sera and those who died and were confirmed by virus isolation.

** Patients who had an antibody titer of 1280 or greater in the acute serum.

Table 2

Dengue Virus Isolations from Serologically Confirmed Dengue Hemorrhagic Fever Patients in Jakarta by Month

Month	No. of Patients	No. of Isolations	%
September 1975	1	0	0
October 1975	7	4	57.1
November 1975	9	3	33.3
December 1975	7	2	28.6
January 1976	12	4	33.3
February 1976	3	1	33.3
March 1976	7	3	42.9
April 1976	13	8	61.5
May 1976	14	8	57.1
Total	73	33	43.8

Table 3

Duration and Magnitude of Viremia in Dengue Hemorrhagic Fever Patients

from Jakarta, Indonesia, by Dengue Serotype

Dengue Type 1			Dengue Type 2			Dengue Type 3		
Patient No.	Probable* duration of viremia	Titer (MID_{50}/ml)	Patient No.	Probable* duration of viremia	Titer (MID_{50}/ml)	Patient No.	Probable* duration of viremia	Titer (MID_{50}/ml)
62	4	$4X10^4$	139	10	$6X10^3$	83	6	10^4
91	5	$6X10^{3**}$	208	2	$2X10^4$	93	5	$6X10^3$
178	6	$2X10^4$	271	6	$2X10^8$	494	4	$2X10^6$
183	2***	$2X10^7$	312	4	$6X10^4$	497	5	10^6
469	6	$2X10^6$	456	4	$2X10^4$	517	2***	$4X10^4$
						1267	4***	$6X10^3$
						1277	4	$6X10^5$

* In days. Estimated from the day of onset given on admission to the hospital and the last day of illness virus was detected in the blood by intrathoracic inoculation of mosquitoes.

** Level of viremia detectable by mosquito inoculation.

*** Patients died - only 1 blood sample obtained.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, JOHN CURTIN SCHOOL OF MEDICAL RESEARCH, AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA, AUSTRALIA.

No cases of Murray Valley encephalitis have been reported since the epidemic of 1974. Study sites selected during the epidemic have been sampled for mosquitoes and bird sera in the Spring (November), Summer (February) and Autumn (April-May) for the past two years.

TABLE 1

	Feb 74	Nov 74	Feb 75	April 75	Nov 75	Feb 76	May 76
Catch/light trap/night	C.2692	214	87	20	236	260	27
% C. annulirostris	99	42	95	8	55	94	17
Total catch (light traps and aspirators)	C.140,000	4,985	3,635	813	13,028	13,285	1,281
Virus Isolations	Sindbis (22) RRV (7) MVE (15) Kunjin (56) Edge Hill (5) Kowanyama (2) Koongol (5)	nil	nil	nil	nil	Sindbis(7) other (2)	nil

Heavy flooding occurred throughout the region during the Spring of 1973, 1974 and 1975. Surface water persisted on flood plains and beyond through February 1974 and to a lesser extent in 1976; there was very little in 1975. These conditions are reflected in the mosquito catch per light trap night in Table 1, which are already tending to confirm subjective observations that the epidemic coincided with a quite abnormally large mosquito population. All viruses

recovered to date have been from pools of Culex annulirostris, except one MVE isolate from the blood of a white faced heron. The seasonal prevalence of C. annulirostris in the species spectrum is remarkably constant despite yearly variations in overall abundance of mosquitoes; about 50% in Spring, 95% in Summer and 10% in Autumn. The low yield of MVE virus during the epidemic is disturbing, and the possibility that the epidemic was in part due to the closely related Kunjin virus has been suggested elsewhere (Doherty et al. 1976, Aust. J. Exp. Biol. Med. Sci. 54, 237-243). Mosquito activity during daylight hours was virtually non existent in February, but was intense during December and January when the first confirmed cases were reported.

Serological investigations during the epidemic drew suspicion to the nankeen night heron as having a special relationship with MVE (and/or Kunjin) virus. With 22/25 specimens positive for flavivirus antibody, this was twice the rate of the next highest species (little black cormorant, 11/26) and more than four times the overall mean percentage incidence. This was confirmed in 1974-75 when 75/77 adult and 82/170 immature NNH were positive. Despite the presumed addition of birds hatched since the epidemic, the percentage positive remained fairly constant in November, February, and April for both adult (88%, 100%, 95%) and immature birds (52%, 46%, 52%). Sindbis antibodies occurred at a lower but relatively constant incidence across a wider range of species.

Water birds were again very plentiful throughout the region in 1975-1976, but inexplicably NNH were scarce (this was NOT due to our predation!). There was no convincing evidence of recent MVE-Kunjin infection in the few NNH obtained, but a very low incidence of high titres in other species. High titred Sindbis antibodies were again fairly common, which is in accord with the virus isolations from mosquitoes in February 1976.

A large breeding colony of NNH and little white egrets about 15 miles from one of the regular study sites was visited in April 1976 and 40 NNH nestlings were captured and returned to Canberra for study of change in plumage with age, virus growth cycles, antibody development and decay etc. Little is known of the life history of the NNH, but the main breeding season is reported to be in Spring with some breeding continuing throughout the year. It was estimated that there were over 2,000 NNH in this breeding colony when visited in autumn. This, and possibly other heronries will be included in future arbovirus surveillance.

Ian D. Marshall

Gwen Woodroofe.

REPORT ON CSIRO DIVISION OF ANIMAL HEALTH LABORATORIES AT:

PRIVATE BAG NO. 1, PARKVILLE, VICTORIA, 3052, AUSTRALIA

PRIVATE BAG No. 1, GLEBE, NEW SOUTH WALES, 2037, AUSTRALIA

PRIVATE BAG NO. 3, INDOOROOPILLY, QUEENSLAND, 4068, AUSTRALIA

Akabane Disease in Australia

Akabane virus, a bunyavirus in the Simbu serological group, has been associated with congenital infections of cattle and of sheep in Australia. Epizootics of bovine congenital arthrogryposis (limbs locked in flexed or extended position) and hydranencephaly (replacement of the cerebral hemispheres of the brain by a fluid filled cavity) have been reported to occur in south-eastern New South Wales at 2 to 3 year intervals since the mid-1940's. The deformed calves are usually born between July and October, and it would appear the infection occurred during the previous February and March. In 1974, a large epizootic occurred in which in excess of 5,000 calves were lost. Pre-suckling serum from all affected calves with arthrogryposis and hydranencephaly possessed serum neutralizing antibodies against Akabane virus, as ruminants do not receive antibodies across the placenta but at their first suckling of colostrum from their mothers. Virus could not be isolated from tissues of these deformed calves, possibly due to the presence of antibody.

An Australia-wide serological survey was undertaken to define the distribution of cattle which had been infected with Akabane virus. In excess of 80% of all cattle in northern Australia (Queensland, Northern Territory, and Western Australia) possessed antibodies. In the epizootic area in New South Wales, up to 100% of animals in herds possessed antibodies. The Australian distribution of antibodies would fit the distribution of the biting midge Culicoides brevitarsis, from which the virus has been isolated on three occasions. It is suggested that in northern Australia the disease is enzootic, and animals become infected early in life and hence immune when they become pregnant, which would account for the few isolated cases of deformed calves seen in this area. Extended warm, humid summer (as occurred in 1974) would provide excellent conditions for the southward spread of virus-infected insects, which could meet non-immune pregnant animals. The area in which epizootics occur, southward from Sydney to Bega, appear to be at the lower limit of distribution of C. brevitarsis during these periods.

Lambs showing signs of micrencephaly (small brains) have also been observed in the epizootic area in New South Wales. Neutralizing antibodies to Akabane virus has been found in the pre-colostral sera of deformed lambs and in the sera of their mothers. In Israel and Japan, the virus has also been reported to cause deformities in goats. We have also found antibodies present in the sera of horses, but not of pigs, chickens, and wallabies.

Experiment Studies in Sheep

Studies in pregnant ewes using mouse adapted Akabane virus (strain B8935), have shown that this virus can cross the placenta and cause congenital deformities in lambs. Ewes that were infected by the intravenous route at 30 to 36 days gestation of pregnancy showed no signs of illness but produced 5 congenitally deformed lambs (an incidence of 31%). Two of these lambs had arthrogryposis and hydranencephaly; one had micrencephaly; and the other two had porenecephaly (cavitations in the brain). Four of these five lambs possessed serum neutralizing antibodies against Akabane virus, at birth.

The infection in cattle and in sheep is inapparent. Both develop a good neutralizing antibody response to the virus, the maximum titres being in the range of 1 in 64 to 1 in 512. The antibody levels appear to persist for long periods, the titres being in the range of 1 in 2 to 1 in 16 up to 2 years after infection.

(A.J. Della-Porta, I.M. Parsonson, W.A. Snowdon, M.L. O'Halloran, M.D. Murray, D.H. Cybinski)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF WESTERN AUSTRALIA, PERTH, WESTERN AUSTRALIA, AUSTRALIA

Virus isolations from mosquitoes, and serological studies in humans, cattle and birds in the Ord River area of the Kimberley, Western Australia.

This report continues from the results presented in earlier issues of the Information Exchange. The six tables are self-explanatory and record our observations up to July 1976. The figure locates the study site areas referred to in Table 2.

(N.F. Stanley, M.P. Alpers, K.H. Chan, S. Paul, A. Wright, N.P. Hamilton)

TABLE 1.

130 virus isolations from 23,872 mosquitoes collected in the Ord Valley.

Field collection date	No. of mosquito pools	Mosquito species	No. of pools per species*	No. of pools yielding virus
May-June 1972	33	*Culex annulirostris*	29	7
Nov.1972 - Jan.1973	69	*Aedeomyia catasticta*	7	1
March-May 1973	113	*Culex annulirostris*	93	37
		Aedeomyia catasticta	14	3
		Aedes tremulus	1	1
Nov.-Dec. 1973	63	*Culex annulirostris*	40	3
		Aedeomyia catasticta	9	1
March-April 1974	207	*Culex annulirostris*	150	64
		Culex fatigans	41	11
		Aedes normanensis	3	2
Total	485		387	130

*Species from which no viruses were isolated are not recorded in this table.

TABLE 2.

Characterization of 45 isolates from mosquitoes collected in Kununurra and Parry's Creek areas.

Period of mosquito collection	Group	Virus	Strain	Vector	Collection site	Map site no.
May-June 1972	Flavivirus	MVE	OR 1	*C. annulirostris*	Dunham River/Little Lily Creek	25
"	"	"	OR 2	"	E.S.D.D.W.[1]	11
"	"	"	OR 3	"	Golf Course	13
"	"	Kunjin	OR 4	"	E.S.D.D.W.	11
"	Koongol	Wongal	OR 5	"	E.S.D.D.W.	11
"	"	"	OR 7	"	Packsaddle Swamp	2
"	Alphavirus	Sindbis	OR 6	"	E.S.D.D.W.	11
Nov.-Mar. 1973	Non-haemagglutinating		OR 115	*Aedomyia catasticta*	10 sites: 19, 18	
Apr.-May 1973	Flavivirus	MVE	OR 155	*C. annulirostris*	Boat Ramp	15
"	"	"	OR 156	"	Boab behind Kununurra Hospital	18
"	"	Kunjin	OR 130	"	W.E.D.D.W.[2]	1
"	"	"	OR 134	"	Picnic Area	25
"	"	"	OR 161	"	Boab behind Kununurra Hospital	18
"	"	"	OR 165	"	Kelly's Knob	19
"	"	"	OR 166	"	" "	19
"	"	"	OR 192	"	Parry's Creek	
"	"	"	OR 193	"	" "	
"	"	"	OR 237	"	Race Course	28
"	"	"	OR 205	*Aedes tremulis*	Lily Creek	24
"	Koongol	Koongol	OR 133	*C. annulirostris*	Picnic Area	25
"	"	"	OR 136	"	" "	25
"	"	"	OR 182	"	Lily Creek	24
"	"		OR 234	"	Boat Ramp	15
"	"	Wongal	OR 137	"	Picnic Area	25
"	"	"	OR 177	"	Entrance to Hidden Valley	
"	"	"	OR 188	"	Parry's Creek Run	
"	Non-haemagglutinating		OR 126	"	W.E.D.D.W.	1
"	"		OR 135	"	Picnic Area	25
"	"		OR 140	"	" "	25
"	"		OR 151	"	Boat Ramp	15
"	"		OR 153	"	" "	15
"	"		OR 158	"	Boab behind Kununurra Hospital	18
"	"		OR 159	"	"	18
"	"		OR 233	"	Boat Ramp	15
"	"		OR 194	*Aedomyia catasticta*	Helicopter Pad	
"	"		OR 195	"	Picnic Area	25
"	"		OR 199	"	Lily Creek	24
"	Mapputta	Trubanaman	OR 189	*C. annulirostris*	Parry's Creek Run	
Nov.-Dec. 1973	Non-haemagglutinating		OR 278	*Aedomyia catasticta*	Boab behind Kununurra Hospital	18
"	"		OR 281	"	W.E.D.D.W./Picnic Area	1/25
"	"		OR 285	"	Lily Creek/Little Lily Creek/Hidden Valley	24/23/23
Mar.-Apr. 1974	Flavivirus	MVE	OR 324	*C. annulirostris*	Boat Ramp	11
"	"	Kunjin	OR 311	"	W.E.D.D.W.	1
"		"	OR 317	"	Picnic Area	25
"		"	OR 354	"	Airport	12

[1]East side of Diversion Dam wall.
[2]West side of Diversion Dam wall.

TABLE 3.

Arbovirus antibodies in human sera according to length of residence in the Study Site.

Years of residence in Study Site	No. of sera	% positive by H-I tests with MVE	Sindbis	Ross River
<3 years	31	26	0	23
>3 years	39	64	8	41

TABLE 4.

*Arbovirus antibodies in cattle sera from the East Kimberley Study Site, the West Kimberley and South-West Australia.**

Site of cattle station	No. of sera tested	% positive by H-I tests with MVE	Sindbis	Ross River
Kununurra (Study Site area)	316	80	4	2
Kimberley (remote from Study Site)	564	37	4	2
South-West of Australia	200	1	0	0.5

*Permission granted from Department of Agriculture for release of these results.

TABLE 5.

Arbovirus antibodies in avian sera from the Ord River area.

Bird species	No. of sera tested	No. positive by H-I tests with MVE	Sindbis	Ross River
Magpie goose *(Anseranas semipalmata)*	79	42	17	2
Black duck *(Anas superciliosa)*	24	9	3	
Water whistle duck *(Dendrocygna arcuata)*	9	8	3	3
Green pygmy-goose *(Nettapus pulchellus)*	2	2	2	
Rainbow-bird *(Merops ornatus)*	2	1		
Common sandpiper *(Tringa hypoleucos)*	2	2	1	
Royal spoonbill *(Platalea regia)*	3	3	1	
Yellow-billed spoonbill *(Platalea flavipes)*	2	1		
Little grebe *(Podiceps novae-hollandiae)*	1	1		
Pied heron *(Ardea picata)*	1	1	1	
Mangrove heron *(Butorides striatus)*	1	1		
Whitefaced heron *(Ardea novae-hollandiae)*	4	3	1	
Whitenecked heron *(Ardea pacifica)*	2	1		
Little egret *(Egretta garzetta)*	1	1	1	
White egret *(Egretta alba)*	3	1	1	
Little black cormorant *(Phalacrocorax sulcirostris)*	1			
White ibis *(Threskiornis molucca)*	3			
Glossy ibis *(Plegadis falcinellus)*	2			
Black-winged stilt *(Himantopus leucocephalus)*	1	1	1	
Masked plover *(Lobibyx miles)*	1	1		
Yellow honeyeater *(Meliphaga flava)*	14	11	2	
Red-throated honeyeater *(Conopophila rufogularis)*	4	4		
Black kite *(Milvus migrans)*	2	1		
Little corella *(Kakatoë sanguinea)*	105	58	10	
Pink and grey galah *(Kakatoë roseicapilla)*	9	6		
Red-tailed black cockatoo *(Calyptorhynchus banksi)*	9	4		
Red-winged parrot *(Aprosmictus scapularis)*	1	1		
	288	164	44	5

TABLE 6.

Comparison of arbovirus antibody in sera of water birds and others.

	Total	No. positive by H-I tests with		
		MVE	Sindbis	Ross River
Water birds	144	79	32	5
Others	144	85	12	0

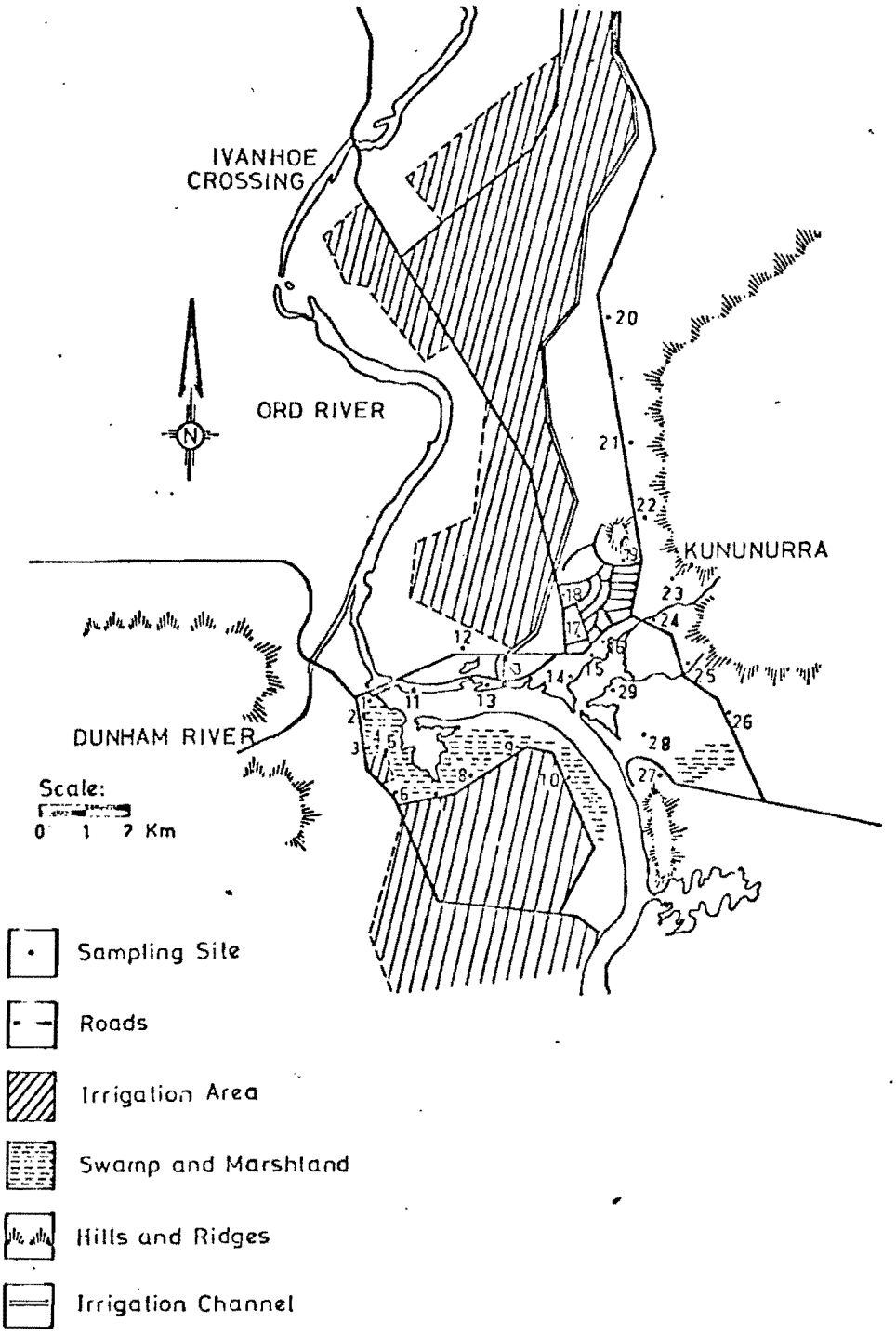

FIGURE. Sampling sites in the Ord River study area, East Kimberley.

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

Protection of mice by Japanese encephalitis vaccine against a locally isolated strain of JE virus.

An imported lot of Japanese encephalitis vaccine was assayed for its potency and efficacy in mice. The potency of the Test vaccine was found to be satisfactory as compared to that of a Reference Vaccine.

The efficacy of the Test Vaccine against a locally isolated Calcutta strain of JE virus was compared with that of the Nakayama strain of the virus. All the dilutions of the Test Vaccine conferred cent per cent protection against the Nakayama strain, while the protection against the Calcutta strain was hundred per cent with 1 in 4 dilution, 80 per cent with 1 in 16 dilution and 70 per cent with 1 in 64 dilution of the vaccine. The results of the experiments are shown in Table-1.

(B.C. Das, S.K. Chakrabarty, J.K. Sarkar & K.K. Mukherjee)

TABLE - 1.

Showing the protection of mice immunised with Test Vaccine against challenge by Nakayama strain and the Calcutta strain.

Dilutions of the Test Vaccine	Challenge virus	Died/survived	Percentage survival
1/4	Nakayama strain	0/10	100
	Calcutta strain	0/10	100
1/16	Nakayama strain	0/10	100
	Calcutta strain	2/10	80
1/64	Nakayama strain	0/10	100
	Calcutta strain	3/10	70

REPORT FROM THE VIROLOGY SECTION, SCHOOL OF PUBLIC HEALTH

UNIVERSITY OF TEHRAN, IRAN

Studies on the epidemiology of sandfly fever in Iran

During the summer of 1975, field studies on the epidemiology of sandfly fever were conducted in 4 villages in Isfahan Province, Iran. These studies were in collaboration with the Pacific Research Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Honolulu, Hawaii. Approximately 40,000 sandflies were collected in one of the villages between July and September. Of this total, 12,485 Phlebotomus papatasi (10,615 females and 1,870 males) were processed for virus isolation in tube cultures of Vero cells. From these specimens, 62 virus isolates were obtained. Two isolates (one each of Sicilian and Karimabad viruses) were made from pools of male sandflies; the remainder were from females. The overall virus isolation rate from female P. papatasi was 1 per 177 insects processed, demonstrating the vector potential of this species. Identification of the sandfly virus isolates indicated that 49 were Sicilian virus, 11 were Karimabad, and 2 were an apparent new member of the vesicular stomatitis virus serogroup, designated as Isfahan virus.

Blood specimens were obtained from humans, domestic animals and gerbils in the study area and were examined by plaque reduction neutralization test for antibodies against 5 Phlebotomus fever virus serotypes (Naples, Sicilian, Karimabad, Salehabad and I-47), known to occur in Iran. These serologic results are summarized in Table 1. Many of the human residents of the study area had neutralizing antibodies to Sicilian and Karimabad viruses. A smaller number, mostly adults, had evidence of Naples virus infection. All sera were negative against Salehabad and I-47 viruses.

Of the animal sera tested, only gerbils (Rhombomys opimus) showed evidence of infection. Both Sicilian and Karimabad neutralizing antibodies were found in gerbil sera, suggesting that these ubiquitous rodents might serve as amplifying hosts of the viruses. Studies on the experimental infection of gerbils with these two viruses are in progress. R. opimus will be experimentally infected with both Sicilian and Karimabad viruses to determine the level and duration of viremia.

Blood meals from 575 P. papatasi collected in the study area were examined by microplate precipitin test. The majority of the sandflies (58%) had fed on birds, mostly chickens and pigeons. The remainder had fed on humans, equines, bovines, sheep and goats. The results indicate that P. papatasi has a much wider natural host range than previously reported and suggest that this species is an opportunistic feeder and is not strongly anthropophilic.

Results of our studies indicate that both Sicilian and Karimabad viruses are endemic in Isfahan Province. We suspect that continual virus activity probably occurs in this region each summer during the sandfly season. Since Phlebotomus fever viruses appear to be maintained in the sandfly population by vertical (transovarial) transmission, they therefore are not dependent upon the presence of susceptible vertebrate hosts and their activity is uneffected by the immune status of the local human or animal populations. Among the indigenous population, many of whom have already been infected, sporatic cases of sandfly fever probably occur mainly in young children.

Our studies also suggest that the ecology of Sicilian and Karimabad viruses involve P. papatasi (and possibly other sandfly species), gerbils and man. Interesting, this same epidemiologic pattern also has been reported for cutaneous leishmaniasis in Isfahan Province. A more detailed report of our studies will be published shortly.

S. Saidi

Table 1

Prevalence of Naples, Sicilian and Karimabad virus neutralizing antibodies among residents of 4 villages* in Isfahan Province, Iran

Age group (yrs.)	Total sera tested	Percentage positive		
		Naples	Sicilian	Karimabad
1-5	87	2.3	1.2	41.4
6-10	136	2.9	14.7	64.0
11-15	38	7.9	26.3	79.0
16-20	16	56.3	25.0	75.0
21-30	32	28.1	59.4	78.1
31-40	36	33.3	61.1	88.9
41-50	32	59.4	46.9	78.1
51-60	17	41.2	47.1	76.5
>60	8	50.0	37.5	87.5
Total	402	17.2	25.4	66.4

* Dormian, Ali-Abad, Komchesheh and Shapurabad villages

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

I. Status of Dengue Virus Antibodies in Egypt

Epidemics of Dengue have been reported in Egypt in 1927, 1928, and 1937. These epidemics were diagnosed on epidemiological and clinical bases, without virological studies to determine the etiological agent. It is now known that many arboviruses, including West Nile (WN) which was isolated in Egypt in the 1950's, can produce dengue-like illness. Lately, when a serologic survey was carried out on 1133 serum specimens collected in 1969 from certain Egyptian Governorates, hemagglutination - inhibiting (HI) antibodies against Dengue type I (D-1) virus were encountered in 4 (0.3 %) positive sera (Darwish and Ibrahim, 1971).

It was decided to extend the search for Dengue antibodies by testing a large number of serum specimens by HI test. Out of 1590 sera tested, 416 reacted with Dengue-1 antigen (26.2 %). When excluding the cross-reactions resulting from WN infections, the reactive sera to D-1 antigen were reduced to 98 (6.2 %). The following table shows the distribution of these 98 reactive sera :

Type of D-1 reaction	No.	Percent of D-1 reactive sera (98)	Percent of total tested sera (1590)
Specific	17	17.4	1.1
Superinfection	50	51.0	3.2
Undiagnosable	31	31.6	1.9
T o t a l	98	100.0	6.2

If we neglect the sera with undiagnosable titers and overlook those exhibiting superinfection HI-titers, the specimens demonstrating specific titers cannot be ignored. All the specifically reactive sera were free from HI antibodies against WN virus. Four of those 17 "specific" sera belong to persons from Assiut Governorate (Upper Egypt) and exhibited a low HI titer of 1 : 10 against Dengue-1 virus. The remaining 13 "Dengue specific" sera were from school children (12 - 15 years) at Alexandria Governorate and exhibited variable HI titers 1 : 10 (4 sera), 1 : 20 (4 sera), 1 : 40 (3 sera), and 1 : 80 (2 sera). In fact, to have 17 sera (1.1 %) demonstrating HI titers against Dengue virus without concomitant WN, HI antibodies, particularly when 13 of them belong to children from the same school at Alexandria, may be indicative of Dengue - or a closely related virus - activity in the locality. More sera are now being collected from Alexandria and planned to be tested for Dengue HI and neutralizing (NT) antibodies.

II. Antibodies Against Abu Hammad and Abu Mina Viruses in Mammalian Sera From Egypt

Abu Hammad (AH) and Abu Mina (AM) viruses of the Dera Ghazi Khan serogroup of Arboviruses were first isolated in Egypt. Abu Hammad virus was isolated in 1971 from Argas (A) hermanni ticks from pigeon houses in Abu Hammad (Sharqiya Governorate). Abu Mina virus was isolated in 1963 from a northward migrating dove trapped in Abu Mina near Bahig (Matruh Governorate), and in 1969 from Argas (Persicargas) streptopelia from a date palm in which doves and herons rested in Dakhla Oasis (El-Wadi El-Dedded Governorate).

Information about AH virus was submitted to the International Catalog of Arboviruses in April 1972, but AM virus has not yet been registered (Hoogstraal, 1973; Converse et al., 1975; Berge, 1975).

The purpose of this work was to determine whether complement-fixing (CF) antibodies against these two bird-and bird-tick-associated viruses can be detected in mammalian sera from Egypt.

Sera from man (717) and different animals (774) were explored for the first time, for CF antibodies against the 2 viruses. The 717 human sera from 4 Governorates (two in Upper Egypt and 2 in Lower Egypt) did not react with either of the 2 antigens. The animal sera tested and results with the 2 viruses are shown in the following table :

Animal species	No. sera tested	Abu Hammad Virus		Abu Mina Virus	
		No. positive	%	No. positive	%
Buffalo	95	2	2.1	0	-
Pig	93	1	1.1	0	-
Sheep	195	2	1.0	1	0.5
Camel	113	0	-	0	-
Cow	59	0	-	0	-
Horse	9	0	-	0	-
Dog	65	0	-	2	3.1
Rodent	145	0	-	3	2.1

Sera of the camel, cow, and horse were free of CF antibodies against both antigens, even at 1 : 4 dilution. Among the 5 sera which reacted with AH virus, 2 from buffaloes exhibited a high titer (1 : 32). Antibodies to AM virus in sera of the sheep, dog, and rodents were at low titer (1 : 4).

These data demonstrating the infrequancy of CF antibodies against the 2 viruses are neither inclusive nor conclusive. Samples of human and animal sera from different localities will also be surveyed for possibly more durable neutralizing antibodies against these 2 viruses. Ticks from serologically suspect animals can also be tested for possible virus isolation. The fact that these two viruses, particularly AM, have been isolated in Egypt more than once at distant times and places should encourage this investigation.

Although the percentage of positively reacting sera is low (0.5 % to 3.1 %), the fact that some mammalian sera did react against these two bird-and-bird tick-associated viruses suggests that the range of virus reservoirs and vectors is probably wider than has been recognized.

III. <u>A Serological Survey to Certain Arboviruses in Human Population in Libya</u>

Libya is an African Country fronting on the Mediterranean, bounded with Egypt in the east, Tunisia and Algeria in the west, Niger, Chad and Sudan in the south. The incidence of disease in Libya was not known, except perhaps for the last few years. Available reports may give a rough indication of the prevalence of certain parasitic and bacterial diseases. Information regarding arboviral infection is lacking for Libya, as well as many of the adjacent countries, apart of Egypt and Sudan. The presence of several types of mosquitoes-anopheles, culex and Aedes-sand-flies,

as well as certain arthropod-transmitted diseases as malaria, kala-azar and cutaneous leishmaniasis, make one suspect arboviral activity in Libya.

Human sera were made available from 2 governorates : Sebha (148 sera) in Southern Libya and Benghazi (93 sera) in the north. The sera were tested by HI test for antibodies against Sindbis (SIN), O'nyong - nyong, Western Equine Encephalitis (WEE), WN, and Dengue type-1 viruses. Complement fixing antibodies against 13 tick-borne viruses (or possibly so) which do not usually produce hemagglutination were also looked for. The idea was to test these sera against the same viruses which are known to be active in Egypt or which have been searched for in Egypt.

The results showed that no CF antibodies were detected against the tested viruses viz : Quaranfil, Qalyub, Chenuda, Nyamanini, Wad Medani, Wanowrie, Dhori, Abu Mina, Aou Hammad, Bahig, Matruh, Matariya, and Burg El-Arab. HI antibodies were positive only for WN, SIN, and Dengue as shown in the following table :

Governorate	No. of sera tested	West Nile		Sindbis		Dengue-1	
		No.	%	No.	%	No.	%
Sebha	148	110	74.3	62	41.8	-	-
Benghazi	93	11	11.8	1	1.1	9	9.7
T o t a l	241	121	50.2	63	26.1	9	3.7

These results indicate the following :

1. It represents, most probably, the first indication of Arboviral activity in Libya and serves as a base line for the antibody pattern in this rapidly developing and changing country.

2. The survey indicates an overall intense infection with WN and SIN viruses viz : 50 % and 26.1 % respectively. The positivity rates for both WN and SIN at the southern Sebha Governorate were higher than those of the northern coastal Benghazi Governorate (74.3 % and 41.8 % versus 11.8 % and 1.1 %). The HI titers for WN ranged from 1 : 20 to 1 : 640 but those of Sindbis did not exceed 1 : 160. In fact, the prevalence rates of these 2 viruses in Libya are comparable with those of Egypt, and also the north-to-south increase in antibody rates is noticed in both countries.

3. The antibodies against Dengue virus was of the specific type. None of the 9 sera were simultaneously positive for WN and the Dengue titer ranged from 1 : 20 to 1 : 80. The positivity of sera from Benghazi for Dengue antibodies is similar to the situation in Egypt where Dengue antibodies were detected in Alexandria. The nature of the antibodies in the 2 coastal governorates (Benghazi and Alexandria) can be Dengue or a Dengue-related virus.

4. The negativity of sera to antibodies against the other Arboviruses tested represent the same as was previously found in Egypt except for Quaranfil and Qalyub viruses for which CF antibodies were found in man in Egypt.

(Medhat A. Darwish and Imam Z. Imam).

REPORT FROM NAMRU-5 ADDIS ABABA, ETHIOPIA, APO NEW YORK 09319

OR C/O AMERICAN EMBASSY, P.O. BOX 1014, ADDIS ABABA, ETHIOPIA

Congo Virus Isolated in Southwest Ethiopia

A virus (Eth AR 838) resembling Congo was isolated from a pool of 20 partially engorged Hyalomma impeltatum ticks collected from sheep in the village of Omo Ratay on 17 March 1975. Omo Ratay is a police post and government ferry station on the Omo River about 35 km upstream from Lake Rudolph (4°47'N, 36°02'E). The area is ecologically semidesert except for the rather narrow flood plains of the Omo where a few trees and 200 meter wide belt of bush line the river banks. The elevation of the village is about 500 m and the villagers support themselves principally by raising cattle, sheep, and goats. The ticks were collected at the end of a rather extensive dry season when most cattle were absent, having been taken to pastures bordering Lake Rudolph. Hemorrhagic disease in the human population of the immediate area has not been reported, although an outbreak of hemorrhagic disease occurred among the Turkana just across the border in Kenya in 1972. (D. Metselaar personal communication).

Additional collections of ticks were made at Omo Ratay and the Kelam mission 10 km to the south and included the following species: Amblyomma variegatum, Hyalomma anatolicum, H. marginatum rufipes, H. truncatum, Rhipicephalus evertsi, R. pravus, R. pulchellus, R. sanguineus, and R. simus.

The virus (Eth AR 838) was tested at NAMRU-5 against the prototype Congo Strain Ib Ar 10200 and its antiserum, obtained from the Institute Pasteur in Dakar and YARU. The complement fixation test gave reactions of identity. Eth AR 838 virus was sent to YARU and screened by complement fixation against 55 tick-borne viruses. It reacted only with Congo antigen and immune ascitic fluid. Dr. Casals then performed complete titrations which gave the following results (Table 1) confirming that (Eth AR 838) is Congo virus.

(O. L. Wood, V. H. Lee and J. S. Ash)

Antigen:	Serum: Eth Ar 838							
Eth Ar 838	1/8	16	32	64	128	256	512	1024
1/8	4	4	4	3	0	0	0	0
16	4	4	4	4	4	0	0	0
32	4	4	4	4	1	0	0	0
64	4	4	4	4	1	0	0	0
128	2	2	2	1	0	0	0	0
256	0	0	0	0	0	0	0	0
512	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	0	0	0

Antigen:	Serum: Congo (Ibar 10200)							
Congo	1/8							1/1024
1/8	4	4	4	4	4	0	0	0
	4	4	4	4	4	0	0	0
	4	4	4	4	4	1	0	0
	4	4	4	4	4	3	0	0
	4	4	4	4	4	3	0	0
	4	4	4	4	4	4	0	C
	4	4	4	4	4	4	1	0
1/1024	4	4	4	4	4	4	2	0

Antigen:	Serum: Eth Ar 838							
Congo	1/8							1/1024
1/8	4	4	4	0	0	0	0	0
	4	4	4	1	0	0	0	0
	4	4	4	4	0	0	0	0
	4	4	4	4	1	0	0	0
	4	4	4	4	0	0	0	0
	4	4	4	3	0	0	0	0
1/1024	4	4	4	1	0	0	0	0

Antigen:	Serum: Congo (IbAr 10200)							
Eth Ar 838	1/8							1/1024
1/8	4	4	4	4	4	2	0	0
	4	4	4	4	4	3	0	0
	4	4	4	4	4	4	0	0
	4	4	4	4	0	4	1	0
	4	4	4	4	4	2	0	0
	2	2	2	0	0	0	0	0
	0	0	0	0	0	0	0	0
1/1024	0	0	0	0	0	0	0	0

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

A virus strain isolated from a pool of Anopheles funestus mosquitoes, collected in the coastal area of Kenya in November 1972, was identified as Akabane virus at the Pasteur Institute of Dakar (Director Dr. Y. Robin).

The virus was first isolated in Japan from Aedes vexans niponii and later from Culex tritaeniorhynchus by Oya and co-workers. Doherty and co-workers isolated the virus from Culicoides brevitarsis in Queensland in Australia.

Japanese and Australian workers produced evidence that Akabane virus is the aetiological agent in outbreaks of abortions and epidemics of the congenital arthrogryposis-hydranencephaly syndrome in cattle.

In the Pasteur Institute of Dakar, using mouse ascitic fluid, the isolate could not be differentiated from Akabane virus in the complement fixation test. There was a slight one way difference in the neutralization test.

D. METSELAAR

Preparation of haemagglutinating & precipitin antigens to wanowrie (G 700) Virus

This virus was originally isolated from adult male and female ticks of the Hyaloma species (Hyaloma marginatum) in Wanowrie, India, by VRC, Poona, India.

The study reported here aims at: (1) finding erythrocytes capable of being haemagglutinated by this virus, (2) producing a potent HA antigen from this agent, and (3) looking for methods other than CFT and NT (which are possible with this agent) e.g. precipitin antigen, for quick diagnosis of the agent.

(i) HA and HI Tests:

From infected mouse brain suspension, HA antigen was prepared according to the method of Clarke and Casals, 1958. This antigen did not haemagglutinate erythrocytes from geese, Cercopithecus aethiops, Arvicanthis rats, chickens and guinea-pig. However, when the extracted antigen was concentrated X20 with Polyethylen-glycol (PLEG) - HO $(C_2 H_4 O)_n H$- and tested with goose r.b.c. for HA activity, agglutination was positive at pH 5.8, and the titre was 1/80.

HI test carried out with homologous antiserum and with virus grouping antiserum, Polyvalent 10, containing Upolu, DGK, Dhori and Wanowrie, was positive with both antisera. HA activity however, was lost after a short storage period (a little over one month) at 4°C.

Haemagglutination capacity of Wanowrie Virus (G 700) to 4 other Erythrocytes

Haemagglutination capacity of Wanowrie Virus to four species of

animal erythrocytes was investigated. The results are set out in the Table below:

Erythrocytes Species	Suspending fluid	% Concentration of RBCs	HA titre reciprocals obtained with Diluent used.	
			0.4% BPA/BSB	0.85% NaCl
Goose	Saline	0.5	20	40
	DVG PH 5.8	0.5	80	80
	DVG PH 5.8	1.0	80	80
Grey Monkey (Cercopethicus ethiops)	Saline	0.5	20	40
	DVG PH 5.8	0.5	Non-specific Agglutination	Non-specific Agglutination
	DVG PH 5.8	1.0	"	"
Chicken	Saline	0.5	40	"
	DVG PH 5.8	0.5	40	"
	DVG PH 5.8	1.0	40	"
Arvicanthis rat	Saline	0.5	Non-specific	"
	DVG PH 5.8	0.5	Agglutination	"
	DVG PH 5.8	1.0	"	"
Guinea-Pig	Saline	0.5	20	"
	DVG PH 5.8	0.5	80	"
	DVG PH 5.8	1.0	80	"

From these results it is apparent that apart from Goose erythrocytes normally used for Wanowrie HA tests, chicken and Guinea-pig erythrocytes may also be employed.

It is also clear from the above account that the agent posseses haemagglutinins against goose, chicken and guinea-pig erythrocytes. The best method of storing the HA antigen is being sought.

(ii) Agar Gel Diffusion Precipitation Test (AGDPT).

A 1/5 dilution of this concentrated material was tested by AGDP method against 2 - fold dilutions of its homologous antiserum. The antiserum titred 1/8, whilst the reverse, i.e. constant concentration of antiserum against titrated antigen gave a titre of 1/64.

From the above finding which proves the presence of Wanowrie virus precipitin antigen, it is suggested that AGDP test can be used in determining the presence of both antigen and antibody to Wanowrie virus in tissues, tissue extracts and in sera of man and animals infected with the agent. It is a rapid test and may lend itself to surveillance work.

(Addy, P. A. K., Sekyalo, E., Mukuye A., and Mujomba, E.)

Immunity status of populations of selected localities in Kenya, Tanzania, and Uganda to Chikungunya, O'nyong-nyong and Semliki Forest VIRUSES:

During the months of August through December, 1975, a total number of 828 serum samples were collected from neonates, infants, children, young adults and adults in Bwamba, Kampala, Entebbe in Uganda, Malindi, Kilifi and Mombasa in Kenya and from Tanga and Dar-es-Salaam in Tanzania.

Antigens used in the survey were, O'nyong-nyong (JAR Strain), Chikungunya (Strain E 103) and Semliki Forest (Strain Aedes 42) viruses.

The test method employed in this survey was the HI test, and HA antigens were prepared according to standard methods. Four to eight haemagglutinating units were used throughout the test. The indicator system was goose erythrocytes.

The method described by Porterfield, (1961), was applied in interpreting the results which are set out in a tabular form (see accompanying table). Only sera with HI titres of 1:20 and higher were considered positive.

Analysis of the data in relationship to donors born before and during the 1952 - 53 Chikungunya epidemic and after, showed that 35.58% of those born after the epidemic and 30.40% of the ones born before or during the epidemic were immune to Chikungunya. It was further found that those born after the epidemic had low antibody titres ranging from 1:20 to 1:160 to Chikungunya as against 1:320 - 1:2560 recorded for the epidemic populations. On the whole it can be seen from the table, that the proportion of individuals with antibodies to Chikungunya, as compared to O'nyong-nyong and Semliki forest viruses, was highest; Semliki Forest virus converters were the least in all three countries.

HAEMAGGLUTINATION INHIBITION TEST RESULTS OF SERA TO 3 GROUP A ARBOVIRUSES (CHIKUNGUNYA, O'NYONG-NYONG AND SEMLIKI FOREST VIRUSES)

Area of Specimen Collection	Number of Sera Tested	HAI Positive (≥ 1:20) serum samples to					
		Chikungunya		O'nyong-nyong		Semliki Forest	
		Number	Per cent	Number	Per cent	Number	Per cent
Uganda:							
Bwamba	331	130	39.27	69	20.85	18	5.44
Kampala	20	1	5.00	0	0.00	1	5.00
Entebbe	30	4	13.33	1	3.33	1	3.33
Sub-total	381	135	35.43	70	18.37	20	5.25
Kenya:							
Mombasa	297	79	26.60	51	17.17	8	2.69
Kilifi	20	3	15.00	3	15.00	0	0.00
Malindi	30	9	30.00	4	13.33	0	0.00
Sub-total	347	91	26.22	58	16.71	8	2.31
Tanzania:							
Tanga	30	8	26.7	5	16.67	2	6.67
Dar-es-Salaam	70	19	27.14	11	15.71	2	2.86
Sub-total	100	27	27.00	16	16.00	4	4.00
GRAND TOTAL	828	253	30.57	144	17.39	32	3.86

Furthermore, seroconverters tothe three Group A arboviruses in the survey countries showed no marked variations.

From these results, therefore, it is postulated that Chikungunya is endemic in East Africa. O'nyong-nyong is still present in an endemic form but not to the same extent as Chikungunya.

(Addy, P. A. K., and Sekyalo E.)

A comparative study of Yellow Fever and other selected Group B arbovirus antibody incidence in a known YF endemic inland country (Uganda) and some coastal areas in Kenya and Tanzania.

During August through December, 1975, 828 serum samples were collected from/*individuals resident in Uganda, Kenya and Tanzania.

These were tested by HI against YF antigen, and 5 other Group B arboviruses. The results are given in Tables 1 and and 2. Samples with titres of 1:20 and above were taken as positive. This gave 41.99% positive YF for Uganda, 17.23% for Kenya and 9% for Tanzania.

From table 2, it is apparent that the incidence of Group B arboviruses other than Yellow Fever, is highest in Kenya as compared with Tanzania and Uganda.

Age distribution of samples from Dar-es-Salaam and Uganda (Table 3) positive to YF shows that more children in Uganda possess antibodies to YF than their counterparts in Tanzania. YF can, therefore, be considered endemic in Uganda, and for that matter, Bwamba, Entebbe and Kampala.

All sera positive only to YF in HI test were tested by Complement Fixation (CF). There were 115 samples from Uganda and 5 from Kenya and Tanzania together. The 5 Coastal samples gave low titres ranging from 1:8 - 1:16. CF titres of the Uganda samples were between 1:8 and 1:512.

In order to confirm YF specificity of CF and HI positive sera, 36 Uganda samples and 5 Coastal samples were tested by the mouse protection test. None of the coastal samples was protective, whereas all the 36 Uganda samples were protective against 100 MLD_{50} YF challenge dose.

It is concluded that YF is endemic in Uganda; the opposite is true for the Kenya and Tanzania coastal strips.

(Addy, P. A. K., Mukuye, A., Lule, M., Mujomba, E., and Sekyalo, E.)

* YF non-immune

Table 1: Yellow Fever Haemagglutination-Inhibition Test Results

Areas of Sample Collection	Number of Sera Examined	Negative Serum Samples				Positive Serum Samples	
		<1:10		1:10		≥1:20	
		Number	%	Number	%	Number	%
Tanzania	100	76	76.00	15	15.00	9	9.00
Kenya	347	209	60.23	70	20.17	68	19.60
TOTAL	447	258	63.76	85	19.02	77	17.23
Uganda	381	115	30.18	106	27.82	160	41.99

Table 2 Haemagglutination-Inhibition Results (5 Group B Arboviruses other than Yellow Fever)

Area of Sample Collection	Number of Samples Tested	Serum Samples Positive at 1:20 and above to									
		JBE*		WN		BAN		ZIKA		DEN-2	
		Number	%	Number	%	Number	%	Number	%	Number	%
Kenya	341	72	21.11	88	25.81	90	26.39	105	30.79	73	21.41
Tanzania	100	11	11.00	17	17.00	20	20.00	18	18.00	10	10.00
SUB-TOTAL	447	83	18.57	105	23.49	110	24.61	123	27.52	83	18.57
Uganda	381	67	17.59	62	16.27	50	13.12	86	22.57	11	2.89
GRAND TOTAL	828	150	18.12	167	20.17	160	19.32	209	25.24	94	11.35

*JBE = Japanese B Encephalitis; WN = West Nile; BAN = Banzi; ZIKA = Zika; DEN-2 = Dengue Type 2

Table 3: Age Distribution of YF HAI Positive (≥1:20)

Sera from the Coast (Dar-es-Salaam)

and from Uganda

Area of Sample Collection	Adults (16 years and over)			Children (0 – 15 years)		
	Number of Samples Examined	Number Positive (≥1:20)	Percentage Positive	Number of Samples Examined	Number Positive (≥1:20)	Percentage Positive
Dar-es-Salaam	47	1	2.13	23	1	4.35
Uganda	154	40	25.97	227	120	52.86
TOTAL	201	41	20.40	250	121	48.40

Bats and Arboviruses:

523 bats of various species were mist netted in Kenya and Uganda. Distribution of the species according to area and numbers caught are given in Table 1.

In Table 2, a summary of the arboviruses Groups, serotypes and strains employed in the survey is given. Fifteen arbovirus serotypes belonging to 4 Groups were employed.

The method of antibody assay employed in the survey was the HI test as described by Clarke and Casalas, adapted for use in microtitre plates using 4–8 haemagglutinating units of antigen.

Results obtained are depicted in Table 3. In this test all sera with HI titre of 1:10 and above were considered positive.

The proportion of bats seropositive to the Group A arboviruses was only 1.0%.

To the Simbu group (Sango) and the ungrouped arbovirus(Rift Valley Fever), the respective rates of antibody incidence were 5.33% and 10.13%. Epomophorus franquetti appears to be particularly naturally prone to RVF virus infection and may, therefore, serve as a reservior and possible disseminator of this agent. It was further found that the incidence of antibodies to the Group B arboviruses ranked highest. Among the group B arboviruses the rates of incidence ranged from 7.80% for Bukalasa Bat virus (BB) through 57.02% for Entebbe Bat Salivary Gland virus. Rousettus aegyptiaous seems to be the most important reserviour & possible dissseminator of all arboviruses studied in this survey.

(Addy, P. A. K., Tukei, P. M., Killango, A. B. C., Sekyalo, E.)

Table 1: SPECIES DISTRIBUTION OF EAST AFRICAN BATS

Locality	Absolute No of Bats	Pteropodidae						Vespertilionidae	Megalodermatidae
		Epomophorus anurus	Epomops franqueti	Epomophorus wahlbergi	Megaloglossus woermanni	Rousettus aegyptiacus	Micropteropus pusillus	Glauconycteris papillio	Lavia frons
Bugabo	39	17	18	–	–	–	3	1	–
Entebbe (EAVRI)	9	–	–	–	–	9	–	–	–
Kisubi	10	10	–	–	–	–	–	–	–
Lunyo	11	5	6	–	–	–	–	–	–
Masaka	384	–	7	–	–	377	–	–	–
Zika	45	21	16	–	4	1	1	–	1
Sebei	5	5	–	–	–	–	–	–	–
Tororo	5	5	–	–	–	–	–	–	–
Mbale	2	2	–	–	–	–	–	–	–
Soroti	6	4	–	–	–	–	2	–	–
Lira	1	1	–	–	–	–	–	–	–
Mubende	1	1	–	–	–	–	–	–	–
Fort Portal	1	1	–	–	–	–	–	–	–
Bwamba	3	3	–	–	–	–	–	–	–
Kilifi	1	1	–	–	–	–	–	–	–
GRAND TOTAL	523	76	47	1	4	387	6	1	1

Table 2: ARBOVIRUS GROUPS, SEROTYPES AND STRAINS EMPLOYED IN THE SURVEY

Arbovirus Groups	Arbovirus Serotypes	Arbovirus Strain Designation	ACAV Abbreviation
A	Chikungunya	(E 103)	CHIK
	O'nyong-nyong	(JAR)	ONN
B	Dengue Type 2	New Guinea B	DEN-2
	Zika	MR 766	Zika
	Ntaya	Ntaya (1943)	NTA
	Yellow Fever	(FNV)	YF
	Banzi	H 366	BAN
	Japanese B Encephalitis	Nakayama	JBE
	West Nile	Ug B 956	WN
	Wesselsbron	Ug 5969	WSL
	Bukalasa Bat	Ug BP 111	BB
	Entebbe Bat Salivary Gland	Ug 1-L-30	ENT
	Dakar Bat	Dak 249	DP
Simbu	Sango	Ib An 5077	SAN
Ungrouped	Rift Valley Fever	(Lunyo)	RVF

Table 3: PROPORTION OF EAST AFRICAN BATS IMMUNE TO 15 ARBOVIRUSES DISTRIBUTED ACCORDING TO SPECIES

Arbovirus Serotypes	PTEROPODIDAE						Vespertilionidae	Megalodermatidae
	Epomophorus anurus	Epomops franqueti	Epomophorus wahlbergi	Megaloglossus woermanni	Rousettus aegyptiacus	Micropteropus pusillus	Glauconycteris papillio	Levia frons
CHIK	0/76*	0/47	0/1	0/4	5/387	0/6	0/1	0/1
ONN	0/18	0/20	0/1	0/3	1/32	NT	NT	NT
DEN-2	1/58	0/27	0/1	0/1	38/355	2/6	0/1	0/1
ZIKA	13/76	10/47	0/1	1/4	228/387	2/6	0/1	1/1
NTA	18/58	9/57	0/1	0/1	200/355	3/6	0/1	1/1
YF	4/76	3/47	0/1	0/4	106/387	1/6	0/1	0/1
BAN	4/58	2/27	0/1	0/1	126/355	1/6	0/1	1/1
JBE	1/58	2/27	0/1	0/1	84/355	1/6	0/1	1/1
WN	1/18	3/20	1/1	0/3	15/32	NT	NT	NT
WSL	3/18	1/20	1/1	0/3	16/32	NT	NT	NT
RB	1/58	1/27	0/1	0/1	33/355	0/6	0/1	0/1
ENT	14/58	6/27	0/1	0/1	234/355	2/6	0/1	1/1
DB	2/58	0/27	0/1	0/1	66/355	1/6	0/1	0/1
SAN	1/18	1/20	0/1	0/3	2/32	NT	0/1	0/1
RVF	5/76	14/47	0/1	0/4	32/387	1/6	1/1	0/1

*Numerator = Number positive at 1:10 and above. Denominator = Number examined.

REPORT FROM ARBOVIRUS UNIT, NATIONAL INSTITUTE FOR VIROLOGY, JOHANNESBURG, SOUTH AFRICA.

Chikungunya.

Outbreaks of chikungunya have been observed infrequently in southern Africa during the past 2 decades since the virus was first isolated. This was in 1956 during a small outbreak in the Lowveld of the eastern Transvaal province of South Africa. In 1962 a large epidemic occurred in the eastern lowlands of Rhodesia. In 1975 and 1976 further outbreaks were diagnosed in the Transvaal lowveld. The distribution of these outbreaks and the results of antibody surveys in man and wild primates have indicated that in Southern Africa the virus is restricted to the tropical wooded savannah.

After the Rhodesian outbreak circumstantial evidence was obtained, based on feeding habits, prevalence and vector capability, that members of the Aedes (Diceromyia) furcifer/taylori group might have been important vectors in both human and wild primate infection. These suspicions would now appear to have been justified as during the epidemic in the Transvaal in 1976 chikungunya virus was isolated from mosquitoes of this group. There were 17 isolations of this virus from 538 furcifer/taylori tested in 57 pools, yielding an infection rate of 1/32. During the collections 57 furcifer males were obtained as against only 4 taylori males so it is likely that furcifer was the main species involved during this outbreak. Ae. furcifer/taylori were the only species from which virus was isolated, were also the most prevalent and were attracted to man. In previous vector capability trials it has been found that furcifer was readily infected from viraemic monkeys, with a 10% infection threshold of 2,0 logs of virus, and 2 out of 8 infected mosquitoes transmitted virus to monkeys.

The distribution of the virus in southern Africa also correlates well with the distribution of the furcifer/taylori group.

Baboons (Papio ursinus) are present in the area of the Transvaal affected by the recent outbreaks and an effort is being made to determine immune rates among them.

The 2 recent outbreaks were notable for the very severe persistent arthralgia and arthritis in patients. Virus was isolated from 8 persons and 65 cases were identified by antibody studies. While O'nyong-nyong virus also reacted strongly with convalescent sera the virus isolates were typically chikungunya in their pathogenicity for mice.

(B.M. McINTOSH).

REPORT FROM THE NATIONAL VETERINARY RESEARCH INSTITUTE

Vom, Nigeria

Fresian calves born on the Vom home farm have been bled at monthly intervals until such time as a bluetongue serological conversion occurs. The samples are being evaluated at Vom using the group-specific agar gel precipitation test and at Pirbright using the type-specific neutralization test. Results are still at a preliminary stage, but there are indications that a large number of serological conversions take place at the end of the rainy season. It also appears as if sera coinciding with the first exposure to bluetongue in the life of a calf will be of value in identifying the virus types present in the area.

No illness is associated with serological conversion.

(W. P. Taylor)

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUT PASTEUR and ORSTOM

DAKAR-SENEGAL

The results of the investigations of the arbovirus laboratory include virological studies and serological examinations. During the first half of 1976, work was continued at the field station in Kedougou, providing the laboratory with most of the material for virus isolation.

1. VIROLOGICAL STUDIES

1.1. Human blood samples

18 blood specimens collected from febrile patients, mostly children, in Bandia (Senegal) were inoculated into suckling mice without success.

2 strains of chikungunya virus were isolated from the blood of 2 technicians involved in transmission experiments with a local strain of chikungunya virus.

6 blood specimens from patients seen in Dakar, were processed for virus isolation without success. Zinga virus was isolated from the blood of a mosquito catcher.

1.2. Wild vertebrate samples

48 blood specimens from monkeys (47 Papio papio and 1 C. aethiopes) caught in Kedougou were inoculated without success.

1.3. Arthropods

33050 mosquitoes caught in Kedougou were processed in 990 pools.

Numerous viruses lethal for suckling mice were isolated, the majority of wich have now been identified :

- Chikungunya virus (28 strains) from Aedes furcifer taylori (25), Aedes luteocephalus (2), Aedes dalzieli (1).

- Middelburg virus (9 strains) from Aedes dalzieli (8) and Aedes argenteopunctatus (1).

- Wesselsbron virus (2 strains) from Aedes dalzieli and Aedes minutus.

- Bunyamwera virus (2) from Aedes dalzieli and Aedes argenteopunctatus.

- Bwamba virus (3) from Aedes dalzieli (2) and Anopheles funestus.

- Nyando virus (3) all from Aedes dalzieli.

- Tataguine virus (3) from Anopheles gambiae (2) and Anopheles nili (1).

These data indicate that chikungunya virus, which almost disappeared after the 1966 outbreak in Senegal, is again very active.

436 mosquitoes parenterally inoculated for infectivity increments were processed in 154 pools. These studies are still in progress.

2. SEROLOGICAL STUDIES

2.1. Human sera

2.1.1. From Senegal (Bandia and Dakar)

40 sera collected from febrile patients in Dakar and Bandia were examined for the presence of antibodies.

Chikungunya virus antibodies (1/40-1/80) were found in 4 sera. One serum was positive for Wesselsbron at a rather high titer (1/640).

2.1.2. From Togo

750 sera were collected in five districts of Togo during the third quarter of 1975 from school children 5 to 15 years old.

These specimens were examined for HI, CF and neutralizing (plaque reduction test) antibodies. This second survey, carried out after the rainy season, completed the studies of the sera collected in the same area during the first quarter of 1975. The results suggest that yellow fever virus has been active in Togo before the first survey but did not circulate during the 1975 rainy season.

Other findings have to be cleared up : during this survey, specific Koutango HI antibodies were found in some sera. Koutango virus has been isolated only from rodents and vectors are unknown even though it can be easily transmitted by Aedes aegypti in the laboratory.

2.2. Wild vertebrate samples

125 sera from monkeys caught in Kedougou were tested for HI, and CF antibodies.

These studies show flavivirus circulation (especially yellow fever) and confirm increasing activity of chikungunya virus in the area.

(Ch. JAN, J. RENAUDET, Y. ROBIN, Institut Pasteur
J. COZ, M. CORNET, J. L. CAMICAS, ORSTOM.)

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUTO DE HIGIENE E MEDICINA TROPICAL

LISBON, PORTUGAL

Isolation of DHORI virus from Hyalomma marginatum ticks in Portugal

In 1971 we started the study of the tick-borne viruses of Portugal as a part of the research program of our laboratory. During 1971 several thousands of ticks were collected from domestic animals, mainly bovines, from several areas of southern Portugal. During this screening program a group of 177 Hyalomma marginatum ticks (males and females) were taken directly from calves, at the saughter-house of Lisbon, after their arrival from an area called Vidigueira, a small town at 130 km southeast of Lisbon.

Pools of 25 ticks each were processed for virus isolation as usual. From one of the pools (PO Ti 461) an agent virulent for mice was isolated. This agent was compared with the virus isolates from our collection but no serological relationship was found.

The new agent was sent to Dr. J. Casals at the Yale Arbovirus Research Unit (YARU) where it was compared with all the other tick-borne viruses already known. The Vidigueira virus (PO Ti 461) was found to be indistinguishable from DHORI virus as judged by complement-fixation tests.

We are very grateful to Dr. Casals for his cooperation in identifying this virus isolate.

(Armindo R. Filipe)

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

Arbovirus surveillance in France has shown that Tahyna virus infection is still prevalent in the Rhone delta area: 32,8 % HI positive human sera out of 204 sent by Pr.R.Baylet from Montpellier; and in the Rhine valley: 36,7 % HI positive human sera out of 90 sent by Dr.Waller from Colmar. Also 5,4 % of the sera from the Rhone delta area had West Nile HI antibodies.

West Nile virus circulation has been studied in Algeria on 45 human sera collected at Biskra and Ouled-Jellal by Dr. J.P. Dedat of the Pasteur Institute of Algeria: 28,8 % had HI antibodies, half of them with also CF antibodies which is in favor of recent infections.

In Iran, virus infections by West Nile, Tahyna and CHF.Congo were investigated through four serological surveys conducted in 1975-76 in cooperation with Dr.Y.Karimi of the Pasteur Institute of Teheran: 236 sera were collected in the Mazanderan province, 87 in the Gilan province, 83 in Azerbeidjan and 194 in Kermanshah. Antibodies for West Nile were detected in 27,8 % of the tested sera in Kermansnah (more than half of them with CF antibodies), 5,1 % in Mazanderan, 2,4 in Azerbeidjan and 1,1 in Gilan. Antibodies for Tahyna virus were found only in Azerbeidjan: 4,8 % HI positive sera. CF antibodies for CHF.Congo virus were detected only in Mazanderam in 7,3 % of the tested sera.

Arbovirus infections were investigated in Madagascar during a serological and entomological survey done by Dr.F.Rodhain in April 1976 in collaboration with Dr.P.Coulanges of the Pasteur Institute of Madagascar: 96 sera from young military recruits were tested in the east coast of the island. No antibodies were found for Chikungunya, Semliki Forest, Bunyamwera and Tahyna. But 23,0 % of the sera had antibodies for Sindbis, 61 % for West Nile, 56 % for Wesselsbron, 48 % for Uganda S, 23 % for Zika, 22% for Dengue I and 20 % for Dengue II. Most of the positive reactions in group B were of the "secondary infection" type. In the

epidemiological conditions of Madagascar, where yellow fever does not exist, the 61 % positive HI reactions for this antigen must be interpreted as non specific in group B secondary infection reactions.

The serological status for arboviruses of a group of 349 primitive Pygmees of the equatorial forest of the south-west part of the Central African Republic has been investigated in cooperation with Dr.G.Jeager in 1975-76: HI antibodies were found in 5,2 % for Sindbis, 9,5 % for Chikungunya and 13,5 % for Semliki Forest; 11,7 % for Bunyamwera; 6,3 % for Uganda S and 2,3 % for West Nile. This population being <u>not</u> vaccinated against yellow fever; the HI antibodies detected in 8,9 % of the individuals tested indicate some circulation of the virus in the forest (sylvatic yellow fever); all yellow fever positive HI reactions were in adults at least 20 years old or over.

(report from Dr.P.Sureau).

REPORT FROM THE INSTITUTE OF TROPICAL MEDICINE "PRINCE LEOPOLD",
LABORATORY OF TROPICAL BACTERIOLOGY AND VIROLOGY, ANTWERP, BELGIUM

Replication of flaviviruses in mouse organ cultures.

Continuing our studies (1, 2) on the influence of the age of the donor mice on arbovirus multiplication in organ cultures, we investigated the multiplication of wild pantropic YF (YFW), neurotropic YF (YFN), 17D YF (YF17D) and WN viruses in leg muscle, myocardium and brain organ cultures of OF_1 mice of different ages. Organ cultures were infected with $10^{-3.5}$ TCD_{50}/ml of the appropriate virus. After 2 hours adsorption the infected organ fragments were rinsed five times in order to remove all traces of non-absorbed extracellular virus. At different time intervals after inoculation, samples were harvested and infectious virus titrated. Virus was detected in the culture medium of brain at two days and in the other organ cultures at four or five days post-inoculation.

Thermal inactivation rates of all viruses studied in cell free culture medium showed no more infectious virus after two days. Therefore the presence of infectious virus in the culture medium of infected organ fragments two days post inoculation was used as the criterion for multiplication. Brain from mice older than 5 days did not support growth of YFN; growth of both YFW and $YF_{17}D$ ceased at the age of one day and WN at the age of 5 days. In muscle, multiplication of YFN and WN ceased in mice older than 5 days and at the age of 5 days, respectively.

Myocardium cultures supported only the multiplication of WN and YFN until the age of one day and two days, respectively. No virus multiplication was detected in organ cultures of spleen and liver of 1-day-old mice. These results show the rapid decrease of intrinsic organ sceptibility with increasing age of donor mice for the flaviviruses tested, in contrast with the observations

made with alphaviruses, where organ susceptibility is greater than that of the inact animal (1). These results and the delay with which new virus is synthesized in these organ cultures illustrate that the organ cultures are much less susceptible to flaviviruses than to alphaviruses (2). This work was supported by grant No 20 208 F.W.G.O.

(G. Van Der Groen (x), D. A. R. Vanden Berghe (+) and S. R. Pattyn (x+).

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REPORT FROM THE

FEDERAL RESEARCH INSTITUTE FOR ANIMAL VIRUS DISEASES

TÜBINGEN, WEST-GERMANY (FRG)

Studies on the structure of Alphaviruses

It has been shown by electron microscopic analysis of Sindbis and Semliki Forest viruses that these members of the alphavirus group reveal an icosahedral structure. From our data we conclude that the nucleocapsids of Sindbis and Semliki Forest viruses belong to the non-skewed class P=3 of icosadeltahedra. Their triangulation number is T=3 and therefore both nucleocapsids are composed of 32 morphological subunits. The diameter (D) of the Sindbis virus-core is 34 nm. The length of an edge (E) of one of the 20 equilateral triangular facets of the original icosahedron is 21 nm. The center to center spacing (d) of two subunits is 11,5 nm.

The corresponding values for Semliki Forest virus are:
E=20,5 nm; D=33 nm; d=11,5 nm.

From the mechanism of budding it was expected that a possible symmetry of the envelope would be identical with that of the nucleocapsid. Surprisingly the icosadeltahedron of the envelope exhibits a symmetry with a triangulation number of T=4. It can be shown that it is conceivable to have a T=4 arrangement of subunits in the coat and a T=3 arrangement of the subunits in the core without disturbing the mechanism of budding.

(P.J. Enzmann and F. Weiland)

REPORT FROM THE DEPARTMENT OF VIROLOGY, NEUTROLOGY CLINIC

UNIVERSITY OF COLOGNE, FEDERAL REPUBLIC OF GERMANY

Isolation of further Tettnang (TET) virus strains from the tick species Ixodes ricinus in the Federal Republic of Germany.

From 1972 to 1975 another seven strains of TET virus could be isolated from adults (1350) and -- for the first time -- also from nymphs (10,305) of tick Ixodes ricinus. In 1972 and 1973, in the locality of Northrhine Westphalia where one of the first strains of this virus was isolated 1970, attempts to find the virus once more were negative. The isolation of 3 further strains in the following two years revealed the continuous activity of this natural focus.

In two other localities in Bavaria, Central European Encephalitis has been identified as a human disease and TBE virus was isolated from Ixodes ricinus ticks. There four TET virus strains were isolated incidentally.

The newly isolated TET virus strains showed a similar pathogenicity in suckling mice as the strain TET 63 and a close relationship to this prototype virus when tested by CF and immunodiffusion test.

Together with the first isolations, these results demonstrate the widespread occurrence of the TET virus in the federal republic of Germany.

(R. Ackerman, B. Rehse-Küpper and B. Abar).

REPORT FROM THE INSTITUTE OF PARASITOLOGY

CZECHOSLOVAK ACADEMY OF SCIENCES

PRAGUE, CZECHOSLOVAKIA

Clones of Singh's Aedes albopictus mosquito cell line and their sensitivity to the Ťahyňa and Čalovo viruses

In the Singh's line of mosquito cells Aedes albopictus the Ťahyňa and Čalovo viruses, occurring on the territory of Czechoslovakia, multiply quite well. We ascertained however, that also in peak multiplication (i.e. the 3rd, or the 5th day after infection) the virus is replicated by only one third of cells.

In order to estimate whether some cells are insensitive to the viruses, we decided to clone the line. For this reason we needed to be quite sure that the clones were originated from only one cell. Therefore we carefully chose the cloning method. As an optimal method we used the isolation of single cells from few cell colonies grown on pieces of coverslips in Petri dishes by mechanical removing of all cells of the colony except one with a subcutaneous needle. After the isolation of single cells the coverslips were transferred into Leighton tubes. In this manner isolated cells grew very well again into colonies and gave rise to clones. Altogether 25 clones of the original line were obtained. Though we tried to separate morphologically distinct types of cells from the original line, all clones contained all three types of cells after several passages, indicating the fact that morphological differences depend on the developmental stage of single cells.

In all clones the two viruses multiplied well, but with different intensity. We succeeded to obtain clones to both viruses more sensitive than the original line (up to 2 log TCD_{50}/0.1 ml). However, the clone which was the most sensitive to one virus, was not identical with the most sensitive clone to the other virus. The percentage of virus-producing cells of the most sensitive clone to the Ťahyňa virus was only slightly higher (37 %).

On the basis of these results we suppose that all cells of the original line promote multiplication of both viruses and that the fact that all cells are not participating in the process of infection is caused by other mechanisms.

(Zdeněk Marhoul, Marie Weiserová-Lébrová)

x Rating procedure for establishing the role of vertebrates in the circulation of arboviruses

Vertebrates are one of the main links seccuring the circulation of arboviruses in nature. For this reason great attention was paid in the past to their serological testing. Such tests brought evidence showing which vertebrate was in contact with the arbovirus under study but at the same time giving no information at all which of these vertebrates were maintaining and amplifying the circulating amount of virus in nature because the results of serological tests depicted the participation of the vertebrate in the circulation only but in no case the extent of this participation.

To stimulate more experimental studies in the future to answer these questions, it was felt that a "rating procedure" should be adopted for the evaluation of the results of these experimental works.

Our main goal should be to gain knowledge of which vertebrates are "true" /proven/ amplifiers.

The vertebrates to be designated as true amplifiers must fulfill some strict criteria. A guide to these criteria is put foreward enabling us to rate the contemporary results of experimental studies and thus to get information on our knowledge along these lines. In table 1. the guide to this rating is described.

It is hoped that the exact knowledge of those vertebrates which may be designated as"true"/proven/ amplifiers will help us to elaborate some programme of action aimed to control, at least partly, the circulation of arboviruses in nature.

A review of the present state of knowledge of the extent of the role of vertebrates in the circulation of the tick-borne encephalitis virus /western subtype/ in central and northern Europe according to this rating is presented in the attached review.

It is hoped that this proposal will initiate some discussion which in turn will help to work out more precise guides for the proposed rating of vertebrates.

(V.Bárdoš, B.Rosický).

x Virological, serological and clinical follow up of Ťahyňa virus infections of children in South Moravia, Czechoslovakia in 1973-1975

During the summer months of 1973, 1974 and 1975 blood was taken for virological and/or serological studies from febrile children in whom the attending pediatrician supposed to have recognized a virus infection. This was done in one pediatric ward and 3 pediatric outpatient clinics in southern Moravia. In 1974 and 1975, 62 blood samples were inoculated intracerebrally into 2-day old suckling mice. In 1973 virus isolation experiments were done from the stool specimens only. Ťahyňa arbovirus was isolated in 1974 from the blood of 2 children (cf. "Arthropod-borne Virus Information Exchange" no. 30: 180, 1976). Paired samples of sera from 97 children were serologically tested against Ťahyňa virus in haemagglutination inhibition and plaque-reduction tests. Seroconversion was found in 2 patients from whose blood Ťahyňa virus was isolated (Tab.2) and moreover, in 7 other children (3 times in 1974, 4 times in 1975: Tab.3). Influenza-like symptoms were noticed in 6 and meningeal symptoms in 3 of these patients. In 1973 no seroconversion was recorded. In 1974 and 1975, seroconversion was recorded in 14.3% of the tested children with influenza-like symptoms, and in 21.4% of the children with symptoms of a CNS infection.

In 6 children with seroconversion against Tahyňa virus, neutralizing antibodies were detected neither against Coxsackie virus type B 3 nor against four other cytopathic agents which were isolated from the stool specimens of several sick children in 1973. Virus neutralizing antibodies were detected in 2 other children with Ťahyňa infection against Coxsackie virus type B 3 but without any titer increase. No serum was available to test the sera of the 9th child.

/ Z.Hubálek, V.Bárdoš, I.Schwanzerová[1], Z.Juřicová, M.Medek[2], V.Kania[2]/

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x Experimental infection of foxes with Ťahyňa virus

Three foxes /Vulpes vulpes - age approx. 18 weeks/ were subcutaneously inoculated with 193.5 i.c. suckling mouse LD_{50} of Ťahyňa virus. /The extraneurally passaged strain " 236" was used. Blood for isolation experiments was taken on the 3rd, 4th, and 5th day post infection from the vena saphena. Viraemia was tested by intraperitoneal inoculation of heparinized blood in 2 day old suckling mice. The highest infection titers of virus 1,3 ldex resp. 0.83 dex LD_{50}/0,02 ml/ i.pt. were observed 72 resp. 96 hours post infection. Ťahyňa virus neutralizing antibodies of titers 1 : 4096 - 1 : 8192 in the serum of all foxes were detected three weeks post infection in a plaque reduction test. Foxes may be designated as probable hosts and possible amplifiers of Ťahyňa virus in nature. It should be mentioned, however, that foxes are not a numerous species.

(P.Rŏdl, V.Bárdoš, Z.Hubálek)

x Enlarged summaries of articles presented at the congress of Anthropozoones, June 23-25,1976 in Kosice Czechoslovakia.

Tab.1. GUIDE TO THE RATING PROCEDURE OF VERTEBRATES AS HOSTS

POTENTIAL[@]	POSSIBLE[@]	PROBABLE	TRUE (PROVEN)
Seen with feeding ticks in nature	Caught in nature and being serologically positive	Developing viraemia and seroconversion or seroconversion only after experimental infection in the laboratory	From whose blood and/or organs virus was isolated after a natural infection in nature

GUIDE TO THE RATING PROCEDURE OF VERTEBRATES AS AMPLIFIERS

POSSIBLE	PROBABLE	VERY PROBABLE	TRUE (PROVEN)
Only traces of virus were recovered from blood after a natural or experimental infection	The level of viraemia after a natural or experimental infection is considered to be sufficiently high to infect the biological vector	The level of viraemia after an experimental infection was proven to be sufficiently high to infect the feeding biological vector	The biological vector was infected after feeding on a naturally infected vertebrate in nature

@ According to the Webster's New Collagiate Dictionary (1974) "potential" means something that can develop and "possible" something within the limits of realization.

Review of rating of vertebrates as amplifiers of the tick-borne encephalitis virus /western subtype/.

According to this proposed rating based on the results of experimental works which have been published, these species of vertebrates may be designated as probable amplifiers of the tick-borne encephalitis virus /western subtype/.
The following free living mammals are rated as probable amplifiers: Erinaceus europaeus, Sorex araneus, Talpa europaea, Mustela nivalis, Putorius putorius, Vulpes vulpes, Sciurus vulgaris, Clethrionomys glareolus, Pitymys subteraneus, Apodemus flavicollis, Apodemus sylvaticus, Mus musculus, Lepus europaeus and Lepus timidus. From the domestic mammals: Canis familiaris. From the birds: Turdus merula, Fulica atra and Anas platyrhynchos.

Apodemus flavicollis is so far the only vertebrate which can be designated as a very probable amplifiers.

Table 2. Seroconversion in two children from whose blood Tahyňa virus was isolated

			Virus neutralization						
			Cell cultures						
		HIT	PRT			Tube test	Suckling mice		
Virus (antigen):		Ť 92 P6b	T 16	P6b	Ť 92	Ť 92	Ť 92	T 16	P6b
Test dose:		8 units	15 PFU	15 PFU	15 PFU	100 TCD_{50}	40 LD_{50}	500 LD_{50}	100 LD_{50}
Patient (Virus)	Blood taken on	Reciprocal titers							
H.L. (T 16)	27.8.1974	<10	4	-	-	<5	<4	<4	-
	10.9.1974	20	>16	-	-	25	-	-	-
	22.10.1974	<10	512	512	256	125	48	24	-
K.R. (P6b)	10.9.1974	<10	<4	4	<4	<5	<4	-	<4
	16.9.1974	<10	32	32	16	<5	-	-	-
	5.3.1975	10	512	512	256	64	64	-	214

"-" not tested
HIT = haemagglutination-inhibition test PRT = plaque-reduction test

Table 3. Serologically demonstrated Tahyňa virus infections

Patient Sex, age Village	The disease onset (clinical symptoms)	Blood taken on	Serum reciprocal titers PRT	HIT
Š.M. boy, 6 y. Vlasatice	2.9.1974 (mening.)	3.9.1974	<2	10
		17.9.1974	4096	20
H.V. girl, 8 y. Dol. Dunajovice	2.9.1974 (infl.-like)	3.9.1974	<2	10
		17.9.1974	4096	20
V.L. girl, 8 y. Jevišovka	5.9.1974 (infl.-like)	6.9.1974	<2	10
		20.9.1974	4096	20
B.P. boy, 5 y. Mikulov	25.7.1975 (mening.)	28.7.1975	<2	<10
		4.8.1975	NT	10
		28.8.1975	768	10
Š.B. boy, 6 y. Vlasatice	13.8.1975 (infl.-like)	14.8.1975	<2	<10
		5.9.1975	512	10
S.Z. boy, 15 y. Mušov	3.9.1975 (infl.-like)	4.9.1975	<2	<10
		10.9.1975	1024	80
		9.10.1975	2048	10
P.P. boy, 8 y. Drnholec	6.9.1975 (infl.-like)	8.9.1975	<2	<10
		12.9.1975	256	10
		10.10.1975	384	<10

NT = not tested

"infl.-like" = influenza-like symptoms

"mening." = meningeal symptoms

REPORT FROM THE VIROLOGICAL DEPARTMENT

RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY

BRATISLAVA, CZECHOSLOVAKIA

Differences were noted (Wallnerova, 1973) in the courses of infection in 4-week-old mice inoculated with Tahyna virus strain "669" isolated from a sick man in 1972 (Simková, Sluka, 1973) and reference strain "92" isolated in 1958 from mosquitoes (Bárdos, Danielová, 1959). This observation stimulated further investigation of strain "669", especially from the aspect of the importance of the isolation source and the strain passage history.

The study was broadened by employing 3 additional strains of Tahyna virus isolated in 1972 in the same locality of Southern Moravia as strain "669". These were strain "802", another strain of human origin isolated at the Research Institute of Epidemiology and Microbiology, Bratislava (Simková, Sluka, 1976) and strains "6064" and "6075", isolated from mosquitoes which were kindly supplied by Dr. Malková from the Institute of Parasitology, CSAS, Prague. Each of these 4 strains, after an initial 2-3 passages in mice, was propagated simultaneously in two lines: subcutaneously (s.c.) and intracerebrally (i.c.) in suckling and young SPF mice up to the final number of 10 successive passages.

When tested at different passage levels by foot-pad inoculation of 4-week-old mice, these two lines of the same virus strains showed marked differences in some biological properties. The subcutaneously propagated lines were capable of quickly spreading by lymphatic and blood vessels throughout the body of experimental animals, and viremia lasting 3-4 days was accompanied by the replication of the virus in the lymphatic system and in some internal organs. However, peripheral inoculation of the intracerebrally passaged lines of the same virus strains resulted in no spreading of the virus and no detectable

viremia over a 10-day period. Infection was localized only in the regional lymph nodes where limited replication of the virus was detected. This change of the originally pantropic character of Tahyna virus strain occurred at the level of 7^{th} - 9^{th} i.c. passages in mice, and the results obtained with Tahyna virus strains isolated from man and mosquitoes were in this respect comparable.

(Z. Wallnerová)

The aim of experiments were to answer the question as to whether the TAH "802" strain of Tahyna virus, isolated from blood of a man (Simková, Sluka, 1976), reproduces in lung and muscle explants from mice which had been infected in vivo with this virus. One-day-old specific pathogen free mice were subcutaneously inoculated with 1-10 mouse intracerebral LD_{50} of TAH "802" and were killed on the fifth day after inoculation. Blood from five animals was pooled and treated with heparin. From aseptically removed portions of lungs and muscles, 10% suspensions in PBS were prepared. Other parts of lungs and skeletal muscles were cut into fragments. After repeated washing in L-15 medium, the fragments were placed onto scratched areas of 4 cm Petri dishes. One ml of L-15 medium supplemented with 3% calf serum was added per dish. The cultures were incubated at 36-37°C in humified atmosphere. Blood, organ suspensions and medium from explants were assayed for virus in tube cultures of GMK cells.

At the time of bleeding the titres of infectious virus in blood were found to be slightly lower (3.5 to 4.3 $\log_{10}$ GMK CPD_{50}/0.1 ml) than those found in lungs and muscles (4.4 to 5.0 $\log_{10}$ GMK CPD_{50}/0.1 ml). Medium from lung and skeletal muscle explants was assayed for virus on days 1-7 after explantation. In the case of lung tissue the virus titre in the medium grew

gradually and reached maximal values (2.3 - 3.7 $\log_{10}$ GMK CPD_{50}/0.1 ml) between the 4^{th} and 6^{th} day. In medium from muscle explants, the virus titre reached a peak on the 3^{rd} - 4^{th} day after explantation (1.5 - 3.0 $\log_{10}$ GMK_{50}/0.1 ml) and then declined rapidly.

Even though the explantation of tissue fragments was performed at a time of high viraemia, the experiments indicate that the virus in lung and muscle tissue did not merely passively survive in vitro, but reproduced in them. However, it will be necessary to answer the question as to whether the reproduction of virus in the case of lung tissue really took place in vivo or whether the virus was present only in the extracellular fluid, and in tissue culture conditions was adsorbed onto lung cells and reproduced in these. The reproduction of Tahyna virus in striated muscles of suckling mice in vivo was previously demonstrated by the fluorescent antibody technique (Wallnerová, 1969). It will be useful to perform these experiments on mice of different ages and to attempt to isolate the virus in explanted tissue at different intervals after inoculation, not only in the viremic period but also in the postviremic period.

(I. Schwanzerová)

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

1. Investigation of haemagglutination-inhibiting (HI) antibodies to some arboviruses in sera of cattle from the locality of Javorina.

In 1975, sera of 68 cattle from the village of Javorina (Northern Slovakia) were examined for the presence of antibodies to some arboviruses. All serum samples were kept frozen until examined.

Sera were examined by the HI test. Sera were extracted by acetone and adsorbed onto concentrated erythrocytes of goose. Antigens were prepared by sucrose-acetone extraction, and in the HI test 4-8 haemagglutinating units of antigen were used. The following virus antigens were employed: antigen of Sindbis virus (Alfavirus), antigens of tick-borne encephalitis and West Nile viruses (Flaviviruses), and antigen of Quaranfil virus (Quaranfil group). The HI test was performed by the micromethod on plastic dishes.

All examined sera were negative by HI test with all antigens used.

(M. Batiková and M. Gresiková)

2. HI antibodies to some arboviruses in sera of pigeons trapped in Bratislava.

We obtained altogether sera of 83 pigeons, trapped in Bratislava. The sera were inactivated, acetone treated and then examined for the presence of antibodies to Sindbis virus (Alfavirus), tick-borne encephalitis and West Nile virus (Flaviviruses). Results of HI tests showed that 17 of the pigeon sera reacted with the antigen of Sindbis virus, 34 with the antigen of TBE virus and 34 with the antigen of West Nile virus at a titer of 1:10 or

greater (Table 1). Virus neutralization antibodies to Sindbis virus on duck embryo cell cultures were detected in 7% of the sera with a titre of 1:4.

Native serum samples from another 50 pigeons were extracted by acetone and tested for HI antibodies to Sindbis, West Nile and TBE antigens. Antibodies to Sindbis virus were found in 8% of examined sera; to TBE virus, in 20%; and to West Nile virus, in 26%.

For comparison 150 serum samples from pigeon were treated by acetone and then by kaolin. Of all 150 sera examined, 2% reacted with Sindbis virus and 4% reacted with West Nile virus. No TBE virus antibodies were found in acetone- and kaolin-treated sera.

(M. Sekeyová, M. Gresiková, O. Kozuch, presented at 2. Internationales Arbeitskolloquium über "Naturherde von Infektionskrankheiten in Zentraleuropa." Graz, 25.2.-28.2.1976).

Table 1. Haemagglutination-inhibiting antibodies to some arboviruses in 83 pigeon sera collected in Bratislava.

HI antibody titre	Antigens					
	Sindbis		Tick-borne encephalitis		West Nile	
	No. of reacting sera	% of total	No. of reacting sera	% of total	No. of reacting sera	% of total
<10	66	79.5	49	59	49	59
10	5	6.0	23	27.7	15	18
20	0	0	8	9.6	7	8.4
40	6	7.2	2	2.3	8	9.6
80	6	7.2	0	0	4	4.8
160	0	0	1	1.2	0	0

3. Non-infectious TBE antigen.

To avoid laboratory infections TBE non-infectious antigen was prepared.

Four TBE virus strains (a strain isolated from Ixodes ricinus ticks, another strain isolated from the organs of Clethrionomys glareolus, a strain isolated from the blood of Tringa ochropus and the prototype strain, Hypr) were tested for hemagglutination (HA) activity after sucrose-acetone extraction. All strains had HA activity at pH optima typical of group B arboviruses (pH 6.4 - 6.8). After treatment with 0.1% formalin (overnight at +4°C) all antigens differed in patterns of HA activity: a plateau from pH 6.0 to 6.4 was observed and HA titres decreased with increasing pH. After the formalin treatment HA activity was even higher for antigens of the Clethrionomys glareolus, Tringa ochropus and prototype strains.

HI tests were carried out with both types of antigens (sucrose-acetone untreated and sucrose-acetone formalin treated). No differences were found in the number of positive-reacting sera and/or in the HI titres. Therefore, formalin-treated TBE antigens may be recomanded for routine work to avoid laboratory infections.

(M. Gresiková and M. Sekeyová, To be published in Acta virologica).

4. The effect of 2-mercaptoethanol on HI activity of sera of human patients who had been hospitalized with clinical diagnosis of tick-borne encephalitis.

The aim of the present study was to determine the suitability of 2-mercaptoethanol treatment of human patients' sera to determine recent tick-borne encephalitis infection. In a limited serological study, the HI antibody titres in the first samples of human patients sera were treated by 2-mercaptoethanol.

Sensitivity to 2-mercaptoethanol treatment may be used for differentiation of acute cases of tick-borne encephalitis virus. A healthy human population, living in natural foci of tick-borne encephalitis, may have antibodies to this virus acquired in the past, and therefore difficulties may be encountered in determining the etiology of a recent infection. The use of 2-mercaptoethanol treatment of sera is recommended for routine laboratory work. The results obtained are presented in Table 2.

Table 2

The effect of 2-mercaptoethanol on HI titres of human patients' sera, hospitalized with clinical diagnosis of TBE.

The name of patients	No. of the sample	HI titre in acetone extracted sera	HI titre in sera treated with 2-mercaptoethanol
Šiška	1	20	10
Šiška	2	160	40
Targoš	1	1280	40
Targoš	2	1280	80
Janíčková	1	1280	80
Janíčková	2	1280	320
Kovár	1	160	40
Kovár	2	320	160
Benegrafová	1	160	0
Oreščáková	1	640	160
Havran	1	1280	320
Marek M.	1	2560	160
Marek M.	2	640	320
Marek J.	1	5128	320
Marek J.	2	2560	1280
Božek P.	1	640	80
Božek P.	2	640	160
Križma L.	1	320	80
Križma L.	2	160	160
Božik J.	1	1280	320
Božik J.	2	640	320

/M. Grešíková and M. Sekeyová /

REPORT FROM THE NATIONAL INSTITUTE OF HYGIENE

BUDAPEST, HUNGARY

In collaboration with the Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia, mosquitoes were collected in the 2nd half of June 1975 in the South-Western part of Hungary. The mosquitoes were captured by entomological nets and exhaustors in the coastal biotopes of Balaton Lake, in the surroundings of the town Kaposvár, and in the stables of domestic animals (e.g., horse stables, cow-shed, pigsty, duck farms, etc.) of the mentioned area. Altogether 5156 mosquitoes were collected, belonging to 15 species and 5 genera. Catches were effected at the end of spring and at the beginning of the summer season. The most numerous species in collections were Aedes vexans and members of the Anopheles maculipennis group.

Only one arbovirus strain was isolated, from a pool of females of 34 Ae. caspius collected in a pigsty; it was identified as Tahyna virus.

(E. Molnár, D. Málková, V. Minár)

REPORT FROM THE DEPARTMENT OF ECOLOGY OF VIRUSES,

THE D.I. IVANOVSKY INSTITUTE OF VIROLOGY

AMS USSR, MOSCOW

In 1975, 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, in the European part. More than 44,000 ticks were examined, and 65 strains of arboviruses were isolated from them (Table). The identification of viruses isolated in the previous years was continued.

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 DOChNa), and the size of virion (according to filtration through Millipore filters of 220, 100 and 50 nm pore size).

Tamdy virus was isolated in 1972-1974 in the Uzbec SSR and Turkmen SSR from ticks, Hyalomma asiaticum (10 strains), and H. plumbeum (1 strain), collected from sheep and camels, as well as in sheep farms. The virus is not antigenically related to CFT or to more than 60 other arboviruses, the antisera of which we used for identification. It contains RNA and lipids, and the size of a virus is about 90 nm (according to ultrafiltration and electronic microscope data). Electron microscope study of ultrathin sections of newborn mouse brains allow us to refer the virus to the Bunyamviridae family.

Khasan virus was isolated from ticks, Haemaphysalis neumanni (=longicornis), collected in 1971 in the Khasan region of the Primorye territory (1 strain). It is not antigenically related to the other arboviruses. The virus contains RNA and lipids in membrane, and the size is about 90-110 nm. The findings of morphology and morphogenesis in electron microscope study allow us to refer this virus also to the Bunyaviridae family.

Paramushir virus was isolated from the ticks Ixodes putus and I. signatus (4 strains) collected in nesting grounds of seabirds on the Paramushir Islands (the North of Kuril Isls), Tyulenyi Island (Okhotsk Sea), Bering Island (Commodore Islands). 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.

Bhanja virus was isolated in the Azerbaijan SSR from ticks, Rhipicephalus bursa, collected on cattle, and in the Kirghiz SSR from ticks, Hyalomma plumbeum.

Wad Medani virus (Kemerovo group) was isolated in the Tadjik SSR from ticks, H. anatolicum; two strains of Issyk-Kul virus from bats, Vespertilio pitistrellus; and 1 strain of Tahyna virus from mosquitoes, Anopheles hyrcanus.

Reference antisera (IAF) to the arboviruses isolated from ticks were prepared in the laboratory.

(D. K. Lvov)

Table. EXAMINATION OF TICKS IN DIFFERENT REGIONS OF THE USSR IN 1974–1975 (numerator-number of examined ticks; denominator - number of isolated strains)

Families, species of ticks \ Regions		European part				Caucasus			Middle Asia		Far East			Total
		Murmansk region	Ukraine	Leningrad region	Rostov region	Georgia	Armenia	Azerbaijan	Uzbek	Turkmenistan	Khabarovsk region	Sakhalinsk region	Kamchatsk region	According to species
Ixodes	Putus	1252/15										3695/23	4244/13	9191/51
	signatus												2962/2	2962/2
	ricinus							978/0						978/0
	lividus			1080/0										1080/0
	persulcatus												181/0	181/0
Hyalomma	asiaticum						56/0		4033/2	109/0				4198/2
	anatolicum								1557/0	1945/1				3502/1
	plumbeum								85/0	1230/1				1315/1
	detritum							877/0	122/0	70/0				1069/0
	dromedarii									739/0				739/0
Haemaphisalis	punctata						36/0							36/0
	numidiana								50/0					50/0
	concinna										315/0			315/0
Dermacentor	marginatus				95/0		2197/1	25/0						2317/1
	pictus						15/0							15/0
	pavlovskyi								96/0					96/0
	daghestanicus								1956/0					1956/0
	silvarum										36/0			36/0
Rhipicephalus	rossicus		26/0											26/0
	turanicus								4226/0	428/0				4654/0
	pumilio								248/0					248/0
Argas	persicus							2400/0	2163/0					4563/0
	vulgaris								260/0					260/0
Ornithodoros	verrucosus	27/0			308/0									335/0
	tartakovskyi								1155/2	112/0				1267/2
	coniceps								562/2	2857/2				3419/4
	Total	1252	55	1080	95	308	2304	4280	16314	7089	351	3695	7387	44708

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