ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 37 September 1979

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

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Roy W. Chamberlain, Sc.D., Editor
Bette A. Hall, Secretary

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL

TO : Recipients of the Arthropod-borne Virus Information Exchange

DATE: November 13, 1979

FROM : Editor

SUBJECT: Late mailing of issue No. 37

I am very sorry about the late mailing of this issue of the Arthropod-borne Virus Information Exchange. We planned to have it reach you before the end of October, well in advance of the annual meeting of the American Society of Tropical Medicine and Hygiene, which is being held this year in Tucson, Arizona. However, through circumstances beyond our control, the printing was considerably delayed.

Efforts are being made to assure that future printings are not so unduly delayed, as it is realized that the true value of an Information Exchange lies in the currency of the information contained. The delay of issue No. 37 was particularly unfortunate because, for the first time abstracts of arbovirus papers being presented at the Tucson meeting had been included. It was believed that such a preview would have been helpful to many of you.

I hope we can look forward to more prompt service next March.

Roy W. Chamberlain

Roy W. Chamberlain, Sc.D.

★ G.P.O. 1977—744-858 / 2514. REGION NO. 4

ERRATA

Dr. J.J. Salaun has pointed out an error in the report of the Arbovirus Laboratory, Institut Pasteur and Orstom, Dakar, Senegal which appeared on page 115 of Information Exchange No. 36 (March, 1979). Under 1. Virological studies, 1.2 wild vertebrate samples, the seventh line should read, "Andral in Ethiopia in February, 1962" instead of "Andral in Ethiopia in 1968".

Please make this correction in your copy of No. 36.

COMMENTS FROM THE EDITOR

Something new has been added in this issue of the Information Exchange, namely, abstracts of arbovirus papers that will be presented at the 28th Annual Meeting of the American Society of Tropical Medicine and Hygiene, 14016 November, Tucson, Arizona. We hope they will be helpful and informative.

Also included in this issue is the program of a timely symposium, at the Tucson meeting, "Molecular Approaches to Arbovirus Research", being sponsored by ACAV's Subcommittee on Applied Molecular Arbovirology (SAMA).

The deadline for reports for the next issue (No. 38) of the Arthropod-borne Virus Information Exchange is March 1, 1980. The address, as usual:

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

It is not unusual to see reports where a particular virus is shown to multiply in laboratory-infected arthropods following inoculation of the agent and then the assumption is sometimes made that this provides proof of the probable arbovirus nature of the agent. The danger in this interpretation is demonstrated in the work of Hurlbut and Thomas (Virology 12: 391, 1960, J. Med. Ent. 6: 423, 1969) who showed that arboviruses could be propagated readily in a variety of arthropods, many of which have no blood-feeding habits, such as grasshoppers, plant bugs, beetles and moths. Moreover, virus survival was prolonged in a number of instances; for example, larvae of the Black Carpet Beetle hosted JE virus for 10 months, Ntaya 8 months, SLE 11 months and West Nile 3 months.

In other words, the mere demonstration of virus propogation in a mosquito, tick or other vector is not sufficient. It would be more to the point if the energy expended as above could be diverted into other more rewarding channels such as per os infection and transmission. Preferably such experiments would involve viremic donor hosts (this means viremia studies), but where difficulties are encountered, in vitro techniques may be substituted and can produce meaningful results.

For the tick virus "boys" I would like to suggest a method of demonstrating in vitro transmission of virus. For a number of years scientists have been interested in tick saliva in connection with

studies of tick paralysis as well as the phenomenon of tick resistance in cattle. Various methods have been devised for collecting saliva. One of the more interesting involves the use of pilocarpine for stimulating salivation. The biochemists are interested in studying the electrophoretic patterns of proteins, etc., but to me saliva means only one thing, a vehicle for pathogen transfer, and in our world, these pathogens are arboviruses. Thus, when I cameacross a "lost" paper from "down under", I was immediately stimulated to salivate. The paper, "Automatic collection of tick saliva" by K. C. Binnington and M. Schotz (J. Austr. Ent. Soc., 12: 78, 1973) describes the use of a "Fractomat" for automatically collecting saliva from 1,300 pilocarpine-stimulated *Boophilus microplus*; daily quantities of tick saliva varied from 9.5 to 13.0 ml! The use of a "Fractomat" is not pertinent to our work, but the knowledge that one can infect ticks with say a "new" virus and then after an appropriate incubation interval, stimulate them to salivate means that we have the ability to demonstrate *in vitro* transmission of potential tick borne viruses.

Thomas H. G. Aitken
Chairman, SEAS

SAMA-SPONSORED SYMPOSIUM

"MOLECULAR APPROACHES TO ARBOVIRUS RESEARCH"

Introduction - The Future of Molecular Virology in Arbovirus Research.
Dr. Karl Johnson, CDC, Atlanta, GA

Arboviruses RNA - Description of Segmented and Unsegmented Genomes.
Dr. Dennis Knudson, YARU, New Haven, CT

Arbovirus Evolution - Drift and Recombination.
Dr. David Bishop, University of Alabama, Birmingham, AL

Molecular Correlates of Antigenic Variation
Dr. Dennis Trent, CDC, Ft. Collins, CO

Arbovirus Replication in Vertebrate and Invertebrate Cells
Dr. Victor Stollar, Rutgers Medical School, Piscataway, N.J.

Lymphocyte Hybridomas- Production of Antibody to Arbovirus Antigens.
Dr. Joel M. Dalrymple, WRAIR, Washington, D.C.

Summation - The Application of Molecular Virology to Problems in Tropical Medicine
Dr. Philip K. Russell

A symposium sponsored by the Subcommittee on Applied Molecular Arbovirology (SAMA) to be presented at the Annual Meeting of the ASTHM&H, 14-17 November 1979, Tucson, Arizona.

ABSTRACTS OF PAPERS TO BE PRESENTED AT THE ANNUAL MEETING OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE, November 14-16, 1978, Tuscon, Arizona

Abstracts submitted and approved for presentation at the meeting in Tuscon are reproduced here. We hope that this "preview" of the meeting will generate questions and discussion of the papers and that those of you unable to attend the meeting will have an opportunity to learn something of its content.

Abstracts are reproduced with the permission of the authors and of the American Society of Tropical Medicine and Hygiene. Several abstracts are omitted because we did not receive permission to include them prior to the deadline of the Information Exchange.

The presentation of the abstracts here do not constitute publication (see footnote, bottom of front cover of the Arthropod-borne Virus Information Exhchange).

A Method for the Isolation and Identification of Dengue Viruses, Using Mosquito Cell Cultures

Robert B. Tesh

Abstract

An improved method for the isolation and identification of dengue viruses will be described. Viruses are isolated in mosquito cell cultures (C6/36 or AP-61), identified by indirect fluorescent antibody technique, and typed by complement fixation test, using the cell culture fluid as antigen. The sensitivity of this method was compared with mosquito inoculation in comparative titrations of 16 low passage dengue virus strains. Although lower virus titers were obtained by the mosquito cell culture technique, its decreased sensitivity was compensated by the much larger volume (588X) which could be assayed. By incubating the mosquito cells at 32°C, dengue viruses can be identified and typed within 6 days after inoculation.

Characterization of Dengue 1, 2, 3 and 4 viral RNA.

A.C. Vezza, L. Rosen, P. Repik, J. Dalrymple and D.H.L. Bishop.

The University of Alabama in Birmingham,
Birmingham, Alabama 35294,
Walter Reed Army Institute Research in Washington,
Pacific Research Unit, Honolulu.

The 40 S viral RNA species of prototype DEN 1, 2, 3 and 4 viruses have been analysed and compared by ribonuclease T1 resistant oligonucleotide fingerprint analyses using virus grown in *Aedes albopictus* cells (Igarashi clone). The results show that the four viruses are easily distinguished. Analyses of the 5' terminus of DEN 3 RNA indicate that in contrast to the alphavirus Sindbis (5' sequence: m7GpppApUpGp), the flavivirus DEN 3 has a 5' sequence of m7GpppAmpXp. DEN 3 40 S RNA, unlike alphaviruses, does not have any 3' poly A tract or any unusual internal polyprimidine tracts.

LONG TERM PERSISTENCE OF BUNYAVIRUSES IN ARCTIC MOSQUITOES

Donald M. McLean
Division of Medical Microbiology, University of British Columbia,
Vancouver, B.C., V6T 1W5.

Persistence of arctic bunyaviruses for prolonged periods was investigated in arctic mosquitoes incubated at 4°C. Culiseta inornata and Aedes communis adult female mosquitoes were collected near Whitehorse, Yukon Territory (61°N 134°W) during spring 1978 and injected intrathoracically with 300 or 30 PFU of the 75-L-10 larval isolate of snowshoe hare (SSH) virus (California encephalitis group) or the 76-Y-330 adult mosquito isolate of Northway (NOR) virus (Bunyamwera group). Virus titers in mosquito salivary glands were estimated by plaque titrations on monolayers of BHK cells.

After injection of Cs. inornata with 30 PFU snowshoe hare virus and incubation at 4°C, mosquito salivary glands yielded 2.5 log PFU at 211 days, 3.4 log PFU at 285 days and 2.5 log PFU at 329 days, and in each instance SSH virus antigen was revealed by indirect immunofluorescence. After injection with 300 PFU, virus titers were 2.7 log PFU at 196 days and 3.0 log PFU at 218 days. Previously reported results indicated viral persistence to 194 days. Virus infectivity was also detected in salivary glands of A. communis after incubation for 188 days (2.5 log PFU) and 300 days (2.4 log PFU).

Northway virus was detected in salivary glands of Cs. inornata after injection of 300 PFU and incubation for 198 days (2.8 log PFU), 271 days (3.6 log PFU), 293 days (3.2 log PFU), 307 days (4.3 log PFU) and 329 days (3.7 log PFU), and viral antigen was observed by indirect immunofluorescence at 4 of 5 sample intervals. After injection of 30 PFU, virus infectivity was detected at 166 days (3.0 log PFU) and 214 days (3.0 log PFU).

The above results suggest that, in addition to transovarial transfer, overwintering of virus is possible through its long term persistence in adult mosquitoes maintained at near-freezing temperatures.

Enzyme immunoassay (ELISA) tests have been adapted for detection of SSH and NOR antigens in tissue culture supernatants after removal of extraneous material by ultracentrifugation in sucrose density gradients. Employing antisera prepared in rabbits by single intravenous injections of SSH or NOR viruses, anti-SSH serum reacted with SSH antigen at 1:1600 and with NOR antigen at 1:100, but anti-NOR serum reacted with NOR antigen exclusively, but serological cross reactions were not detected in mouse neutralization or plaque reduction tests. Work is in progress to adapt the ELISA technique to detection of CE and NOR antigens in mosquito salivary glands.

RELATIVE IMPORTANCE OF BACTERIAL AND ARBOVIRAL INFECTIONS AS CAUSES OF FEVER IN BALI, REPUBLIC OF INDONESIA

J. Olson[1], T. Ksiazek[1], R. Rockhill[1], A. Cobet[1], J. Mulya[2], M. Soemendra[2], A. Nurul[2], N. Sugitha[2], G. Cok[2] and S. Leimena[2]

[1] NAMRU-2 Jakarta Detachment, APO San Francisco, 96356

[2] Ministry of Health of Indonesia

A group of 104 patients having fever for 5 days or less was studied from two rural health clinics and a city hospital on the island of Bali, Republic of Indonesia between December and March, 1979. An oxgall medium blood culture was inoculated from each clinic patient and liquoid medium blood and rectal swab cultures from the hospitalized patients. Acute and convalescent phase sera were collected from 94 of these patients and tested for antibodies to group A and B arboviruses using the hemagglutination inhibition test. Diagnostic rises in antibody titer were observed in 32 (34%) of those tested; 31 evidenced infection with group B arboviruses and a single patient showed evidence that a group A arbovirus was responsible for his illness. Bacterial species were isolated from the blood of 8% of clinic patients and 13% of hospitalized patients. Salmonella typhi was the most frequently recovered pathogen followed by S. paratyphi A. Other bacteria isolated included S. enteritidis serotypes C and E and S. choleraesuis. Bacterial pathogens were isolated from 31% of the fecal specimens cultures. Salmonella paratyphi B and S. enteritidis were isolated from 18% and S. typhi from only 4% of rectal cultures. No etiologic agent was determined for 47 (50%) of the 94 patients studied by culture and serology. These findings document that group B arboviruses cause a significant proportion of fevers in both rural outpatient and urban hospitalized patient populations of Bali.

Fatal Case of La Crosse Viral Encephalitis -- 1978

ABSTRACT

A case presentation of a 3-year-old child admitted to the La Crosse Lutheran Hospital in July of 1978. Film will show child having a focal seizure 14 hours after admission to the hospital. The seizures characteristically are focal and generally are refractory to the usual anti-convulsant medications. Such was the situation in this case. The child responded poorly and on the second hospital day had a spontaneous bleed in the right frontal lobe from which she did not recover. A countercurrent immunoelectrophoresis test done on the second and fourth day of the disease show a change from negative to positive. This helped to confirm the diagnosis. Subsequently rises in antibody in the hemagglutination inhibition (HI) test, and a virus was isolated from brain tissue by Dr. Wayne Thompson, which on oligonucleotide fingerprint analysis by Dr. David Bishop of the University of Alabama was interpreted to be similar to the prototype virus isolated from the index case of La Crosse viral encephalitis.

A CAT study shows the location of the bleeding. Gross pathology of brain and photomicrograph of brain tissue will be shown demonstrating the lesions.

Dr. Bernard Kalfayan will discuss the neuropathology involved in this case.

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EXPERIMENTAL TRANSMISSION OF ROCIO VIRUS BY MOSQUITOES

by Carl J. Mitchell and Thomas P. Monath
Vector-Borne Diseases Division
Center for Disease Control
Fort Collins, Colorado

ABSTRACT

Rocio encephalitis is an epidemic flaviviral infection of man first described in Sao Paulo State, Brazil in 1975. The virus has been isolated from arthropods collected in nature only once, and the ecology of the viral transmission cycle remains largely unknown. Rocio virus produces high level viremias in suckling mice ($10^{5.2}$ to $10^{5.9}$ Vero PFU/ml at 24 to 48 hrs postinoculation) and young chicks (10^6 to 10^8 Vero PFU/ml at 48 to 72 hrs postinoculation). Chicks develop comparable levels of viremia after being bitten by individual mosquitoes, and virus persists in the blood for up to five days.

We evaluated the susceptibility of certain Culex mosquitoes to infection per os, and determined transmission rates to chicks after different periods of extrinsic incubation. The following species of mosquitoes have been tested thus far: Cx. tarsalis from Colorado, Cx. nigripalpus from Florida, Cx. p. pipiens from Chicago, Illinois, Cx. pipiens complex from Memphis, Tennessee, and Cx. p. quinquefasciatus from Santa Fe Province, Argentina. Wild-caught Cx. tarsalis were readily infected (100% after 19 to 20 days of extrinsic incubation) and grew virus to high titers, but they would not refeed and transmission rates could not be measured. The Chicago Cx. pipiens became infected at a lower rate (71 to 80%) than other members of the complex tested; however, this strain was far more efficient at transmitting virus (71%) than either the Argentina (23%) or Memphis (36%) strains following 20 days of extrinsic incubation. Transmission rates among Memphis Cx. pipiens complex mosquitoes were similar on days 13 and 20 (33% and 36%); however, none of 15 Cx. p. quinquefasciatus from Argentina transmitted virus on day 13.

The majority of Cx. nigripalpus tested had fed on a chick circulating much less virus (4.2 to 4.7 $\log_{10}$ Vero PFU/ml) than had the other mosquito species and infection rates were low (6% on day 14, and 0% on day 22). However, 4 Cx. nigripalpus which fed on a chick circulating 8.1 to 8.2 $\log_{10}$ Vero PFU/ml contained relatively small amounts of virus (3.7 to 4.9 $\log_{10}$ Vero PFU/ml) following 20 days of extrinsic incubation. In contrast, the average titers for Culex mosquitoes which transmitted virus ranged from 6.1 to 7.2 $\log_{10}$ Vero PFU/ml. Results thus far suggest that Rocio virus may be maintained in nature in a cycle involving mosquito vectors and avian hosts. Of the Culex species investigated, two (Cx. tarsalis and certain populations of Cx. pipiens) represent potential vectors should Rocio virus be introduced into the United States. It is interesting to note that the northern subspecies Cx. p. pipiens appears to be significantly more efficient for the transmission of Rocio virus than is Cx. p. quinquefasciatus; the same phenomenon has been shown for the related flavivirus, St. Louis encephalitis. Studies are underway to test the susceptibility of Psorophora ferox (the species from which the isolate in nature was obtained) to Rocio virus and to measure its ability to transmit the virus.

Antibody development in garter snakes (Thamnophis spp.) experimentally infected with western equine encephalitis virus.

L. A. Thomas, E. R. Patzer, J. C. Cory, and J. E. Coe

U.S. Department of Health, Education, and Welfare, Public Health Service National Institutes of Health, National Institute of Allergy and Infectious Diseases, Epidemiology Branch, Rocky Mountain Laboratories, Hamilton, MT 59840

Garter snakes (Thamnophis spp.) have been considered to possibly play an important role in the ecology of western equine encephalitis (WEE) virus. Serological tests (hemagglutination-inhibition, complement-fixation, neutralization test in mice, and plaque neutralization) to detect antibody in these reptiles following laboratory exposure to this virus have, in our experience, been unsatisfactory. A new test, the snake globulin precipitation (SGP) test, has been developed and we consider it to be reliable in detecting antibody in WEE virus-infected garter snakes. Antibody has been detected in these snakes over 4 1/2 years following inoculation with WEE virus. The SGP test should be a valuable tool in obtaining further information regarding the possible role of these cold-blooded vertebrates in the ecology of this important arbovirus.

Ife: A new African Orbivirus isolated from Eidolon helvum Frugivorous bats collected in Nigeria and the Cameroun

Graham E. Kemp, Georges Le Gonidec, Nick Karabatsos, André Rickenbach and C. Bruce Cropp

Independent collections of Eidolon helvum, frugivorous bats, by scientists at the Institute Pasteur at Yaounde, Cameroun and the Virus Research Laboratory, University of Ibadan, Nigeria resulted in the isolation of 7 agents from tissues of 280 bats sampled during 1971. IbAn 57245 virus was subsequently designated as the prototype and Ife is proposed as the name.

These viruses were isolated from a multitude of tissues including serum, brain, salivary gland and mixed tissue pools. A single bat yielded virus from serum, brain and salivary gland.

Two- to four-day-old infant mice which became ill showed symptoms between 7 and 12 days following intracerebral inoculation of original material. On second passage, mice became ill between 5 and 7 days. IbAn 57245 virus, passage 3, killed 4-day-old mice following IC inoculation with an average survival time of 5.2 days.

Chloroform solvent sensitivity tests indicated that Ife virus was relatively insensitive to this lipid solvent. Following chloroform treatment, the infectivity of Ife virus was reduced only 0.4 - 0.8 logs when compared to the control titrations. The agent proved to be sensitive to low pH showing no activity following exposure to pH 3.0 for 3 hours at $4^{o}C$, whereas virus exposed to pH 7.8 titered 5.5 logs per 0.02 ml inoculated IC into infant mice.

The virus was readily filtered through a 0.22 u membrane filter showing about 1 log reduction in titer as compared with the control.

Extensive testing by complement fixation against a variety of arboviruses and other known arboviruses showed no relation to any other agent except YV 177, the Younde agent, also isolated from an *Eidolon helvum* bat.

Electron microscopic examination of infected BHK-15 cell cultures revealed typical orbivirus morphology. Particles were seen singly and in clusters in the cytoplasm of infected cells. No virions were seen in nuclei. Filamentous structures were seen commonly in masses, usually in close association with clusters of virions.

No information is presently available regarding possible arthropod vectors of this new orbivirus or whether any illness or death occurs in bats, as isolations reported here were from presumably healthy animals. To our knowledge only one other orbivirus has been described from bats, Japanaut, from *Syconycteris crassa* in Australia.

EFFECT OF CONCENTRATION OF WEE VIRUS INGESTED BY CULEX TARSALIS ON TRANSMISSION RATE BY BITE

L. Kramer, J. Hardy, S. Presser

It has been well demonstrated that the concentration of virus which mosquitoes ingest affects their ability to become infected--the higher the concentration of virus, the higher the infection rate. It has also been shown by Hardy *et al*. that the concentration of virus ingested is directly related to the ability of infected female *Culex tarsalis* to transmit WEE virus by bite, even after 14 - 21 days extrinsic incubation.

We have now examined 2 strains of *Culex tarsalis*--Yuma and a strain selected for its susceptibility to WEE virus, WS-3--to confirm the correlation between low ingested dose and low transmission rate and to characterize WEE viral infections in transmitting vs. non-transmitting mosquitoes.

Following ingestion of 10^2 - 10^3 pfu WEE virus per mosquito, 44 - 66% of the females became infected, as demonstrated by the presence of WEE virus in their mesenterons. Fifty to 65% of these females failed to transmit to chicks 14 - 23 days following infection. These mosquitoes could be divided into two groups on the basis of viral distribution in their tissues. In the first group, in addition to being found in the mesenterons, virus was also detected in the hemolymph and remnants, but not in the salivary glands by either IFA or plaque titration. In the second group, only the mesenterons were found infected. No virus could be detected in the remnants, hemolymph, or salivary glands. In this latter group, there appeared to be a midgut barrier to the escape of virus as well as to initial infection. While the mean concentration of virus in the individual organs of the transmitting females was usually higher than that in the non-transmitting females (where virus was detectable at all) significant overlapping in titers did occur.

Experiments are currently in progress to determine where the virus is in the remnants of females with salivary gland barriers.

ABSTRACT

EFFECT OF DENGUE ANTIBODY ON DENGUE

VIRUS ISOLATIONS FROM HUMAN SERA

G. E. Sather, M.P.H.,[1] C. G. Moore, Ph.D.,[1] J. P. Woodall, Ph.D.[1]

During the past few years Puerto Rico has experienced a series of dengue epidemics which provided a large number of patient sera. Multiple isolations of each of the three dengue types, 1, 2, and 3, have been made from acute phase sera.

Close to 2,000 sera have been inoculated into mosquitoes, Aedes aegypti or Toxorhynchites amboinensis, for virus isolations. Virus was detected by the direct FA technique with a conjugate prepared with high-titered (flavivirus) Puerto Rican human serum. Viruses were identified by the CF technique utilizing mouse hyperimmune ascitic fluids. Serum antibody was measured by the HI technique.

Viruses were recovered from acute phase sera obtained up to the fourth day of illness. Patients were shown serologically to have either primary or secondary serological responses. The presence of low antibody levels (1/20, 1/40) did not affect the ease of virus isolations, and isolations were made occasionally from sera with higher antibody levels, for example 1/320.

No viruses were recovered from cases that serologically were not cases of dengue.

[1] San Juan Laboratories, Bureau of Laboratories, Center for Disease Control, Public Health Service, U.S. Department of Health, Education, and Welfare, GPO Box 4532, San Juan, Puerto Rico 00936

ABSTRACT

DENGUE VIRUS ISOLATION USING AEDES PSEUDOSCUTELLARIS CELLS

G. Kuno, Ph.D.,[1] G. E. Sather, MPH,[1] and

C. G. Moore, Ph.D.[1]

During the 1977-78 dengue pandemic in the Caribbean, 62 selected sera, taken from human subjects during the acute phase of febrile illness, were simultaneously inoculated intrathoracically into adult mosquitoes and into cultured Aedes pseudoscutellaris (Mos. 61) cells, and the efficiency of dengue virus isolation by the two methods was compared. The presence of virus was detected by fluorescent antibody staining of mosquito heads and by the appearance of CPE (syncytia) in the cultured cells. The viruses were identified by the complement fixation and/or plaque reduction neutralization test.

Of 38 sera found positive, 27 were isolated in both adult mosquitoes and cell culture, 8 in adult mosquitoes alone, and 2 in cell culture alone.

The effect of 2 parameters, cell density and cell passage level, on the development of syncytia was studied. It was found that extremely dense monolayers at inoculation and/or high passage level (65 or more passages) retarded the appearance of syncytia.

The majority of isolates obtained in this comparative study proved to be type 1. The efficiency for isolating types 2 and 3 in Aedes pseudoscutellaris cells from human sera from which these viruses have been obtained is currently in progress. These results will be included.

[1]San Juan Laboratories, Bureau of Laboratories, Center for Disease Control, Public Health Service, U. S. Department of Health, Education, and Welfare, GPO Box 4532, San Juan, Puerto Rico 00936

Evaluation of ELISA in Cross Comparison of Bunyamwera Viruses and in Serologic Surveys for Bunyamwera Group Antigen

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The use of cell culture-derived gradient-purified virus as coating antigen has resulted in an ELISA test with members of the family Bunyaviridae that can be performed with no elaborate equipment and the results evaluated visually. To establish the specificity, the cross ELISA patterns of six members of the Bunyamwera group were determined. BUN, GER, BAT, and ILE were closely related (HO/Ht[a] = 2 to 16) and had minimal cross reactivity with SOR, WYO, and GRO (Ho/Ht = 256 to 10240). Birao showed a slight cross reactivity with BUN, BAT, GER, and ILE (Ho/Ht = 32 to 1024) and a stronger reaction with CV (Ho/Ht = 8). The CV sera was broadly cross reactive (Ho/Ht = 4 to 32) except with GRO (Ho/Ht = >1280) but the antigen reacted weakly with BUN, BAT, BIR, GER, ILE and SOR (Ho/Ht = 16 to 128). There was a cross between WYO and SOR (Ho/Ht = 0.025) and neither antigen showed a strong reaction with any other sera (Ho/Ht = 128 to >10240). The sera of GRO was broadly cross reactive but the antigen reactions showed GRO to be a distant relative of the other viruses (Ho/Ht = 64 to >10240). No appreciable reaction was noted between a control group C Oriboca antigen and the sera of BUN, BIR, GER, BAT, SOR, WYO, and ILE (Ho/Ht = 1024 to >10240) but low level reactions were seen with CV (Ho/Ht = 128) and GRO (Ho/Ht = 32). The cross ELISA test detected the same inter-relationships as did HI and cross neutralization tests reported in the literature.

To establish the relative sensitivity of the ELISA test and to determine if it could be used in routine serum surveys, 60 coded African human sera were tested with BUN, BIR, BAT, GER, ILE, and GRO. HI tests were run concurrently with BUN, BAT, GER, and CV and data from previous HI tests with GER, BUN, and ILE were available for some sera. All sera which were HI positive for Bunyamwera group antigens also were positive by ELISA for at least one antigen in the group. Nine per cent of the determinations were positive by ELISA and negative by HI for any tested antigen. Some of these reactions might be nonspecific but many may be due to a greater sensitivity of ELISA. In most cases ELISA titers (1/10 to 1/640) exceeded their paired HI titers (1/10 to 1/40). The results of this experiment showed that ELISA could be used effectively in routine serum surveys and that ELISA was more sensitive than the HI test.

[a] $\frac{\text{reciprocal of homologous serum titer}}{\text{reciprocal of heterologous serum titer}}$

The Medium-Sized RNA Segment of California Group Viruses Codes for Neutralization of Infectivity

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Reassortant viruses of the California group in the bunyavirus family were produced by coinfection of BHK-21 cells with clones of wild type or temperature sensitive California group virus pairs. Bunyaviruses have genomes with 3 segments: large, medium, and small (L/M/S). The resultant reassortant viruses contained either the Tahyna or LaCrosse M segment in combination with L and S segments of LaCrosse, snowshoe hare, Tahyna, or trivittatus viruses. The reassortant virus segments were identified by oligonucleotide fingerprint analyses.

Plaque reduction neutralization tests of 9 reassortant viruses representing 7 different combinations of L/M/S were carried out with reference sera of LaCrosse, snowshoe hare, Tahyna, and trivittatus viruses. Three reassortants with the Tahyna M segment were specifically neutralized by Tahyna reference antibody irregardless of the serotype of the L and S segments. Six reassortants with LaCrosse M segment were specifically neutralized by LaCrosse reference antibody irregardless of the serotype of the L and S segments. Thus the M segment codes for neutralization of infectivity. Assuming one or both of the glycoproteins of bunyaviruses are responsible for the neutralization reaction, the M segment must then code for one or both glycoproteins.

DENGUE IN CENTRAL AMERICA - 1978

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Epidemic dengue fever has not involved Central America since 1940. In contrast 3 major dengue epidemics involved the Caribbean; den-3 in 1963, den-2 in 1968, and den-1 in 1977. A major dengue outbreak swept through Central America in mid-1978. The first Central American dengue cases occurred on Roatan Island, Honduras during February 1978. The epidemic peaked between April and May and had largely subsided by the end of June. Dengue reached the Honduras mainland in late May-early June, peaked in August, and had declined by September. The clinical attack rate was 43%. We isolated den-1 virus from 25 individuals. Convalescent serum specimens from 8 other patients had unequivocal anti-den-1 neutralizing antibody.

Concurrently with the Honduras outbreak, towns along the Guatemalan Pacific coast and throughout El Salvador experienced dengue. A house to house questionnaire survey conducted in Escuintla, Guatemala documented a 20% clinical attack rate. Of 79 Guatemalan convalescent bloods, representing 3 towns, only 6 lacked anti-den antibody, 4 had equal, low levels of anti-den-1 and -2 antibody, the remaining 69 had significantly elevated anti-den-2 neutralizing antibody titers. We recovered den-2 virus from one Guatemalan patient. Den virus was isolated and seroconversion documented in Santa Ana, San Miguel and Ilopongo, El Salvador. We are analyzing data from a seroepidemiologic survey conducted in all 14 Departments of El Salvador to estimate both infection and clinical attack rates and to determine whether den-1 or den-2 or both were active.

Clinical material collected in Honduras was used to simultaneously compare several den isolated systems; including direct and delayed plaquing in $LLCMK_2$ cells, intrathoracic inoculation of Toxorhynchites mosquitoes with both head-squash assay by IFAT and culture of sonified abdomens, and inoculation onto Aedes pseudoscutellaris (AP-61) cell cultures. Preliminary results indicate that culture of den virus is more sensitive, rapid and technically less difficult in AP-61 cells than the other systems.

Title: LaCrosse virus infection of red fox (Vulpes fulva) following consumption of an infected chipmunk (Tamias striatus)

By: Terry E. Ammdson and T. M. Yuill

Abstract:

Our previous laboratory and field work showed that red fox (Vulpes fulva) were susceptible to infection by LaCrosse virus (LACV) following the bite of infected (Aedes triseriatus) mosquitoes. Since the eastern chipmunk (Tamias striatus), which is commonly infected with LACV in nature, is one of the fox's many prey species; this suggested the possibility that red fox might become infected by eating infected chipmunks.

Individual chipmunks were inoculated with a field isolate of LACV, from the LACV endemic region. The chipmunks were bled three days later to determine the amplitude of viremia and then fed to an individual fox. LACV was isolated from blood samples of two of four red fox, each of which had consumed an infected chipmunk. The viremias in the fox were of four days duration with a peak titer in one fox of 3.4 and the other fox 3.7 $\log_{10}$ SMIC LD_{50}/ml of blood which was comparable to previous experimental mosquito to red fox transmission studies in which fox viremias ranged from 2.7 to 3.7 $\log_{10}$ SMIC LD_{50} ml of blood with a duration of four to five days.

This is the first reported case of an arbovirus being transmitted by a predator consuming an infected natural prey species. These observations suggest that transmission of LACV to a susceptible predator species could occur in nature from July to September when nearly all chipmunks residing in many enzootic foci become infected.

ABSTRACT

RNA segment analyses of American and Australian Bluetongue serotypes

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In 1977, YARU identified an unknown virus from Australia as bluetongue virus by complement-fixation (CF) tests. The new virus, known as bluetongue type 20, was isolated in 1974 from a pool of mixed Culicoides species from the Northern Territory of Australia. Type 20 also exhibited a low level cross-reaction by CF with the epizootic hemorrhagic disease of deer (EHD) virus. This was the first isolation of bluetongue virus from Australia. This finding provided the rationale for the polyacrylamide gel electrophoresis (PAGE) analyses of the double-stranded (ds) RNA segments of bluetongue viruses and other related viruses.

The virus stocks were prepared for dsRNA analyses by growing the isolates in a porcine kidney (MVPK) cell line. The dsRNA profiles for four bluetongue serotypes found in the U.S.A. were compared with the profiles of type 20, EHD, and Ibaraki. The RNA profiles for bluetongue viruses 10, 11, 13, and 17 were similar with only minor variations in the mobility of a few dsRNA segments, and thus, each serotype exhibited a similar but unique RNA profile. The two EHD strains were also distinguishable, but more similar to themselves and to Ibaraki than to the bluetongue serotypes. The RNA profile for type 20 was similar to the other bluetongue types, but it was not the typical bluetongue-like RNA pattern because several segments were more similar to the Ibaraki and EHD. Whether the type 20 virus represents a reassortant species from existing Australian or other orbiviruses remains to be demonstrated.

SEROLOGICAL RELATIONSHIPS AMONG SIMBU GROUP ARBOVIRUSES

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We have compared 22 viruses of the Simbu serogroup; 17 registered and five (Yaba-7, Peaton, Facey's Paddock, Utinga, Bradypus) unregistered viruses. Tests were by cross complement-fixation (CF) and serum dilution-plaque reduction neutralization (N) tests in Vero cells. From CF test results three distinct complexes were identified within the Simbu serogroup.

The first complex includes six viruses from Africa (Simbu, Sabo, Sango, Shuni, Shamonda, Yaba-7), one from India (Kaikalur), one from Africa and India (Sathuperi), two from Australia and Asia (Akabane, Aino), and one from Australia (Peaton). N tests showed that individual members of the complex generally are separable with little difficulty. Shuni, Aino, and Kaikalur viruses are closely related to one another, with Aino virus indistinguishable from Kaikalur virus. Peaton virus shows moderate one-way reactivity with Sabo virus and strong two-way reactivity with Sango virus.

The second complex identified by CF tests includes one virus from Asia and Africa (Ingwavuma), one from North America (Mermet),

and two from South America (Manzanilla and Inini). Ingwavuma, Mermet, and Manzanilla viruses are distinguishable, but closely related, by N tests.

The third complex consists of two viruses from central and South America (Utinga, Bradypus), one from South America (Oropouche), and one from Australia (Facey's Paddock). These four viruses are distinct by N tests.

Buttonwillow virus, from California, is related to a number of New World Simbu group viruses. Finally, one virus from Africa (Nola) and one from Africa and India (Thimiri) appear to be distantly related to other members of the Simbu serogroup and may represent additional complexes.

These results, when combined with the limited available information concerning the natural histories of the Simbu serogroup bunyaviruses, including vector-host relationships, vertebrate susceptibilities, and geographic distributions, lead us to conclude that members of this serogroup may occur principally in avian-mosquito or mammalian (ungulates, primates, logomorphs)-Culicoides cycles in nature. The relationship of geographic distribution to intercontinental movement of certain of these viruses by birds is discussed.

Tacaiuma virus: Lack of identity between variants

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Two viruses of the Anopheles A group, from a human in Brazil (H-32580) and from Anopheles freeborni mosquitoes collected in Arizona (743-366) were shown to be members of the Tacaiuma (TCM) complex. The viruses grew to similar titers in suckling mice and in Vero, PS-2 and PK-15 cell cultures. Neither isolate formed plaques in L-929, BHK-21 or primary duck embryo cells. Plaques of H-32580 in Vero cells were 4 mm in diameter and discrete by day 8; plaques of 743-366 were 3 mm andindistinct on day 14. Other than these slight differences and an apparent lack of stability of isolate 743-366, no significant differences were noted; neither produced goose erythrocyte agglutinin and both were sensitive to the effects of sodium deoxycholate.

Serum-dilution plaque reduction neutralization (N) tests were performed in Vero cells. Both H-32580 and 743-366 viruses were cross-tested with each other and with seven other Anopheles A group viruses. Both field isolates were shown to be closely related to TCM virus. H-32580 was essentially identical to the TCM subtype SPAr-2317; 743-366 appears to be a variety of SPAr-2317 but was easily distinguishable from strain H-32580. Had neither TCM nor SPAr-2317 been available for comparison, both H-32580 and 743-366 would have been considered distinct, newly recognized viruses.

These results affirm that not all subtypes of a given bunyavirus are interchangeable and suggest that comparison of sufficient numbers of strains of a virus would, on geographic bases, show a much higher degree of variation than generally appreciated. We submit these viruses as possible evidence for reassortment or recombination under natural conditions. Additional evidence to support this hypothesis is presented using Gamboa virus isolates.

In addition, Anopheles A group viruses are discussed in regard to their mammal-mammal feeding mosquito cycles in nature.

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ABSTRACT

The effect of transovarially acquired La Crosse virus (California encephalitis group) on the fecundity of *Aedes triseriatus*

by

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Studies were undertaken to determine the effect of transovarially acquired La Crosse virus (LAC) on the fecundity of *Aedes triseriatus*. Parameters measured for each of 4 consecutive generations were, 1) number of eggs laid (1st ovarian cycle only) 2) number of 1st instar larvae, 3) number of 4th instar larvae, 4) male and female wing length, 5) number of progeny adult mosquitoes and 6) LAC virus transmission rate.

Vertical passage of LAC virus in *A. triseriatus* had no discernable effect on the replacement rate of infected females through 4 generations. The transmission of LAC virus to suckling mice by vertically infected female mosquitoes was consistent over the 4 generations with a mean of 53% (103/195).

Two conclusions were drawn from these data, 1) the relative fertility rate of vertically LAC virus infected mosquitoes was comparable to uninfected mosquitoes, 2) the relative survival rate to 1st oviposition (7-14 days) under laboratory conditions of transovarially infected mosquitoes was similar to that of their uninfected counterparts.

HUMAN IMMUNE RESPONSE TO DENGUE-2 VACCINE MEASURED BY A SOLID PHASE RADIOIMMUNOASSAY

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Sera from human volunteers inoculated with an attenuated dengue-2 (DEN-2) vaccine were assayed by various serological tests including a solid phase radioimmunoassay (SPRIA). Various SPRIA test parameters were studied to find optimal conditions for detection of low levels of DEN-2 antibodies. Antigen derived from detergent-extracted cells was less sensitive for detecting DEN-2 antibodies than was a preparation containing partially-purified virions. Virus strain was also important for test sensitivity and the homologous strain PR-159 virions gave optimal binding of DEN-2 antibody. S. aureus protein-A (pr-A) labelled with I^{125} was compared to I^{125}-labelled-IgG as an indicator of antigen-specific antibody complexing. Both labelled reagents gave similar results when serum endpoint titers were compared; however, pr-A is limited to detecting only IgG-specific immunoglobulins.

Selected human sera were tested by SPRIA to measure IgG and IgM responses at various intervals post immunization. Human volunteers with no previous history of flavivirus infection stimulated detectable IgM concurrently with IgG antibodies. The IgM response was relatively short-lived and could not be detected six months after immunization. Yellow fever-immune individuals who received DEN-2 vaccine responded more rapidly to immunization and mounted a high titer IgG response without detectable IgM antibodies.

The SPRIA is a sensitive immunological test that can be used to measure specific immunoglobulin responses after DEN vaccination. Incorporation of labelled pr-A into the SPRIA broadens the utility of the conventional SPRIA that uses labelled secondary antibodies.

Infection and transmission thresholds of an enzootic strain of Venezuelan encephalitis virus in Culex (Melanoconion) opisthopus from an enzootic focus in Guatemala

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Cu. (Mel.) opisthopus was found to be a mosquito vector of Venezuelan encephalitis virus at an enzootic focus in Guatemala during 1977. This is the third mosquito species to be so established as an enzootic vector of VE virus, the others being Cu. (Mel.) aikenii in Panama and Cu. (Mel.) portesi in Trinidad and Brazil. Experiments to determine infection and transmission thresholds of an enzootic strain of VE virus in Cu. (Mel.) opisthopus were begun in Guatemala during 1978. Wild-caught, mostly nulliparous Cu. (Mel.) opisthopus were infected by very small amounts of VE virus (<5 plaque forming units) circulating in bloods of hamsters; the 50% infection threshold was between <5 and 10 pfu when measured 12-19 days after engorgement of viremic blood. Transmission also occurred after oral infection with small amounts of virus. Preliminary estimation of the 50% transmission threshold was about 20 pfu of ingested virus. Cu. (Mel.) opisthopus survived for several months in cages and sometimes secondarily engorged even when still gravid within two weeks of the first blood meal. Thus this species of mosquitoes seems to be an efficient vector of VE virus at this focus in Guatemala.

Effect of Temperature on the Vertical Transmission of California Encephalitis Virus in *Aedes dorsalis*

Michael J. Turell and James L. Hardy

Most reported studies on the vertical or transovarial transmission of arboviruses in mosquitoes were conducted at ±26-27°C. Since larvae in the field often are exposed to cooler or warmer temperatures, the relationship was investigated between larval rearing temperature and the rate of vertical transmission for California encephalitis (CE) virus in *Aedes dorsalis* from a laboratory colony. Three viral stocks were used: BFS-283, a strain adapted to grow at 40°C (CE-TA), a temperature sensitive (ts) mutant of BFS-283 (CE-TS), and a pool comprised of 6 isolates of CE wildtype (CE-WT) virus made from *Aedes melanimon* collected in the Sacramento Valley. Each of these viral stocks was used to infect inseminated females by intrathoracic inoculation. Progeny obtained from these females were reared at 18, 26, 30.5, 32, and 34°C.

The rate of transstadial transmission of CE virus to the F_1 adults was influenced by the larval rearing temperature, and this effect depended on the strain of CE virus used. While CE-WT and CE-TA viruses were recovered from transovarially infected larvae at all temperatures tested, CE-TS virus could be recovered from larvae only at 18 or 26°C. Even though larvae were infected with CE-TS virus at both 18 and 26°C, the ts mutant was recovered from the F_1 adults only at 18°C. Similarly, while larvae were infected with CE-WT and CE-TA viruses at all temperatures tested, both of these viruses could be recovered from F_1 adults only at 30.5°C or less.

The effect of larval temperature on the transmission of virus to the F_1 adults must be considered when investigating vertical transmission of CE virus, and possibly all arboviruses. In particular, if these findings apply to other ts viruses, then they will not be recovered from F_1 adults if the larval temperature is 26-27°C or above. However, ts virus is transmitted to the F_1 adults at slightly lower water temperatures. These results also could explain the failure to isolate CE virus from ove 25,000 adult *Ae. melanimon* reared from field collected larve and pupae. The water temperature in the flooded fields where these mosquitoes occur may reach day time temperatures in excess of 36°C.

ABSTRACT

One-System Isolation--Identification of Dengue Viruses*

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Methods currently in use for the isolation and type-identification of dengue viruses are time-consuming and require the employment of more than one system. We are now testing Varma's Aedes pseudoscutellaris cell line grown in Lab-Tek slides as a single system for rapid isolation and typing. Our results to date show that this is a sensitive and relatively rapid method for this purpose.

A culture of Aedes pseudoscutellaris cells, received from Dr. Goro Kuno of the San Juan Laboratories, Puerto Rico, was selectively adapted for adherence to glass surfaces. The adapted cells are pipetted into four-chamber Lab-Tek slides and reach 70-80 percent confluency usually within 24 hours. An appropriate dilution of the specimen, from which virus is to be recovered, is then added to each of four chambers. Human serum, which we have found to be toxic to mosquito cells in low dilutions, is used at a dilution of 1:20, which eliminates the toxic reaction. After incubation at 27°C, the media are poured off, the plastic chambers removed from the glass slides, which are then fixed for 20 minutes in cold acetone. The fixed slides may be stored frozen or used immediately in the indirect fluorescent antibody technique. Mouse immune sera for each of the four dengue virus types, previously titrated for fluorescent endpoints on dengue-infected control slides, are added to the fixed slides, one chamber for each type-specific serum. After incubation and washing, anti-mouse goat serum conjugate is applied with a final incubation and careful washing before examination under the fluorescent microscope. Each test includes positive and negative controls.

Prototype strains of the four types of dengue virus were all readily identified in blind tests. To date, 14 of 15 virus strains have been successfully typed by this method from acute-phase serum specimens of dengue fever patients from Malaysia. The 15th is still in process. These isolates included strains of dengue types 2, 3 and 4. Type 1 strains are yet to be tested.

This method has the advantage of requiring only one system from isolation to final identification. Mouse or mosquito colonies need not be maintained. Observation for cytopathic effects and syncitial formation in the cultures, which may be seen, is not necessary, since the final examination for fluorescence is highly sensitive and clearly seen.

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IXODES DAMMINI AND ASSOCIATED IXODID TICKS IN SOUTH-CENTRAL CONNECTICUT

By: Andrew B. Carey, William L. Krinsky, and Andrew J. Main[1]

Abstract: A survey of wild mammals in south-central Connecticut resulted in the collection of 9,000 ticks of 9 species from 22 species of mammals. Two species of ticks, *Ixodes dammini* and *Dermacentor variabilis*, constituted the the majority of ticks collected. The principal hosts of *I. dammini* were *Peromyscus leucopus*, *Tamias striatus*, and *Odocoileus virginianus*. *D. variabilis* were most abundant on *P. leucopus*, *Microtus pennsylvanicus*, and *Procyon lotor*. Immature *I. dammini* were most abundant in the late seral stages of forest development. Immature *D. variabilis* were most abundant in the early seral stages. The distribution of *I. dammini* was influenced by a coastal climatic gradient. The abundance of *D. variabilis* was inversely related to that of *I. dammini*. The larvae of *I. dammini* were most abundant in the late summer and fall, nymphs, in the spring, and adults, in the spring and fall. Immature *D. variabilis* were most abundant in the spring and summer, adults, in the early summer.

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ABSTRACT

THE USE OF ISOZYME ELECTROPHORESIS IN BLACK FLY IDENTIFICATION

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Isozymes were used to characterize six species of Simulium collected in Chiriqui Province, Panama: S. chiriquense, S. metallicum, S. ochraceum, S. panamense, S. quadrivittatum, S. rubicundulum. Comparative studies were made on 22 enzymes. Each of the six black fly species showed a unique combination of bands when tested for the enzyme aspartate amino-transferase (ASAT). This enzyme could be used to distinguish any of these six species from each of the others. Species that showed only slight differences in isozyme migration for ASAT showed greater isozyme separation when tested for other enzyme systems. For example, ASAT bands of S. chiriquense and S. rubicundulum had only slight differences in migration; however, isocitrate dehydrogenase (IDH) isozyme bands of these species had quite different migration.

Tests for Hardy-Weinberg equilibrium of the phosphoglucomutase (PGM) alleles were made of three sample pools of S. metallicum and one sample pool of each of the other five species. For these tests it was first assumed that the PGM bands recovered from each species were allozymes (allelic isozymes), then phenotypic frequencies were calculated from the electrophoresis data. Chi-square tests were done to determine if the observed numbers fit the expected Hardy-Weinberg proportions. S. metallicum was the only species that showed a highly significant deviation from the expected frequencies (Table 1).

Table 1. Test for equilibrium of the PGM genotypes in the Simulium species indicated. Note: The expected frequencies for chi-square analysis were obtained by multiplying the theoretical frequency by sample size.
P = probability. d. f. = degrees of freedom. n.s. = not significant.
* = significant. *** = highly significant.

Species	Sample size	Number of alleles	Theoretical frequency	X^2 (d.f.)	P
S. chiriquense	187	3	$(a+b+c)^2$	1.9 (3)	$P > .5$ n.s.
S. metallicum (1)	111	5	$(d+e+g+h+i)^2$	43.5 (10)	$P < .001$***
S. metallicum (2)	209	5	$(d+e+g+h+i)^2$	41.96 (10)	$P < .001$***
S. metallicum (3)	162	6	$(d+e+f+g+h+i)^2$	96.0 (15)	$P < .001$***
S. ochraceum	161	5	$(j+k+l+m+n)^2$	18.63 (10)	$P < .05$ *
S. panamense	232	5	$(r+s+t+u+v)^2$	8.4 (10)	$P > .56$ n.s.
S. quadrivittatum	70	3	$(x+y+z)^2$	2.87 (3)	$P > .3$ n.s.
S. rubicundulum	104	2	$(p+q)^2$	0.06 (1)	$P > .7$ n.s.

Morphological variation has been observed in S. metallicum. Trichomes on the pupal thorax may be bifid, trifid or multiple. The pattern of pruinosity on the adult thorax shows variation. These morphological observations together with the isozyme data are consistent with the hypothesis that S. metallicum is a group of morphologically similar but genetically distinct species.

The Cellular Basis of Antibody Mediated Plaque Enhancement of Murray Valley Encephalitis (MVE) Virus

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Our previous report indicated that antibody mediated MVE plaque enhancement on primary chick embryo fibroblast (CEF) monolayers was dependent upon Fc portion of non-neutralized virus-antibody complexes. Taxonomic class homology between animal sources of antibody and the cell cultures used for assay was required in order for Fc-Fc receptor binding to occur. Similar requirements for enhanced infection were also demonstrated in chicken and mammalian peripheral blood leukocyte (PBL) cultures.

In order to further elucidate the role of the Fc portion and its receptor in enhancement of MVE plaque production, the EA rosette method was used to identify Fc receptor-bearing cells in CEF and PBL cultures. Results from 4 experiments showed chicken PBL contained 8.4-12.2% of EA rosettes cells while lightly trypsinized CEF cells contained 5.2-8.3% of such cells. Chicken PBL and CEF were rosetted only by chicken anti-sheep RBC but not rabbit anti-sheep RBC ($<0.5\%$). The percentage of human PBL cells forming rosettes with sheep RBC's sensitized by rabbit antibody was 15.2-20; chicken anti-sheep RBC did not rosette human PBL ($<0.5\%$). These experiments confirm that class homology is required between the Fc terminus and Fc receptor permitting effective binding of antibody to EA rosetting cells.

Chick embryo cell suspensions were rosetted with sheep RBC and then centrifuged on a Ficoll-Hypaque gradient to remove EA forming cells. The derived CEF monolayers produced plaque numbers similar to controls but were incapable of supporting antibody-mediated plaque enhancement. LLC-MK_2 monolayers did not contain a sub-population of rosette forming cells and plaque enhancement was not observed in the presence of mammalian antibody.

Selective elimination of mononuclear phagocytes from chicken PBL by silica at the concentration of 100 μg/ml totally ablated the usual 5-10 fold enhanced virus production in PBL cultures. It is proposed that mononuclear phagocytes are the Fc receptor-bearing cells present in CEF. These cells may originate from bone marrow, blood and spleen of 11 day-old chick embryos used in CEF preparation.

Neutralization of Caribbean and Southeast Asian Dengue 1, 2, 3 Viruses by Convalescent Serums from Puerto Rican Dengue Patients

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In 1977, serums were obtained from residents of Puerto Rico who presented to medical facilities with the symptoms of clinical dengue fever. These were sent to the CDC San Juan Laboratories. Five hundred representative acute and convalescent serum pairs showing a four-fold or greater antibody rise by hemagglutination-inhibition test were examined for neutralizing antibodies in the Department of Tropical Medicine and Medical Microbiology, University of Hawaii. Each serum pair was tested at four-fold dilutions, 1:10 - 1:2560, versus Caribbean dengue strains, D1 (Jamaica), D2 (PR-159) and D3 (PR-6) - virus strains were kindly supplied by the Department of Virus Diseases, Walter Reed Army Institute of Research. Plaque reduction neutralizing (PRNT) titers were calculated at the 50% and 90% levels in acute and convalescent serums using a standard LLC-MK2 plaque assay in 1 ounce prescription bottles (1).

Serum pairs showed conversion to relativity monotypic 50% PRNT antibody as follows: dengue 1 - 26; dengue 2 - 194 and dengue 3 - 138. In addition, 7 serum pairs showed no D1-3 antibodies, 21 showed fixed titers, 46 had secondary-type antibody responses, and 62 had responses suggestive of recent dengue 3 infection.

From serum pairs showing monotypic dengue 1, 2 or 3 responses, twenty representative samples each were selected for further study. These sera were tested against matching Southeast Asian dengue types: D1 (16007), D2 (16681), D3 (16562) strains which were isolated from Thailand or the Philippines in 1963-66.

Convalescent serums always produced significant neutralization with the respective homologous Southeast Asian strain.

Results of the virus neutralization study were as follows:

	Comparative Neutralization vs. dengue strains		
	CARIB = SEA	CARIB > SEA↓	SEA > CARIB↓
Dengue 1	9	6	5
Dengue 2	15	2	3
Dengue 3	2	18	0

↓Neutralization titers considered different when values varied $\geq$ 4-fold.

These results suggest close homology of 1977 dengue 1 and 2 Caribbean strains with 1963-6 Southeast Asian strains, but indicate the continuing circulation in the Caribbean of an antigenically distinct dengue 3 sub-type.

In addition to the study described above, an extensive analysis of the following parameters of neutralization of plaques in LLC-MK2 cells was made:

Container size (1 oz. prescription bottles versus 15 mm wells)
Plaque numbers
50% versus 90% plaque reduction end point
0.2 versus 0.02 ml inocula

Factors which significantly influenced neutralization titers were plaque numbers >70 (depressed antibody titers), plaque numbers ≤ 6 (erratic data), use of 90% end point (12% seroconversions which were observed at 50% were not seen or were equivocal at 90% plaque reduction). Micro vs. macro volumes and use of 15 mm wells vs. bottles generally resulted in similar neutralization titers.

1) Halstead, S.B. et al., Yale J. Biol. Med. 42, 261-275, 1970.

California Group Virus Activity in Illinois-An Emerging Picture

Gary G. Clark, Harvey L. Pretula and Russell J. Martin

ABSTRACT

Since 1966, when the first laboratory confirmed case of California Encephalitis was identified in an Illinois resident, 88 cases have been recognized through laboratory testing. While information on this disease, endemic to the upper midwest, has been reported from several states, Illinois' experience with this disease has not been formally presented. This presentation includes observations on the epidemiology and ecology of this disease caused by LaCrosse virus in the state. The annual distribution of cases, 88 percent of which occurred between 1974 and 1978, and possible reasons for the increase in recognized cases will be discussed. The seasonal occurrence of cases, 44 percent with onset of illness in August, and the age and sex distribution, 50 percent in the 5-9 year-old age group with an overall male: female sex ratio of 2.2:1, will be presented. Ninety percent of the cases have been geographically distributed across the northern half of the state with a concentration (47%) by residence in Peoria County.

Since 1976, when the Illiois Department of Public Health initiated an Arbovirus Surveillance Program primarily directed toward early detection of enzootic transmission of St. Louis Encephalitis virus, limited but productive studies have been conducted in Peoria County. Results include isolation of California group virus from wild-caught and laboratory-reared *Aedes triseriatus* mosquitoes, as well as, detection of California group antibodies in wild mammals.

Protection from Rift Valley Fever (RVF) Virus Infection

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RVF is a persistent problem in sub-saharan Africa and recently has invaded Egypt. If RVF spreads in the Middle East or is introduced into more distant countries existing human or animal vaccine stocks will be inadequate and critical information on protective antibody levels or alternate treatment modes would be useful. Immunization of susceptible rats, mice or hamsters with decreasing doses of formalin-inactivated vaccine showed that minimal plaque reduction neutralizing antibody titers (eg, 1:40 to 1:10 or less) were protective. Passive serum transfer experiments in mice confirmed this. Sheep with post-vaccination serum antibody titers of 1:20 to 1:40 did not develop viremia after challenge with Virulent RVF virus. These findings demonstrate the need for sensitive tests of neutralizing antibody to assess protection from RVF infection and suggest that passive transfer of immune serum or gamma globulin prepared in endemic areas might be useful in prophylaxis of RVF. We also studied several pharmacologic agents in the prophylaxis of murine RVF. Rimantidine, isoprinosine, and thiosemicarbazone were ineffective. A macrophage activator (glucan) was of some utility but the antiviral drug ribavirin and the interferon inducer polyriboinoinic-polyribocytidilic acid (PIC) produced solid protection. Ribavirin also protected hamsters and rats from the lethal effects of RVF and suppressed viremia in the non-lethal rhesus monkey model. Therapeutic studies were carried out in the mouse model using immune serum, PIC, or ribavirin. Mice treated as late as day 3 (a time when controls are already dying of massive liver necrosis) had prolongation of life and increased survival. Late encephalitis was common in treated mice but probably represented inadequate therapy rather than a specific complication of treatment since (1) it was associated with all 3 treatment modalities, (2) it was also seen in the occasional untreated animal surviving the acute liver disease, (3) it was most prominent when therapy was delayed or marginal doses were used (4) none of the agents used in treatment protected against intracranial inoculation. These findings suggest that ribavirin or interferon might be considered in the prophylaxis of RVF or in the therapy of RVF hemorrhagic disease but there is no experimental rationale for their use in RVF encephalitis.

Search for a Sub-human Primate Model of Human Disease for Korean Hemorrhagic Fever

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Development of an animal model for KHF in humans is an essential goal of our research in that it is the key to future studies in prophylaxis, chemotherapy and transmission. In this study nine species of sub-human primates have been evaluated for this purpose. Animals studied included three species of Macaca, the rhesus, Cynomologus and pigtail monkeys; Saimiri sciureus the squirrel monkey; Aotus trivigatus, the owl monkey; Cercopithecus aethiops, the African green monkey; Callithrix jaccus, a marmoset, and Papio anubis, the olive baboon. Detailed results of clinical and laboratory findings in all species will be reported. In brief, all species of primates tested are susceptible to infection in that one or more members developed KHF antibody in response to inoculation with infectious material. None of the primates tested, with the possible exception of the squirrel monkey, developed frank clinical signs of disease during the observation period. Squirrel monkeys appear to be uniformly susceptible to infection and offer the best opportunity for developing a useful model. Recent results in this species have been encouraging and warrant continuation of this approach.

ABSTRACT

Isolation of Keystone Virus from *Aedes infirmatus* D. & K. in the Chincoteague National Wildlife Refuge, Virginia

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Previous attempts to isolate viruses from mosquitoes in the Chincoteague National Wildlife Refuge (CNWR) on Assateague Island, Virginia have yielded no California group viruses. However, antibody to both Keystone (KEY) and Jamestown Canyon (JC) viruses have been commonly found in cottontail rabbits trapped on the island. The vector or vectors of these viruses is still unknown. During the summer of 1978 a large breeding population of *Aedes infirmatus* was found which stimulated investigations into its possible role as a vector.

Mosquitoes were collected by CDC light traps supplemented with dry ice and by hamster-baited hanging net traps. Sentinel hamsters were located near each light trap. Three light traps and three net traps were placed in a pine forest bordered by marshes. Two light traps and two net traps were placed in a freshwater swamp. Sentinel hamsters were replaced semiweekly and held for an additional two weeks before being bled to determine the presence of KEY or JC antibodies.

Light trap collections yielded 4519 *Ae. infirmatus* and 59 *Ae. atlanticus*. Hamster baited net traps yielded 909 *Ae. infirmatus* and five *Ae. atlanticus*. KEY virus was isolated from a pool of 36 *Ae. infirmatus* collected by light trap on 8 August. Neutralizing antibody to KEY virus was detected in two sentinel hamsters which had been exposed between 17-30 August. The virus isolation and the seroconversions showed a temporal relationship to the 2nd generation of *Ae. infirmatus* on the CNWR. The minimum infection rate for KEY virus in *Ae. infirmatus* was 1 per 5428 mosquitoes tested.

Approximately 3000 3rd and 4th instar larvae were tested for virus with no mouse-lethal agents isolated.

Abstract

SELECTION OF MOUSE LYMPHOCYTE HYBRIDOMAS PRODUCING MONOCLONAL ANTIBODY TO SINDBIS VIRUS STRUCTURAL PROTEINS

by

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Lymphocyte hybridoma cell cultures were successfully obtained from the fusion of immune BALB/C mouse spleen cells and mouse myeloma cells P3-X63Ag8. Mice immunized with either infectious suckling BALB/C mouse brain or purified CER cell culture propagated virions were given a booster immunization and spleens harvested 3 days later. Spleen cell suspensions were mixed with P3 myeloma cells (10:1) and fused using 30% polyethylene glycol 1000. Approximately 600 microtiter well cultures were prepared for each mouse spleen resulting in from 6-15% viable cell lines. Cultures of particular interest were cloned on soft agar utilizing a feeder monolayer of human fibroblasts and representative clones frozen in liquid nitrogen. Antibody to Sindbis virus antigen(s) was detected in approximately 5-20% of all growing cultures. Experiments employing purified virion immunogens frequently produced greater numbers of growing cell cultures and an increased frequency of Sindbis virus antibody production. Hybridoma supernatants were screened for antibody production using a radioimmune assay (RIA) system that employed Sindbis virus infected CER cell lysates as antigen and either iodinated anti-mouse antibody or protein A from Staphylococcus. Antibody specificity was examined by RIA using purified and separated Sindbis virus nucleocapsid and each of the envelope glycoproteins. The majority of the hybridoma antibodies characterized reacted specifically with nucelocapsid; however, four cultures possessed anti-E_1 glycoprotein specificity and a single culture reacted with the E_2 glycoprotein. Significant increases in antibody titer were observed following injection of hybridoma clones into Pristane treated BALB/C mice and harvesting the resultant ascitic fluid. Examination of immune ascitic fluids from 10 hybridoma clones revealed only one with complement-fixing activity, another hybridoma product inhibited hemagglutination but none have yet been found with neutralizing activity. Procedures for the preparation of lymphocyte hybridoma cultures and methods for detecting and evaluating monoclonal antibody production will be described in detail.

Infection of *Aedes triseriatus* mosquitoes with California group reassortant viruses

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The tripartite genome of California group viruses is composed of a large, a medium, and a small (L/M/S) RNA segment. The RNA segments can be reassorted in vitro, providing new genomes of known segment composition which can be used to ascertain the in vivo biologic contribution of the respective segments. The following California group viruses and reassortants thereof were compared for their ability to orally infect *Aedes triseriatus* mosquitoes: La Crosse (LAC/LAC/LAC), Snowshoe hare (SSH/SSH/SSH), SSH/LAC/LAC, LAC/LAC/SSH. The identity of each of the segments was determined by RNA oligonucleotide mapping. The virus stocks were serially diluted in defibrinated rabbit blood, and approximately 15 mosquitoes were allowed to engorge on each 10-fold dilution. After 14 days extrinsic incubation, mosquito heads and abdomens were severed, squashed on slides, fixed in acetone, and stained with FITC-conjugated anti-La Crosse immunoglobulins. Smears were then examined for the presence of viral antigen, the positive abdominal smears indicating infection of the gut and the positive head smears indicating a dissemination of infection from the midgut. Representative results are shown in Table 1.

Table 1

California group wild-type and reassortant viruses

	LAC/LAC/LAC	SSH/SSH/SSH	SSH/LAC/LAC	LAC/LAC/SSH
Titer in log pfu/ml	8.3	7.9	7.9	7.8
Oral infectious $dose_{50}$	5.5	5.5	5.6	>7.8
Oral dose resulting in disseminated infection in 50% of the mosquitoes	5.8	>7.9	6.1	>7.8

The titer of the 4 viruses was comparable in BHK-21 cells. As expected LAC/LAC/LAC virus infected and produced disseminated infection in *Aedes triseriatus*, its natural vector; SSH/SSH/SSH virus infected but did not disseminate from the mid-gut. SSH/LAC/LAC reassortant infected and disseminated like the wild-type LAC/LAC/LAC indicating that the large genome segment probably does not by itself code for dissemination. The LAC/LAC/SSH reassortant did not infect *Aedes triseriatus* by the oral route indicating either that the small segment codes for infectivity or that a mutation occurred in one or more of the RNA segments accounting for the attenuation of this reassortant for mosquitoes.

Mosquito studies with rabies-related Mokola virus

By

T.H.G.Aitken, S.M.Buckley, B.J.Beaty and R.E.Shope

A B S T R A C T

The Nigerian shrew virus, Mokola, has never been isolated from naturally-infected arthropods, but it readily produces a persistent infection in Aedes albopictus cell culture.

Earlier attempts in this laboratory to infect mosquitoes parenterally resulted in failure when a mouse-brain strain of virus was used. More recently, success has been achieved by utilizing a virus strain passed through cultures of Aedes albopictus and Vero cells.

Virus has been passaged 5 times through Aedes aegypti mosquitoes and thus maintained continuously in arthropod bodies for a period of 8 months. Contrary to most arboviruses, Mokola virus multiplies very slowly in the arthropod host. Utilizing the direct FA technique, Mokola antigen cannot be demonstrated with any degree of regularity in mosquito head squashes much before the 4th week post infection. Virus has been recovered from various organs, including nerve cord and ganglia and dorsal vessel. Repeated confirmation of antigen in salivary glands is noted but oral transmission of virus has not been documented with certainty.

Detection of La Crosse Viral Antigen in Skin Biopsies

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Diagnosis of arboviral encephalitides is typically accomplished by either serologic techniques or virus isolation. Neither of these techniques provide the physician with early information which could be of value in prescribing patient care. Immunofluorescent techniques have been used to detect viral antigen in cases of Herpes encephalitis and Rabies. Since a diagnosis can be attained in a matter of hours, the physician is provided valuable diagnostic information during the early phase of the clinical disease.

Studies were conducted to determine if La Crosse encephalitis virus antigen could be detected in cryostat sections of skin biopsies obtained from infected mice. The experiment was designed to determine the effect of long and short incubation periods on the detection of viral antigen in the skin biopsies. Two cohorts of mice (2 to 3 days of age and 3 weeks of age) were infected either IC or SC with either 2.0 or 6.0 $\log_{10}$ $SMICLD_{50}$ per ml of La Crosse virus. When possible 5 normal, 5 sick, and 5 dead mice were harvested daily from each experimental group. Brains of mice were also cryostat sectioned and examined. Virus antigen was detected in brain and skin biopsy sections of mice from each experimental group. Virus was isolated from skin biopsies and identified by CF.

The pattern of specific fluorescence differed in the 2 cohorts; in the 2 to 3 day old mice, antigen was widely distributed in a variety of tissues, while in the 3 week old mice it was restricted. The actual tissue tropisms are as yet undetermined, but vascular and neural tissues are suspected.

The efficacy of the technique for the diagnosis of human cases will be tested at the Lutheran Hospital in La Crosse, Wisconsin during the upcoming encephalitis season.

PERSISTENCE OF NEUTRALIZING ANTIBODY TO YELLOW FEVER 30 TO 35 YEARS FOLLOWING IMMUNIZATION WITH 17D YELLOW FEVER VACCINE.

J.D. Poland, C.H. Calisher, T.P. Monath, W.G. Downs and K.F. Murphy

In 1975 and 1976 sera were obtained from 149 World War II (WWII) veterans and tested for antibodies to 17D and French neurotropic strains of yellow fever virus (YF). The study participants were divided into immunized (with 17D YF vaccine) and unimmunized based on whether or not they served during WWII, in a geographic area, or a branch of service, or a period of time when YF vaccine was administered. None had received YF vaccine since WWII.

Of 116 veterans classed as immunized, 78.4% had persistent antibody ($\geq$2) to 17D YF virus 30 to 35 years later; 18.2% of those classed as unimmunized in WWII also had antibody to 17D virus. Examination of immunized Navy and Air Corps veterans revealed that 97% of those immunized had persistent antibody, compared to only 60% of Army veterans. Eighty percent of Army veterans who potentially had multiple opportunities to receive YF vaccine were found to be seropositive.

These findings lead us to the following conclusions: 1) the probability is high (80% to 97%) that PRNT antibody to 17D YF vaccine persists for 30 years or more; 2) that Army personnel supposedly immunized between 1941 and 1948 were more likely to have missed being vaccinated when their service assignments included only a single administratively designated opportunity for YF vaccination; and 3) that 88% or more of veterans with PRNT antibody to 17D virus also had mouse protective antibodies against YF (FNV) 30 years later.

A Description of the Structural Proteins of Some Representative Sandfly Fever Viruses

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Seven Phlebotomus Fever Group viruses were selected for study because of their previously recorded association with human disease. Each of these viruses; Karimabad (KAR), Chagres (CHG), Sandfly fever Sililian (SFC), Sandfly Fever Naples (SFN), Punta Toro (PT) Itaporanga (ITP), and Candiru (CDU) were plaque purified by selecting plaques from VERO cell monolayers under agar overlay containing DMSO on three consecutive plaque to plaque cloning passages. Plaque size varied from 1 to 5 mm in diameter and seed virus preparations propagated in VERO cells ranged from 10^6 to 10^9 pfu/ml.

Conditions for optimal propagation of intrinsically radiolabeled virions were found to vary depending on the particular virus studied. Although the most sensitive cells for plaque assay appeared to be CER cells for KAR, BHK cells for ITP and CDU, and VERO cells for all others, maximum virus production was obtained from infected VERO cells for all viruses except KAR which replicated to higher titers in BHK cells. Intrinsic radiolabeling was best accomplished using Actinomycin-D inhibition of host cell protein synthesis, ^{35}S-methionine and methionine deficient medium. Supernatants from infected cells were concentrated by precipitation with polyethyleve glycol 6000. Virion purification was accomplished using potassium tartrate-glycerol, and sucrose or metrizamide gradients under both density and rate zonal centrifugation conditions. Virion stability to various purification methods differed markedly between viruses.

Structural proteins were analyzed using discontinuous polyacrylamide slab gel electrophoresis and fluorography for detection of radioactive polypeptides. Polypeptide profiles differed for at least 5 of the viruses examined. Differences were observed both in the apparent molecular weight (migration distance) of the polypeptides and the number of structural proteins observed. Only 3 polypeptides could be detected in purified PT virus preparations as compared to 5 proteins in CHG virions. Structural protein analysis may prove valuable in Phlebotomus fever virus identification.

Live Attenuated Dengue Type 2 Vaccine in Human Volunteers

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An experimental, live, dengue type 2 (DEN-2) vaccine (PR-159/S-1) was inoculated subcutaneously into 25 people in initial studies of vaccine reactogenicity, immunogenicity and dose response. The vaccine recipients included 6 yellow fever immune (YFI) and 19 yellow fever nonimmune (YFN) volunteers. The undiluted vaccine had a titer of 2.5 - 4.5 x 10^5 pfu/0.5ml dose at 35C, produced only small plaques in LLC-MK2 cell culture and did not replicate at 39C. Following inoculation, 5/6 YFI and 5/19 YFN developed detectable viremia. Over 40 virus isolates from serum of vaccinees retained the growth characteristics of the vaccine virus.

Seroconversion by complement fixation (CF), hemagglutination inhibition (HI) and/or plaque reduction neutralization test (PRNT) to DEN-2 occurred in 6/6 YFI and 8/19 YFN volunteers within 30 days. Antibody responses in YFN and YFI volunteers were similar to those seen in naturally acquired primary and secondary flavivirus infections.

Use of the vaccine made it possible to compare the clinical and laboratory responses of YFI and YFN volunteers infected with the same strain of DEN-2 virus. Among recipients of undiluted vaccine, evidence of DEN-2 infection occurred in 6/6 YFI and only 2/5 YFN. Six other YFN were infected with lower doses of vaccine.

The following clinical changes occurred in the 6 YFI and 8 YFN infected volunteers: viremia (5 YFI, 5 YFN) leukopenia (4 YFI, 2 YFN), oral temp > 100F (2 YFI, 0 YFN) and an erythrmatous rash (1 YFI, 2 YFN). One YFI developed fever, headache, myalgia and fatigue associated with viremia. One YFN developed headache, myalgia, photophobia and rash with viremia. None of the vaccinees developed local reactions at the site of injection or any bleeding tendencies. The principle differences observed between the 2 groups of vaccinees were an increased frequency of viremia, leukopenia and higher antibody titers in the YFI volunteers. The PR-159/S-1 vaccine is attenuated for humans, does not revert to wild type virus *in vivo* and provides a means for studying primary and secondary flavivirus infection in humans.

Biochemical Characterization of Isolates of Rift Valley Fever Virus

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Recent evidence from our laboratory has suggested that Rift Valley Fever virus (RVFV) isolates share similar biochemical properties with some members of the Bunyaviridae family. Molecular weights of structural protein subunits of these isolates were determined by discontinuous SDS polyacrylamide gel electrophoresis (PAGE). Preliminary results demonstrate the presence of three major subunits with molecular weights of 70, 63 and 23×10^3. These proteins closely correspond to the 75, 65, and 25×10^3 MW moieties reported in Uukuniemi, an unclassified member of the Bunyaviridae family. In addition to electrophoretic analysis, RVFV has been examined utilizing polyacrylamide gel isoelectrofocusing, both with direct detergent disrupted virus and following SDS-PAGE analysis. Studies of RVFV RNA using 2.4% PAGE and sucrose gradient centrifugation have demonstrated the presence of 3 distinct RNA species of 0.6, 1.7 and 2.6×10^6 MW. These values are consistent with members of the Bunyaviridae family which have been shown to contain a segmented genome with small, medium and large RNA. In addition to protein and RNA characterization studies, a method for analysis of intact RVFV isolates utilizing a granular gel isoelectrofocusing technique will be discussed.

Title: THE ARBOVIRUSES OF INDIANA: A STATEWIDE SEROLOGICAL SURVEY OF 10,000 HOOSIERS FOR EEE, WEE, SLE, AND CE GROUP ANTIBODIES.

Authors: Paul R. Grimstad, Charles L. Barrett, Michael J. Sinsko, and George B. Craig, Jr.
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During the past year a screening program has been conducted to determine the antibody prevalence rates in humans in Indiana for CE group, EEE, SLE, and WEE arboviruses. Samples have been obtained from residents of virtually all of Indiana's 92 counties through the cooperation of Board Pathologists with informed consent of patients and donors by the Indiana State Board of Health. The testing was conducted by Notre Dame's Laboratory for Arbovirus Research and Surveillance.

A total of 9670 human serum samples (with 350+ to come through the month of May and early June) were screened at 1:2 in tissue culture microneutralization tests. Seropositive samples were titered in serum dilution microneutralization tests. California encephalitis group seropositive samples were further tested against La Crosse, trivittatus, and Jamestown Canyon viruses.

Antibody prevalence rates for CE group agents county by county ranged from less than 1% to over 15%; rates for SLE ranged from less than 1% to over 10%. Both groups were found over the entire state in essentially every county sampled.

The results are significant for they demonstrate the widespread problem this area of the United States has with these viruses. La Crosse, Jamestown Canyon, and trivittatus are all maintained, presumably, locally overwintering via transovarial transmission. The widespread occurrence of SLE antibodies cannot be adequately explained on the basis of the epidemics on record in Indiana, particularily 1975. The suggestion of recent evidence for the widespread and continued overwintering of SLE in this area of the United States is supported by our antibody prevalence rate data suggestive of widespread silent endemicity.

Testing of samples for EEE and WEE antibodies are not complete at this time but will be complete by early September. Since EEE has occurred only very rarely in Indiana, primarily in the Northwest corner of the state, and WEE is likewise relatively rare, we expect antibody prevalence rates to be much lower than observed for CE group and SLE viruses.

LA CROSSE X SNOWSHOE HARE REASSORTANT VIRUS INFECTIONS IN CHIPMUNKS AND SNOWSHOE HARES

Charles Seymour and Thomas M. Yuill
(Abstract for 1979 ASTMH Meeting)

Viable genetically defined reassortants have been isolated from mixed infections of snowshoe hare (SSH) and La Crosse (LAC) viruses. We will infect chipmunks (*Tamias striatus*) and snowshoe hares (*Lepus americanus*), the natural hosts of LAC and SSH viruses respectively, with three reassortant strains and the two progenitor viruses. This experiment is designed to answer two questions:

(1) Are the reassortants strains more pathogenic for the natural hosts than are the progenitor viruses?

(2) Are the viremia profiles and median infectious doses of the reassortants similar to those of either progenitor?

To study viremia profiles, animals will be bled daily for a week after subcutaneous inoculation of 100 plaque-forming units; their blood will be titrated for virus content in BHK-21 cells and in suckling mice. To determine median infectious doses, animals will be inoculated with serial ten-fold virus dilutions of the reassortant and progenitor strains; presence of antibody in sera taken three weeks after infection will be evidence of infection. These two procedures, together with gross and microscopic examination of sick or dead animals, will enable us to evaluate virulence.

Studies on the Development of a Live, Attenuated Chikungunya Virus Vaccine

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Chikungunya virus strain Number 15561, previously used to prepare a formalin-inactivated vaccine, has been investigated for possible use in the development of a live, attenuated vaccine for human use. During the course of eleven serial passages in primary African green monkey kidney (PGMK) cell culture, some evidence was accumulated suggesting that the virus had become less virulent for suckling mice and rhesus monkeys. For example, early passages of PGMK-grown virus exhibited 10^3 - fold or greater lethality for suckling mice inoculated via the intra-cerebral route, in comparison to a population derived from the eleventh PGMK-passaged material. With regard to rhesus monkeys, using similar virus preparations, those animals given the higher passaged virus exhibited markedly reduced viremias and normal WBC levels throughout the five day post-inoculation study period. In contrast, monkeys receiving low passage virus had viremias of two to four days duration that reached a maximum level of ca 10^4 pfu per ml; moreover, these latter animals showed significant decreases in WBC levels, not unlike those observed in monkeys receiving wild Chikungunya virus. Using virus grown in MRC-5 human diploid cell culture from the pass eleven virus described above, direct plaquing temperature sensitivity (ts) studies have been conducted. Although this pass 12 virus appears to be a mixed population in terms of ts and plaque size, some clones have been derived which will not plaque at the non-permissive temperatures of 39.3°C or greater. The significance of the observed biological markers, such as plaque size, ts, mouse virulence, monkey virulence and genetic stability in terms of the safety of selected clones for human use will be discussed.

ABSTRACT

Winter Survival and Ovarian Development of *Culex pipiens*

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Comparisons were made between five groups of *Culex pipiens* with different physiologic histories to test their ability to successfully overwinter under field conditions. On 14 December 1978, each group of mosquitoes was marked with a distinctive fluorescent dust and released inside an abandoned ammunition bunker at Fort Washington, Maryland. To insure that dead mosquitoes could be dissected and information obtained on their ovarian development, a sample of females from each physiologic group was also released into a plexiglas cage that was attached to an inside wall of the room. The physiologic histories of each mosquito group were as follows: 1) "wild caught", those which had entered the bunkers prior to the release date, 2) "lab-reared diapausing non-bloodfed", 3) "lab-reared diapausing bloodfed", 4) "lab-reared non-diapausing bloodfed" and 5) "lab-reared non-diapausing non-bloodfed".

Within the first week after release the numbers of marked mosquitoes in the room had decreased from 20% to 45% of their original numbers, After the initial sharp decline in populations, the numbers of mosquitoes in each group continued to decrease throughout the sampling period, but at a much slower rate. At least part of these differences can be attributed to emigration from and predation within the room environment.

On 8 March 1979 those females remaining in the room and cage were removed and counted. Those mosquitoes which had a known history of bloodfeeding were dissected to determine parity. The number of females remaining alive within the room varied from 0%, for both "lab-reared non-diapausing bloodfed" and "lab-reared non-diapausing non-bloodfed" groups, to a range of 14-24% for the "wild caught", "lab-reared diapausing bloodfed" and lab-reared diapausing non-bloodfed" groups. The survival of the mosquitoes within the cage ranged from 0-2% for the "lab-reared non-diapausing", "bloodfed" and "non-bloodfed" groups and was 7% for the "lab-reared diapausing bloodfed" group; which developed eggs from their bloodmeal. The percent survival in the "lab-reared diapausing bloodfed" group was 57% compared to 62% and 52% for the "wild caught" and "lab-reared diapausing non-bloodfed" groups respectively. These data support the theory that a significant number of *Cx. pipiens* which have taken a prehibernation bloodmeal do not develop eggs and can survive the winter.

ABSTRACT

Identify of Crimean hemorrhagic fever-Congo (CHF-C) virus strains by neutralizing antibody assays

by

G.H. Tignor, A.L. Smith, C.D. Ezeokoli, J. Okoli and J. Casals

We describe a practical and generally applicable test for quantitative assay of neutralizing antibodies for Crimean hemorrhagic fever-Congo virus (CHF-C), based on the use of CER cell cultures and enumeration of fluorescent foci of infection. Thermostability of the virus was markedly pH dependent. Neutralizing antibody titers derived from the fluorescent foci inhibition test (FFIT) with 90% focus reduction are closely correlated with titers obtained from inoculation of mice with a 50% endpoint. Antibody titers from reciprocal FFIT with four strains of CHF-C from widely separated geographic regions suggested that minor antigenic differences existed among the strains tested. However, statistically significant antigenic differences were not detected between strains of this virus using the kinetic or timed neutralization of fluorescent foci of infection test with one-inoculation immune sera. This fact confirms the identity of CHF-C strains as a single antigenic entity but does not contravene a biological difference among strains which could be detected by other laboratory techniques. Nevertheless, in its antigenic uniformity over the whole range of its wide distribution as measured by the kinetic neutralization test, Crimean hemorrhagic fever-Congo virus apparently differs from other arboviruses with similar or even less distribution.

REPORT FROM THE ARBOVIRUS DEPARTMENT
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MOSCOW, USSR

Passive hemolysis-in-gel technique was developed by G.C. Schild et al. for the influenza virus. Later it was reproduced with rubella, para-influenza, mumps and coronaviruses. There were no reports of its application to arboviruses.

Attempts to reproduce HIG with arboviruses in accordance with the original technique or the modifications applied to other viruses failed. Therefore we modified the conditions of sensitization of erythrocytes, with regard to the ability of arboviruses to agglutinate goose erythrocytes in strictly defined pH zones. The desirable pH was achieved by mixing borate buffer pH 9.0 and acid phosphate buffers, prepared in the same way as for HA. Besides rooster erythrocytes, employed according to G.C.Schild, we tried the most sensitive for arboviruses, goose and some other erythrocytes, which also can be agglutinated by certain arboviruses. As is seen from the table, the results were positive with goose and sheep erythrocytes, the reaction being more distinct with the latter. Hamster erythrocytes gave indistinct reaction with Chikungunya virus, mouse and human erythrocytes gave non-specific hemolysis, and guinea pig ones proved to be insensitive.

The specificity of the reaction was studied in experiments with heterologous IAFs. Each IAF was tested with every antigen. Alphavirus antigens had no cross-reactions either between themselves or with Flaviviruses. Flavivirus antigens did not react with Alphavirus IAFs, but there were cross-reactions between dengue and West Nile viruses. Cross-reactions between these viruses are matter-of-course. The time of storage of antigens did not influence the results of HIG. The experiment with Chikungunya virus shows, that the BPL-inactivated lyophilized antigen kept for 5 years and the fresh non-activated antigen react equally well.

The titration of IAF with homologous antigen in HIG showed correlation with the titre in HI. The diameter of the hemolysis ring decreased by 1-2 mm with each dilution. The reaction was considered positive if the diameter of the hemolysis ring was not less than 4 mm.

Positive results of investigation of convalescent sera after dengue infection suggest the possibility of HIG application for serologic diagnosis.

(S.Ya.Gaidamovich, E.E.Melnikova)

TABLE

HIG with erythrocytes of different animals

Generae	Virus	Time of preservation	Titre in HA	Erythrocytes: rooster	goose	sheep	guinea pig	hamster	mouse	human O gr.
ALPHA-VIRUS	Chikungunya	Dry, inactivated, 6 years	1:640	slight	clear	very clear	negative	negative	negative	negative
	Sindbis	Liquid, infectious, 5 weeks	1:2560	slight	clear	very clear	- "	- "	- "	- "
	VEE	Dry, inactivated, 7 months	1:2560	slight	clear	very clear	- "	- "	- "	- "
FLAVI-VIRUS	JE	Liquid, infectious, 2 months	1:1024	slight	clear	very clear	- "	- "	- "	- "
	TBE	Liquid, inactivated, 2 months	1:160	-	clear	very clear	- "	- "	- "	- "
	WN	Liquid, infectious, 2 months	1:320	slight	clear	very clear	- "	- "	- "	- "
	dengue 1	Liquid, infectious, 2 months	1:1024	slight	clear	very clear	negative	clear	negative	negative

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

Comparative studies on West Nile virus strains isolated in Slovakia

This investigation was based on results indicating the presence of West Nile virus in Central Europe as resulted from serological surveys in Slovakia and in Austria as well as from isolations of West Nile virus from mosquitoes and birds in Slovakia.

In our experiments two virus strains isolated in Slovakia were used: a strain isolated from Aedes cantans mosquitoes, designated as K99, in the 8th mouse passage and a strain isolated from the blood of Tringa ochropus, designated as E13, in the 8th mouse passage. As prototype West Nile virus strains were employed the African strain Eg 101 kindly provided by the Rockeffeller Foundation Virus Laboratory, New York, USA, and the Indian strain IG 2266 obtained by the courtesy of Professor S.Ya. Gaidamovich from the Ivanovskii Institute of Virology, Moscow, USSR.

The virus growth and the kinetics of production of haemagglutinin and complement-fixing antigen were followed in PS cell cultures. The PS cells were cultivated in the Eagle's basal medium enriched by 10 % inactivated calf serum, except experiments determining haemagglutinating and complement-fixing activities of the virus grown, when no serum to the medium was added.

When comparing in PS cells the virus growth, the expression of cytophatic effect and the kinetics of haemagglutinin and complement-fixing antigen production of three West Nile virus strains /K 99, E 13 and Eg 101/, no substantial difference in their growth characteristics was observed. All 3 strains under study caused a chronic infection of PS cells, strain Eg 101 being detectable from the 1 st to the 10th, strain K 99 from the 1st to the 12th and strain E 13 from the 1st to the 11th day post-infection.

Differences were found, however, in the production of haemagglutinin and complement-fixing antigen /Table 1/. The growth in PS cells of newly isolated West Nile virus strains K 99 and E 13 was accompanied by high titres of hemagglutinin /from 32 to 64/ on days 1 through 4 post-infection, hemagglutinin being detectable up to the 8th of infection. On the other hand, in PS cells infected with the African strain Eg 101 the haemagglutinin was not detected on day 1, but only on days 2 through 5 post-

infection. The complement-fixing antigen was detected from the 1st to the 9th day following infection with the African Eg 101 strain, but from the 1st to the 7th day and from the 1st to the 4th day, respectively, after infection with K 99 and E 13 strain. Hence, the African strain Eg 101 displayed the shortest production /4 days/ of haemagglutinin and the longest production /9 days/ of complement-fixing antigen. By contrast, infection of PS cells with newly isolated strain E 13 was accompanied by longer production of haemagglutinin /9 days/, whereas no production of complement-fixing antigen was detected from days 5 post-infection.

Investigation of pathogenicity for suckling white mice of 3 strains of West Nile virus /Eg 101, K99 and E 13/ did not reveal any significant differences following the intracerebral, intraperitoneal and subcutaneous inoculation. As similar as the strains under study did not exhibit any difference in the pathogenicity for Syrian hamsters /weighing 100 g/ inoculated intracerebrally, intraperitoneally and subcutaneously.

Comparison of haemagglutinating activity of these West Nile virus strains revealed that optimum for haemagglutinating activity of the African Eg 101 strain and the newly isolated strain K 99 was at pH 6.8 while for the strain E 13 it was from pH 6.0 to 7.0.

Later on we compared the newly isolated West Nile virus strains with the African strain Eg 101 and the Indian strain IG 2266 in HIT and by the kinetics of HIT.

As shown in Table 2, antigen of IG 2266 strain reacted in a high titre /1280/ with the homologous serum, but in low titres /160 and 320, respectively/ with heterologous sera /Eg 101, K 99 and E 13/. Control titrations of individual sera /Eg 101, K 99 and E 13/ showed high titres with homologous antigens. Therefore, results of HIT indicate the antigenic relatedness of newly isolated strains of West Nile virus to the African strain, but the antigenic diversity from the Indian strain.

These antigenic relations were confirmed by results of the kinetics of HIT /Table 3/. When incubated for 30 min. at 4°C, the monospecific mouse serum Eg 101 diluted 1:320 reacted best with homologous antigen and did not react at all with antigen of the Indian strain IG 2266. The immune serum K 99 diluted 1:640 reacted best with homologous antigen and with antigen of Eg 101 strain, fair with antigen of E 13 strain and did not react with antigen of the Indian strain. The immune serum E 13 diluted 1:640 reacted best with homologous antigen and with antigen of Eg 101 strain, less with antigen of K 99 strain and did not react again with antigen of the Indian strain. Similar results were obtained when incubating sera with antigens for 2 hours. The Indian strain

IG 2266 could be also distinguished from the African strain Eg 101 and from the newly isolated strains K 99 and E 13, when the incubation was prolonged up to 18 hours and dilutions of 1:1280 of sera were used.

As follows from results of our experiments, strains K 99 and E 13 of West Nile virus isolated in Slovakia can be distinguished from the Indian strain IG 2266 of the virus by the kinetics of HIT, Results indicate that newly isolated West Nile virus strain are related to the African strain Eg 101. Similar results were obtained by Soviet authors, who found that West Nile virus strains isolated in the territory of USSR /Astrachan region, Azerbaidjan SSR/ differed from an Indian strain of West Nile virus in serological tests.

We did not notice any difference between some biological properties /the growth characteristics in PS cells, the pathogenicity for white mice and Syrian hamsters/ of newly isolated strains and the African Eg 101 strain of West Nile virus.

Some differences between newly isolated strains and the African Eg 101 strain of West Nile virus were determined by the kinetics of production of haemagglutinin and complement-fixing antigen, when growing the virus strains under study in PS cells.

Batíková, M., Grešíková, M., Sekeyová, M. Presented at International Symposium New aspects in Ecology of Arboviruses, Smolenice, June 11-15, 1979.

Tab.1

Complement-fixation and haemagglutination tests with antigens of WN virus strains grown in pig embryo kidney cell cultures.

days post inoculation	Eg 101		K 99		E 13	
	CF	HA	CF	HA	CF	HA
1st	8/16	0	16/16	64	8/8	32
2nd	16/8	16	16/16	64	16/16	64
3rd	8/8	32	8/8	16	16/32	32
4th	16/32	32	16/16	16	16/16	64
5th	8/8	4	16/4	8	0	8
7th	8/4	0	8/4	0	0	8
9th	8/4	0	0	0	0	4
11th	0	0	0	0	0	0
13th	0	0	0	0	0	0

CF = complement-fixation test with ascitic fluid of mice immunized with E 13 virus strain; numbers indicate reciprocal of serum titre/reciprocal of antigen titre.

HA = haemagglutination test; numbers indicate titres of virus antigen

Tab. 2

Antigenic relationships between WN virus strains in HI test

Immune serum	HI titre with homologous antigens	HI titre with IG 2266 antigens
IG 2266	1280	1280
Eg 101	1280	160
K 99	5120	320
E 13	5120	320

Tab. 3

Comparison of West Nile virus strains by the kinetics of haemagglutination-inhibition test.

Serum dilution	Time of exposure	HI titre with Egypts antiserum x				HI titre with K 99 antiserum				HI titre with E 13 antiserum			
		antigens				antigens				antigens			
		WN/E/	K99	E13	WN/I/	WN/E/	K99	E 13	WN/I/	WN/E/	K99	E 13	WN/I/
40		32	32	32	-	32	32	32	-	32	32	32	-
80		8	16	16	8	32	32	32	16	32	32	32	16
160	30 min.	8	8	8	2	32	32	32	8	32	4	16	8
320		4	2	2	0	8	16	4	2	16	2	8	2
640		0	0	0	0	4	4	2	0	8	2	8	0
1280		0	0	0	0	2	2	0	0	2	0	0	0
40		32	32	32	-	32	32	32	-	32	32	32	-
80		16	16	16	16	32	32	32	16	32	32	32	16
160	2 hrs	8	8	8	8	32	32	32	16	32	8	32	8
320		4	4	4	2	16	16	16	8	32	4	16	2
640		4	0	2	0	8	4	4	2	16	2	8	0
1280		0	0	0	0	2	4	2	0	4	0	4	0
40		32	32	32	-	32	32	32	-	32	32	32	-
80		32	32	32	16	32	32	32	32	32	32	32	32
160	18 hrs	16	16	16	8	32	32	32	16	32	32	32	16
320		8	8	16	8	32	16	32	8	32	4	32	8
640		4	8	8	2	8	16	16	8	32	2	16	8
1280		2	2	8	0	4	8	4	2	16	2	4	0

x = units of antigens inhibited by antibody

REPORT FROM THE DEPARTMENT OF VIROLOGY, VACCINATION CENTER (LANDESIMPFANSTALT), AUF'M HENNEKAMP 50, D-4000 DUESSELDORF, WEST GERMANY

At the beginning of arbovirological work in the institute 15 arbovirus strains have been propagated in the brain of baby mice in order to get a stock of freeze-dried virus suspensions for further diagnostic and scientific work. The next step was the preparation of acetone-treated antigens and of mouse ascitic fluids after different virus inoculations. A large quantity of slides has been prepared with acetone-fixed spots of virus infected cell suspensions for the indirect fluorescence antibody test. About 400 human sera from southern Yugoslavia are now being checked for arbovirus antibodies mainly by the indirect fluorescence antibody test. The scientific projects mentioned in No.36 of this Information Exchange are going on. The institute will be incorporated into the Medical Institute of Environmental Hygiene (Director: Professor Dr. SCHLIPKÖTER) after January 1st, 1980.

(Jürgen Pilaski)

REPORT FROM THE SIR WILLIAM DUNN
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The use of "macrophage-like" continuous cell lines to demonstrate enhancement of virus replication by specific antibody.

In the course of their investigations into the pathogenesis of dengue haemorrhagic fever, Halstead et al (1,2), observed that yields from dengue virus infected peripheral blood leukocytes of human or rhesus monkey origin could be enhanced up to one hundredfold by the addition of subneutralizing concentrations of anti-dengue antibodies. On the basis of their adherance to glass and susceptibility to treatment with colloidal silica, they suggested that the permissive cell subpopulation in the peripheral blood leukocytes was made up of cells of the mononuclear phagocytic series.

One problem with these studies has been the variability inherent in the use of peripheral blood leukocytes. We have investigated the possible use of cell lines having "macrophage-like" properties, including two cell lines of mouse origin, $P388D_1$ (3), and J774 (4), and one of human origin, U937 (5).None of these cells is permissive to dengue virus replication, either with or without the addition of "enhancing" antibody, but by making use ofanother flavivirus, West Nile virus, we have been able to reproduce the antibody mediated enhancement phenomenon. When $P388D_1$ cells were infected with West Nile virus, and virus yields assayed in pig kidney (PS) cells by a plaque method, we found that a rabbit anti-West Nile antiserum having a 50% plaque reduction neutralization titre of 1:64, would increase virus yields one hundred fold when a 1:1000 dilution of antiserum was added to the cell-virus mixture. Furthermore, the same "enhancement" could be demonstrated directly in $P388D_1$ cells by counting West Nile induced plaques which appeared in the presence of different concentrations of West Nile antiserum. At high concentrations of antibody (1:10), neutralization of plaque formation occurred; at lower concentrations of antibody (1:1000 - 1:10,000), a 30-40 fold increase in plaque numbers was observed as compared with controls.

Antisera against all flaviviruses which have been examined are capable of producing enhancement of West Nile virus when added at appropriate concentrations, and it seems that this assay, which has been adapted to Linbro plates (Flow Laboratories), is a highly sensitive and reproducible assay for the detection of antibodies directed against group-specific flavivirus antigens, in addition to being a good model system for the study of "antibody mediated enhancement of virus replication".

1. Halstead S.B. & O'Rourke E.J. J. Exp. Med. 146, 201-217.
2. Halstead S.B., O'Rourke E.J. & Allison A.C. J. Exp. Med. 146, 218-229.
3. Koren H.S. Hardwerger B.S. & Wunderlich J.R. J. Immunol. 114, 894-897.
4. Ralph P. Prichard J. & Cohen M.J. J. Immunol. 114, 898-905.
5. Sundstrom C. & Nilsson K. Int. J. Cancer 17, 565-577.

(J.S.M. Peiris and J.S. Porterfield)

REPORT FROM THE MICROBIOLOGICAL RESEARCH ESTABLISHMENT
PORTON DOWN, SALISBURY, WILTS, UK

The specification and differentiation of strains of virus, which may range from unmodified field-strains to candidate vaccines, depends upon the interpretation of the details of the processes in the animal host of infection, replication, stimulation and regulation. As an approach to this requirement, we have used athymic nude mice and their sex matched litter mates in an assessment of the T-lymphocyte dependence of antibody-stimulation, regulatory immunity (expression of virulence) and protective immunity against challenge.

The required mice at 35 to 65 days old were infected i.p. with 10^4 to 10^6 infective units (p.f.u. or SM i.c. LD50) of distinct 'avirulent' strains of Semliki Forest virus (A774.C3), Venezuelan equine encephalomyelitis virus (TC83.C1 and MUCAMBO) or Yellow Fever virus (17D; ASIBI:1927; SUAREZ, COLOMBIA:1936). After observation of clinical signs and regular bleeding for antibody assay for 21 to 30 days, the mice were challenged i.p. (SFV and VEEV) or i.c. (YFV) with 10^3 to 10^4 infective units of homologous 'virulent' strains of virus for assessment of protective immunity. These procedures and virus strains have been described (BRADISH, FITZGEORGE, TITMUSS and BASKERVILLE, 1979; WALDER and BRADISH, 1979; BRADISH et al in INFORMATION EXCHANGE No 36 of March 1979).

In any comparisons with the responses to avirulent infections of normal adult mice, the severity of clinical signs or incidence of deaths (D) following primary infection indicates the poor-stimulation or impairment of regulatory immunity with the consequent enhancement of virulence. Similarly, the reduced incidence of survivors following the secondary challenge infection and its antigenic boost indicates the impairment of protective immunity (P) which renders the majority of mice in the test group susceptible (S) to the secondary lethal challenge. Furthermore, since individual mice may show high (+) or undetectably low (-) levels of neutralizing activity in serum before challenge, it follows that host-responses may be classified finally as D, +P, -P, +S or -S. These are the five categories of host-group responses shown in Table 1 for the various conditions of infection or immunization. Mixed responses of intermediate type are shown by D/S and P/S.

The above assessments of host-responses were made in normal or athymic-nude mice under a variety of conditions of active or adoptive immunization. The spleen-cell transfers from normal donors to sex matched litter mates were made at the level of one spleen (1 to 4 x 10^8 cells) at up to 4 days before the primary infections. Spleen-cell transfers from immunized donors were made 2 to 3 weeks after the indicated primary, immunizing infections. The results summarized in Table 1 show that athymic mice maintained regulatory immunity and so survived primary infection by all virus strains except D2.M1 (YFV) and MUCAMBO.C1 (VEEV): for these strains regulatory immunity was not restored by prior transfer of normal spleen cells. All other virus strains tested stimulated regulatory immunity in the athymic mouse but nevertheless failed to stimulate normal antibody synthesis or normal protective immunity. These impairments of antibody synthesis and protective immunity were restored by prior transfers of either normal or immune spleen cells in the tests with strains of SFV and VEEV, but not in those with strains of YFV.

These direct tests show that, according to virus strain, thymus processed lymphocytes may or may not be effective at three distinct levels of host-response. Thus a gradient of virus strain dependence is matched against a gradient of T-cell competence. This is indicated by the layout of Table 1. The infections of athymic mice by the TC83.C1 vaccine strain of VEEV are the least aggressive in deficient mice and respond most sensitively to reconstitutions that fail in other cases. At the other end of the scale, the strains D2.M1 (YFV) and MUCAMBO.C1 (VEEV) are avirulent and immunogenic only in normal mice. The other strains of SFV and YFV show intermediate features. These effects are probably related to the efficiency of virus information transfer through T-lymphocytes so that the numbers of normal or primed spleen cells effective in reconstitution may be sufficient for restoration of immune functions only if the virus strain involved is adequately immunogenic.

Closer definitions of these critical balances between the pathogenicity and immunogencity of virus strains is likely to require detailed histological/immunological examination at the response-change levels indicated by 1, 2, 3 in Table 1. However, it appears that T-dependent functions may be expressed in sequence at several different phases of the host-response to infection and that, in consequence, a more sensitive statement of virus strain 'virulence' or of host 'susceptibility' may be available. These results will be discussed more fully in a paper to be published in the Journal of General Virology.

References

C J BRADISH, R FITZGEORGE, D TITMUSS & A BASKERVILLE (1979)
Journal of General Virology 42, 535-566

R WALDER & C J BRADISH (1979)
Journal of General Virology 44, 373-382

(C.J. Bradish, R. Fitzgeorge and D. Titmuss)

TABLE 1: THE RESPONSES OF NORMAL AND ATHYMIC MICE IN ACTIVE AND ADOPTIVE IMMUNIZATION

HOST AND PRIOR TREATMENT	STRAIN OF VIRUS USED AS PRIMARY INFECTING AND IMMUNIZING AGENT			
	VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS: STRAIN TC83.C1	SEMLIKI FOREST VIRUS STRAIN A774.C3	YELLOW FEVER VIRUS STRAINS 17D.C1, ASIBI, SUAREZ	VEEV STRAIN MUCAMBO.C1 AND YFV STRAIN D2.m1
i. NORMAL MICE (HETEROZYGOUS LITTER MATES OF ATHYMIC-NUDE MICE)	+P	+P	+P ② ③	+P ①
ii. ATHYMIC MICE AFTER TRANSFER OF SPLEEN CELLS FROM NORMAL MICE	+P	+P	±S	D
iii. NORMAL MICE AFTER TRANSFER OF SPLEEN CELLS FROM IMMUNIZED MICE (ADOPTIVE IMMUNITY)	+P ③	+P ② ③	-S	
iv. NORMAL MICE AFTER TRANSFER OF SPLEEN CELLS FROM IMMUNIZED ATHYMIC MICE (DONOR RESTRICTED ADOPTIVE IMMUNITY)	+S	-P/S	-S	
v. ATHYMIC MICE AFTER TRANSFER OF SPLEEN CELLS FROM IMMUNIZED NORMAL MICE (RECIPIENT RESTRICTED ADOPTIVE IMMUNITY)	②	①		
vi. ATHYMIC-NUDE MICE	-P/S	-S/D	-S	D

1 Deletion of regulatory immunity
2 Deletion of competence in serum antibody synthesis
3 Deletion of protective immunity

REPORT FROM THE MEDICAL RESEARCH COUNCIL VIROLOGY UNIT
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ecombination in the Bunyamwera Complex

Gentsch and Bishop (J. Virol. 20, 351-354, 1976) have shown that recombination can occur by reassortment of genome segments in cultured cells infected with related bunyaviruses belonging to the California Encephalitis Complex.

We are investigating three members of the Bunyamwera Complex of the Bunyaviridae Family, which exhibit different degrees of serological relatedness, to determine whether viral genes can be freely exchanged between viruses in this taxonomic grouping. For this purpose we have isolated temperature-sensitive (ts) mutants from mutagenised stock of each virus. The three viruses are Batai virus, Bunyamwera virus and Maguari virus, all of which were obtained from Dr. N. Karabatsos, Vector-borne Diseases Division, Center for Disease Control, Fort Collins, Colorado, and subsequently cloned and propagated in BS-C-1 cells. The mutants of each virus could be classified into the two recombination groups listed in Table 1.

Table 1. Number of ts mutants isolated and their classification into recombination groups.

VIRUS	GROUP I	GROUP II	TOTAL
Batai	1	4	5
Bunyamwera	5	3	8
Maguari	14	31	45

Table 2 shows that recombination, assayed as the frequency of non-temperature-sensitive virus produced in mixed infection, was obtained in crosses involving all three viruses in pair-wise combinations. In general the recombination frequency was lower in heterologous crosses. Table 2 shows that even in heterologous crosses recombination was restricted to parental combinations of group I and II mutants. Recombination was reciprocal, i.e. group I of Batai virus recombined with group II of Bunyamwera virus, and group II of Batai virus with group I of Bunyamwera virus, etc.

Each of these three viruses can be distinguished by differences in the electrophoretic mobilities of their Gl and N polypeptides in polyacrylamide gel. In crosses of mutants of Bunyamwera and Maguari viruses we have confirmed that the non-ts clones isolated from mixed infections are indeed recombinant from the electrophoretic profiles of their polypeptides. This analysis will be published in detail shortly.

We conclude that different members of the Bunyamwera Complex can hybridise, in cultured cells at least, and that two of the three genome segments can be exchanged. Therefore barriers to the hybridisation of these viruses in nature are likely to be geographical or ecological, rather than genetical.

C. IROEGBU
C.R. PRINGLE

Table 2. Homologous and heterologous recombination in the Bunyamwera Complex**

Recombination Frequency*	Batai		Bunyamwera		Maguari	
	Gp I	Gp II	Gp I	Gp II	Gp I	Gp II
Batai Gp I	0.07	54.5	(0.002)	53.6	0.006	0.2
Gp II		0.05	6.5	0.05	0.3	0.0002
Bunyamwera Gp I			0.003	(49.4)	0.05	0.66
Gp II				0.005	0.79	0.003
Maguari Gp I					0.03	27.1
Gp II						0.001

*The recombination frequency =

$$\frac{(AB)^{38} - (A + B)^{38}}{(AB)^{31}} \times 2 \times 100,$$ where AB, A and B are the yields at 31°C, and the superscript is the temperature of assay.

**Batai virus was represented by ts B54 (gp I) and ts B7 (gp II); Bunyamwera virus by ts Bun 137 (gp I) and ts Bun 67 (gp II); Maguari virus by ts M 1370 (gp I) and ts M 290 (gp II), except for the two bracketted combinations in which ts Bun 61 represented group I of Bunyamwera virus.

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

Newly identified viruses

Information is available on a miscellaneous group of recent and not so recent virus isolates from Southern Africa. AR 9216, originally isolated in 1967, has been obtained on several occasions from Culicoides spp. It is a member of the Simbu group, probably identical to Shamonda. Another Culicoides isolate, AR 11022, is a member of the Palyam group, different from other African viruses of this group. Three strains of the same virus, isolated from aborted bovine foetuses, which we received from Onderstepoort Veterinary Laboratories, are flaviviruses, closely-related to Bagaza. These are the first isolations of Bagaza in South Africa and the first intimation, as far as we are aware, that Bagaza is a possible bovine pathogen. Another virus, received from our Rabies Unit, was isolated from the brain of a dog with rabies-like symptoms in Botswana. It is also a flavivirus, close but probably not identical to West Nile. Three isolates, obtained recently from Anopheles and Culex mosquitoes, are also flaviviruses new to South Africa. Preliminary tests indicate a close relationship to Bouboui. All three flaviviruses multiplied in Aedes aegypti and Culex quinquefasciatus after inoculation and the Bouboui-like virus was transmitted to hamsters by bite by both mosquitoes.

Transmission of chikungunya virus

Laboratory transmission tests were undertaken to assess the vector capability of one tick and five mosquito species with CHIK virus. First and second nymphal instars of Ornithodoros savignyi were not infected after feeding on a viraemic rodent circulating 6.6 log. of virus. Aedes furcifer was readily infected after feeding on viraemic monkeys. 25% and 32% of the mosquitoes infected transmitted to monkeys and hamsters respectively. Aedes fulgens was readily infected at 5.7 log. and 3 infected mosquitoes transmitted virus to a Mistromys rodent. Only 26% of Mansonia africana were infected after feeding on a monkey circulating 4.7 log. 29% infected after a feed containing 5.2 log. transmitted to monkeys. The virus failed to infect Culex quinquefasciatus and infected only 1 of 17 Culex horridus. Neither species transmitted virus. Ae. furcifer, Ae. fulgens and Cx. horridus are common species in tree holes in epidemic areas in the savanna. Ae. furcifer has been incriminated as the main vector in baboon and human infections in the area.

(B.M. McIntosh, P.G. Jupp and I. dos Santos)

REPORT FROM ARBOVIRUS LABORATORY, INSTITUT PASTEUR and ORSTOM, OI B.P 490 ABIDJAN OI-IVORY COAST

The Institut Pasteur in Ivory Coast set up the Arbovirus Laboratory in 1972 for yellow fever research and surveillance purposes in West Africa. The Ivory Coast Laboratory works in close connection with the Arbovirus Laboratory in the Institut Pasteur in Dakar, Senegal.

In 1977, a medical entomology laboratory near by the ORSTOM research organisation joined forces with the Institut Pasteur Arbovirus Laboratory. Before this date, all research work on mosquitoes and all trapping of mosquitoes was carried out by ORSTOM'S entomological services working with the OCCGE (Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endemies) establisment in Bobo-Dioulasso, Upper Volta.

This paper reports the results obtained from 1973 to May 1979.

I - SEROLOGICAL SURVEY IN IVORY COAST

Serological examinations were conducted on human serums collected in several geographical zones, special attention being paid to children under 15 years old ; mass yellow fever vaccination was stopped in 1960, but partial campaigns have been conducted on different occasions, particularly in the big urban centres and in the border regions.

The methods used are those of the Institut Pasteur in Dakar (IH, CFT and NT by plaque reduction in PS cells). The flavivirus antigens were as follows : Yellow fever (YF), Zika (ZIK), Uganda S (UgS), Wesselbron (Wes) West Nile (WN) and N'TAYA (NTA).

Other antigens were Chikungunya (CHIK), Sindbis (SIND) and Bunyamwera (BUN). 600 to 2,000 serums from children under 15 years old, in principle not vaccinated, were examined in 17 bio-geographical zones (cf map I).

In IHA, the percentage of carriers of YF antibodies only went from 5,8% (Man, Touba) to 0.3% (Bouaké), and the percentage of yellow fever antibodies associated with other flaviviruses went from 1.6 (Man, Touba) to 22.8 (Odienné).

MAP I

Legend :

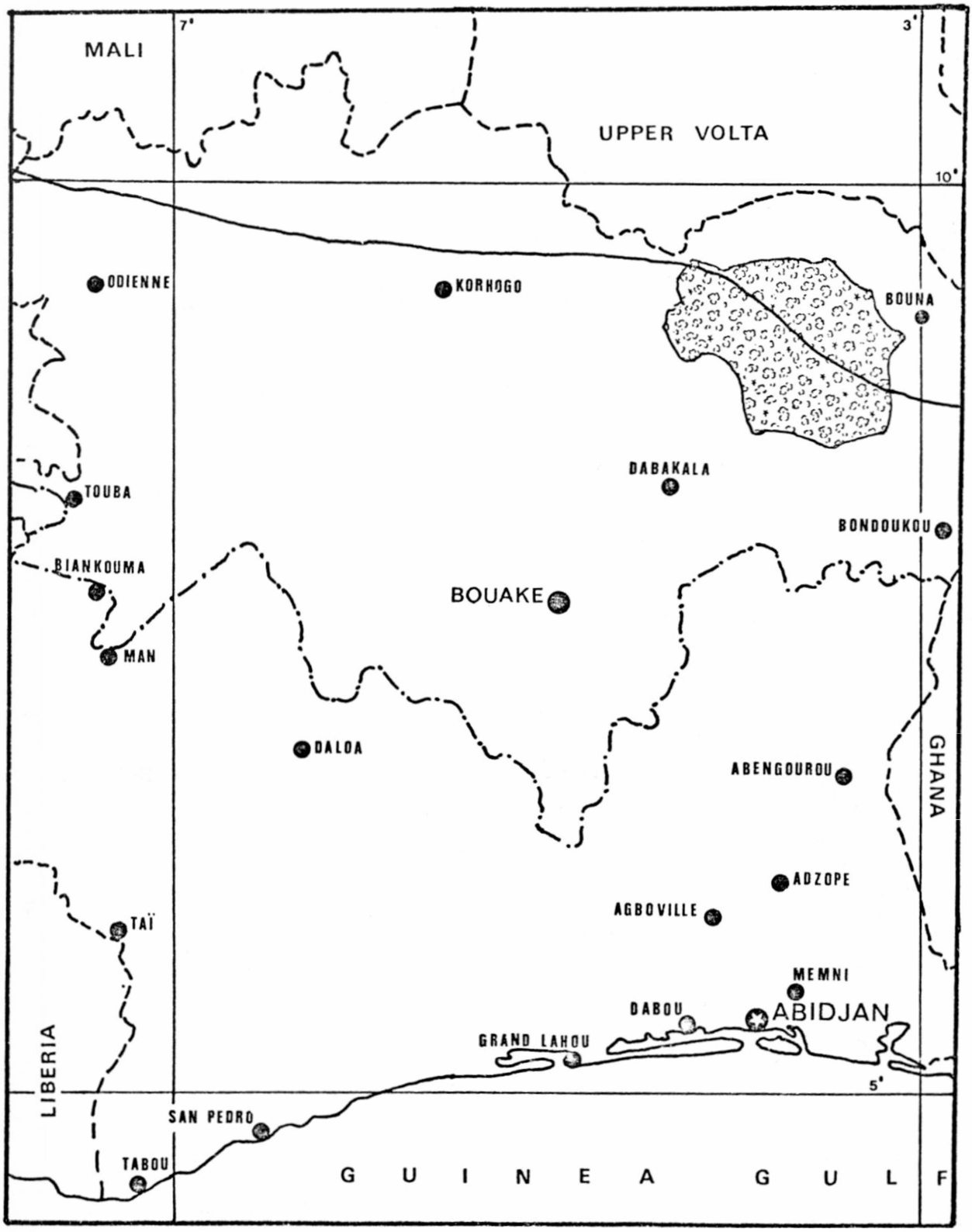
National Park

o-o-o Northern limit of the rain forest

——— Southern limit of the Sudan-type savannah zone

● Places where blood samples were taken.

MAP n°1 IVORY COAST



In the Touba area, Yellow Fever virus was isolated from Aedes africanus in August 1973, and a vertical survey was carried out in several villages where Complement Fixing antibodies (≥ 64) were found on several occasions. 60 to 98% of the children were receptive to yellow fever and, taking into consideration the possibilities of contact between man and vector and the ecological data, the zones at risk where surveillance will be more strictly persued were defined. These zones are mainly rural savannah areas immediately to the north of the forest belt (Dabakala, Touba, Korhogo)

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2 - OUTBREAK OF JAUNDICE WITH SUSPECTED YELLOW FEVER ACCOMPANIED BY SEROLOGICAL EVIDENCE OF THIS. (Department of Dabakala, September - December 1977)

More than 100 serious cases of hepatitis accompanied by haemorrage and resulting in more than 15 deaths occurred in the Department of Dabakala between September and December 1977.

This period corresponds to the second part of the rainy season. Ten villages and approximately 15,000 people were involved ; two liver specimens removed during post mortem examination showed undeniable signs of yellow fever hepatitis. On the request of the health authorities, an epidemiological enquiry was conducted in these villages. From the 1,637 mosquitoes, potential vectors of yellow fever, wich were captured in September before the first cases appeared, and the 42 specimens collected in December at the end of the epidemic, no strain was isolated.

The serological results obtained are given in Table I. There were therefore undoubtedly cases of rural yellow fever with a concomitant epidemic of infectious hepatitis. Immediate and massive vaccination of the population, the disappearance of the mosquitoes at the end of the rainy season and the low population density prevented the occurrence of a full-scale epidemic.

TABLE I

YELLOW FEVER SEROLOGICAL DIAGNOSIS DURING JAUNDICE OUTBREAK IN DABAKALA DEPARTMENT. (Sept-Dec 1977)

	N° TESTED	SEROLOGICAL CASES OF YELLOW FEVER	POSSIBLE CASES of YELLOW FEVER	PROBABLY NOT YELLOW FEVER	DEFINITELY NOT YELLOW FEVER
SERUM PAIRS FROM PATIENTS	29	4	4	-	21
SERA TAKEN FROM PEOPLE IN CONTACT WITH PATIENTS	402	8	10	6	378
SERA TAKEN FROM SCHOOL CHILDREN (in the same villages)	49	2	8	3	36
SERA TAKEN FROM YOUNG SOLDIERS SERVING IN THE REGION	52	3	4	4	41
TOTAL	532	17	26	13	476

3 - VIRUSES ISOLATED IN IVORY COAST

3-I - MOSQUITOES :

I973 -	potential YF vectors	: I08	pools,	I,I70	specimens,	I strain
	other mosquitoes	: 82	" ,	I,695	-"-	0 -"-
I975 -	potential YF vectors	: 224	" ,	7,009	-"-	6 -"-
	other mosquitoes	: 83	" ,	I,I92	-"-	2 -"-
I976 -	potential YF vectors	: I52	" ,	I,484	-"-	0 -"-
	other mosquitoes	: I80	" ,	I,029	-"-	0 -"-
I977 -	potential YF vectors	: 432	" ,	6,I55	-"-	0 -"-
	other mosquitoes	: 252	" ,	I,056	-"-	0 -"-
I978 -	potential YF vectors	: 233	" ,	I,990	-"-	I -"-
	other mosquitoes	: 8	" ,	5I	-"-	0

The strains were as follows

I973 - Yellow fever (27 *Aedes africanus* collected in the Touba area)

I975 -	3 ZIKA	(*Aedes africanus*)	Touba - Odienné
	I ZIKA	(*Aedes luteocephalus*)	
	I ZIKA	(*Aedes tarsalis*)	
	I CHIK	(*Aedes africanus*)	
	I SHOKWE	(*Anopheles brohieri*)	
	I SHOKWE	(*Aedes gr.tarsalis*)	
I978 -	I UNIDENTIFIED	(*Aedes africanus*)	

3-2 - Other isolations

Uganda S (human febrile syndrome)

SHOKWE (human febrile syndrome)

DAKAR An B 277 (organs of Praomys).

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4 - ARBOVIRUSES ISOLATED FROM MOSQUITOES IN UPPER VOLTA

(August-November 1978) cf Map 2

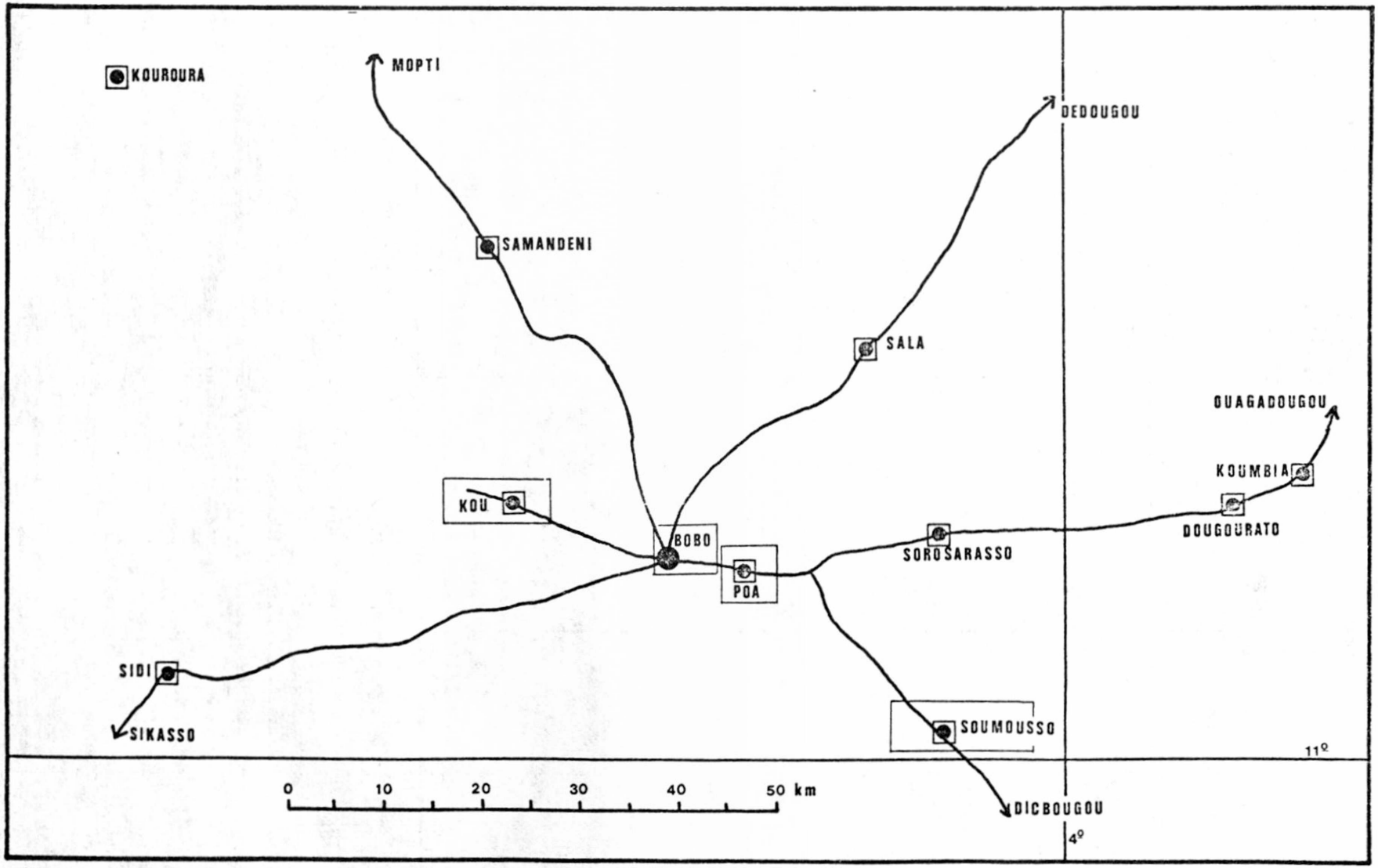
SPECIES	N° TESTED			N° STRAINS ISOLATED		
	pools	Specimens	Collected in	Y.F.	ZIKA	?
Aedes aegypti	5	21	SOUMOUSSO	-	1	-
" "	49	1,123	BOBO-DIOULASSO	-	-	-
" "	1	2	POA FOREST	-	-	-
" africanus	1	9	SOUMOUSSO	-	-	-
" "	5	75	KOU FOREST	-	-	-
" opok	9	172	SOUMOUSSO	-	1	-
" "	2	24	POA FOREST	-	-	-
" luteocephalus	106	3,100	SOUMOUSSO	3	18	7
" "	24	707	KOU FOREST	2	-	-
" "	5	112	POA -"-	-	3	2
" "	4	10	BOBO-DIOULASSO	-	-	-
" vittatus	1	1	SOUMOUSSO	-	-	-
" "	2	9	POA FOREST	-	-	-
" "	8	85	BOBO-DIOULASSO	-	-	-
" gr.furcifer-taylori	10	115	SOUMOUSSO	-	-	-
" "	2	8	POA FOREST	-	-	-
" jamoti	2	6	SOUMOUSSO	-	-	-
" "	4	31	KOU FOREST	-	-	1
Other mosquitoes	24	297	SOUMOUSSO	-	-	-
" "	4	22	KOU FOREST	-	-	-
" "	18	376	BOBO-DIOULASSO	-	-	-
Total	286	6,305		5	23	10

Note: Bobo-Dioulasso is situated in the Sudan-type zone.

The yellow fever strains were isolated during the second part of the rainy season. In 1978, this was below normal, following on several years of exceptionally low rainfall. No suspected human clinical case of yellow fever was found in this zone. The 1969 epidemic occured in the cantons or districts surrounding the capital, Ouagadougou, situated at a distance of 330 kilometres to the north east of Bobo-Dioulasso (cf Map.2)

MAP n° 2 UPPER VOLTA

SITUATION DES LOCALITES DE CAPTURE EN 1978



The five yellow virus strains were all isolated from Aedes luteocephalus captured at two different sites (the forest gallery of the Soumousso, a small tributary of the Volta, and the Kou forest, the last remaining part of the once important Kou forest); these sites are separated from each other by distance of 50 kilometres.

I strain was found in mosquitoes captured at Soumousso on 23 rd August, I strain on 25 th found September and I strain on I6 th. October in the Kou forest, and 2 strains at Soumousso on 25 th October.

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5 - FUTURE INTENTIONS

Routine serological surveys will be made of young children in the high-risk zones. Mosquito capture and the study of vectors and Man/mosquito contacts will be continued and intensified in two regions :

- the Department of Dabakala in the sub-Sudan savannah region, where a permanent research station has just been set up ;
- the forest zone of south-west Ivory Coast (Tai) in connection with the UNESCO "Man and Biosphere" Project (the study of the effects of the increase in human activities on the forest in the south-west part of Ivory Coast).

Mosquitos/monkey contacts will be studied in both these regions. A 24 metres tower for this purpose has already been erected in the Tai forest, and three smaller towers will shortly be erected in two forest galleries in the region of Dabakala.
A study of forest monkeys is also being conducted in Tai (ORSTOM Mammal Laboratory).

A.CHIPPAUX, Institut Pasteur de Côte d'Ivoire OI BP.490 Abidjan OI.
R.CORDELLIER, ORSTOM Adiopodoumé - OI BP. V5I Abidjan OI.
J.P.HERVY, Mission entomologique de l'ORSTOM auprès de l'OCCGE
BP. I7I - BOBO-DIOULASSO (Haute-Volta).

REPORT FROM THE ARBOVIRUS LABORATORY INSTITUT PASTEUR AND ORSTOM

INSTITUT PASTEUR, BP 220, DAKAR, SENEGAL

During the first half of 1979 virological and serological studies were carried out on samples from Senegal (Kedougou, Bandia) and from Gambia.

I - Virological studies

1.Human blood samples

83 human blood samples harvested from febrile children at the clinic of Bandia were inoculated to the suckling mice for virus isolation with negative results.

It was the same thing with 4 samples sent by the hospitals of Dakar.

2.Wild vertebrate samples

213 blood samples have been collected from wild vertebrate in Kedougou country.

From the vertebrates :

- 3 strains of yellow fever were isolated from 44 blood samples of Erythrocebus patas
- 18 blood samples of Cercopithecus were negative.

With this three new isolations of yellow fever virus, this isolation raiser to five the strains of yellow fever isolated during the 1978 rainy season, two strains have been isolated from Cercopithecus in the second half of 1978.

Among the samples from the other vertebrates in the same county, a strain of group B virus, which is not yellow fever was isolated from the blood of a galago. The identification is in progress.

3.Arthropods

a) mosquitoes

Kedougou : 7311 female of mosquitoes in 459 pools and 67 batches of 2910 males were inoculated during the first half of 1979. From the batches of females 35 strains of yellow fever virus were isolated from 876 mosquitoes. Finally the laboratory isolated 53 strains of yellow fever during the rainy season of 1978 (32 from Aedes furcifer-taylori, 19 of Aedes luteocephalus 1 from Aedes neo-africanus, 1 from Aedes vittatus).

It should be observed that the first isolations of yellow fever virus happen later than in 1977 (12 August 1978).

Gambia : During an WHO entomological investigation in Gambia for the particular case of the outbreak of yellow fever 632 mosquitoes were caught (33 pools of female and 9 of 58 males). Two strains of yellow fever virus were isolated from two batches of 20 and 21 females of Aedes aegypti.

b) ticks

After the 5 isolations of Bandia virus from rodents in the second half of 1978, 18 pools of 174 ticks were inoculated. Five strains of the same virus were isolated from Alectorobus sonraî.

II - Serological studies

1.Human sera from Kedougou

During a serological investigation in May 1979, 320 sera of young children have been harvested. These serum spécimens were studied by H.I and C.F tests for group A, group B and Bunyamwera antibodies. In spite of many antibodies at a low titer from yellow fever vaccine, serological pattern of recent infection by yellow fever was found in some villages :

- Village of	Fongolimby	: 2 children
	Tomborocoto	: 1 child
	Saraya	: 1 child
	Bandafassi	: 4 children
	Mako	: 2 children
	Samekouta	: 2 children

more a serological conversion at a high titer 1/2560 in H.I 1/32 in C.F isolated for yellow fever was found in the sera of a young child of 5 years in the village of Tomborocoto. These finding confirm a recent activity of yellow fever virus in the area.

2.Wild vertebrate sera

From january to june of 1979, 50 samples of wild vertebrate sera were tested for H.I, C.F tests antibodies for group A, group B and Bunyamwera viruses.

Several species were concerned : monkeys, galago but also bat, hare, wild boar. Among 25 monkeys 12 have antibodies indicating a rather recent infection by yellow fever virus.

Patas : 5/8 positive sera (62,5%)
Cercopithecus : 3/10 positive sera (30%)
Papio papio : 4/7 positive sera (57%)

The other species were negative.

(J.Renaudet, J.J.Salaun,J.P.Digoutte, Institut Pasteur,
M.Germain and J.L.Camicas, ORSTOM,Dakar,Senegal)

REPORT FROM THE WHO COLLABORATING CENTRE FOR VIRUS REFERENCE AND RESEARCH (INCLUDING ARBOVIRUSES), EGYPTIAN ORGANIZATION FOR BIOLOGICAL AND VACCINE PRODUCTION, AGOUZA-CAIRO, EGYPT

Preliminary Report on the Serological Survey and RVF in Egypt

Preliminary results of the serological survey on 40000 human sera for RVF collected from all over Egypt showed a strong correlation between the human and animal surveys in all provinces. The provinces which were known to have a high incidence of acute cases of RVF showed a high percentage of positive sera of man and animals to RVF. Even within the province the villages which were known to have high incidence of acute cases showed a high percentage of positive human sera to RVF.

Details of the study shall be sent in a later communication.

(Dr. Imam Z.E. Imam and Rifky M. El Karamany)

Susceptibility of CER cell line and primary goat kidney cells to Rift Valley Fever Virus and its uses for antigen and vaccine production

Sharkia strain of RVF was successfully propagated by serial passage in primary goat kidney cell culture which is extremely susceptible to this strain, giving higher infectivity titers in mice. On primary goat kidney tissue culture the titers were about $10^{8.4}$ mouse intracerebral median lethal doses ($MICLD_{50}$). The infectivity titers were lower in chicken fibroblast ($10^{7.3}$) and in hamster kidney cell line ($10^{6.7}$).

Comparative infectivity titers in mice after infecting CER, BHK and Vero cell lines indicated that CER cell culture is highly susceptible to RVF virus giving higher infectivity titer $10^{8.78}$ $MICLD_{50}$/ml which is higher than that of the original strain (H/S/280) Sharkia strain passage 7 in mice to $10^{8.68}$ $MICLD_{50}$/ml. The RVF virus gave low infectivity titer in mice when passed in BHK $10^{7.2}$ $MICLD_{50}$/ml and in Vero $10^{6.4}$ $MICLD_{50}$/ml. RVF virus produced cytopathic effects on CER and primary goat kidney cells.

It was possible to prepare a very good CF antigen on the CER cell line. The antigen is formalin killed, a matter which makes it very safe.

RVF killed vaccine prepared on the CER cell line for immunization of animals proved to be safe and potent.

(Rifky M. El Karamany, Imam Z. Imam, Ahmed H. Farid & Mohammed S. Saber)

REPORT FROM THE PAKISTAN MEDICAL RESEARCH CENTER
UNIVERSITY OF MARYLAND, LAHORE, PAKISTAN

In continuing studies on the genetics of susceptibility of Culex tritaeniorhynchus to infection with West Nile virus, we have attempted to produce mosquito strains with reduced and/or increased susceptibility by selective inbreeding and by treatment with the mutagenic agent ethyl methanesulfonate (EMS). In all of these experiments, the female mosquitoes were infected by feeding on a defibrinated blood-virus suspension through animal skin membranes attached to glass feeding chambers.

Ten different colonies of Cx. tritaeniorhynchus have been selected for increased and/or decreased susceptibility to infection with the Egypt 101 strain of West Nile virus (Table 1). To select for increased susceptibility, females were fed on a 10% infectious dose of virus, and the engorged females were incubated for 6 days at 28±2° C. Progeny were saved as separate family groups from those females that were positive for virus in a direct fluorescent antibody assay or by plaque assay in primary chick embryo cells. When selecting for reduced susceptibility, females were fed on a 90% infectious dose, and progeny were saved from those females that remained virus negative after 10 days incubation. The 10% and 90% virus infectious doses were based on the previously determined susceptibility profile of the Balloki "wild-type" colony of Cx. tritaeniorhynchus. The Balloki stock was also included as a control in every feeding experiment.

For EMS treatment, males from the Balloki or in a few cases, the Tulamba "wild-type" stocks were exposed to a 0.005 M, 0.025 M or 0.05 M concentration of the mutagenic agent in a sugar solution (Sakai and Baker, 1974). The treated males were mated with marker females homozygous for the recessive traits rose eye (re), ebony (e) and abnormal eye (ae). The daughters of this cross were either tested individually for virus susceptibility, or they were backcrossed to re; e; ae males for 1-3 generations before testing as family groups.

Results: None of the strains selected for reduced susceptibility have shown a consistent change when compared to the unselected Balloki control females. However, two strains, Taipei and Changa Manga II, have shown a significant increase in susceptibility (Table 2). After selection of the Balloki stock for reduced susceptibility for 4 generations, three lines homozygous for chromosome 1 and three lines homozygous for chromosome 3 were produced from the inbred males (Sakai and Baker, 1972), and tested for susceptibility to infection with WN virus. Although the number of females tested was small, 5 of the 6 homozygous chromosome strains appeared to be less susceptible than the controls (Table 3).

A total of 29 individual females and 253 family groups derived from EMS treated males were tested for susceptibility to WN virus infection. None of the females or family groups carrying the EMS treated chromosome were significantly less susceptible than the control females in the comparative infectivity assays.

(C. G. Hayes and R. H. Baker)

Table 1. Stocks of Culex tritaeniorhynchus selected for increased and/or reduced susceptibility to infection with West Nile virus.

Stock	Origin	Year	Generations in lab.	No. generations selected for increased susceptibility	reduced susceptibility
Balloki	Pakistan	1968	>100	13	15
Khulna	Bangladesh	1968	>100	--	3
Taipei	Taiwan	(1978)[2]	>100	12	3
Sendai	Japan	(1976)	>100	4	--
Sattoki	Pakistan	1978	10	4	4
Khano-Harni	Pakistan	1978	9	8	8
Changa Manga I	"	1978	6-12	7	1
Changa Manga II	"	1978	5-7	8	3
re;e;ae[1]	"	--	>50	4	2
e;ma[1]	"	--	>50	--	6

[1]Genetic marker stocks maintained in Balloki stock background.
[2]( ) = date colony established at PMRC; original colonization date unknown.

Table 2. Selection of Taipei and Changa Manga II stocks of Culex tritaeniorhynchus for increased susceptibility to infection with WN virus.

Generation	Taipei* infected/total (%)	Control infected/total (%)	Changa Manga II infected/total (%)	Control infected/total (%)
P_1	11/87 (12.6)	2/28 (7.1)	26/78 (33.3)	2/20 (10)
F_1	18/98 (18.4)	6/22 (27.3)	14/64 (21.9)	1/21 (4.8)
F_2	20/70 (28.6)	6/20 (30)	16/31 (51.6)	4/20 (20)
F_3	14/88 (15.9)	2/20 (10)	72/111 (64.9)	3/25 (12)
F_4	21/99 (21.2)	2/20 (10)	27/39 (69.2)	3/20 (15)
F_5	45/82 (54.9)	5/21 (23.8)	23/36 (63.9)	1/15 (6.7)
F_6	50/102 (49)	2/21 (9.5)	70/92 (76.1)	3/26 (11.5)

* Number of females positive for WN virus/ total number of females tested. All females had engorged on a blood-virus dose infectious for about 10% of the unselected Balloki control females and had been incubated at 28±2° C for 7 days.

Table 3. Susceptibility to infection with WN virus of *Culex tritaeniorhynchus* selected stocks homozygous for chromosome 1 or 3.

Stock Family number	Virus dose[1]	No. uninf./total (%)[2]	Control no. uninf./total (%)
1(Chrom 1)	2.63	4/9 (44.4)	0/11 (0)
2(Chrom 3)	2.25	0/10 (0)	5/23 (21.7)
3(Chrom 3)	2.63	7/9 (77.8)	6/32 (18.8)
3(Chrom 1)	2.63	9/14 (64.3)	0/11 (0)
4(Chrom 3)	2.63	8/8 (100)	6/32 (18.8)
5(Chrom 1)	2.63	2/3 (66.7)	6/32 (18.8)

[1] Expressed as Log_{10} $SMICLD_{50}$ imbibed/mosquito.

[2] Number of females negative for WN virus/total number of females tested. All females had engorged on the blood-virus mixture and had been incubated at 28 ± 2° C for 10 days.

References:

Sakai, R. K. and R. H. Baker. 1972. A method for detecting and measuring concealed variability in the mosquito, *Culex tritaeniorhynchus*. Genetics 71: 287-296.

Sakai, R. K. and R. H. Baker. 1974. Induction of heat-sensitive lethals in *Culex tritaeniorhynchus* by ethyl methanesulfonate. Mosquito News 34: 420-424.

REPORT FROM NATIONAL INSTITUTE OF VIROLOGY
(FORMERLY VIRUS RESEARCH CENTRE) PUNE, INDIA

1. INVESTIGATIONS OF OUTBREAKS OF JAPANESE ENCEPHALITIS (JE) IN INDIA

From the last quarter of 1977 through 1978, the NIV investigated a number of outbreaks of encephalitis. These occurred in widespread geographic areas as detailed in Table 1. The important species of mosquitoes collected in various parts of India during or subsequent to JE epidemics are shown in Table 2.

1.1 INVESTIGATIONS IN KOLAR DISTRICT, KARNATAKA STATE

Seventy two cases of encephalitis occurred in Kolar district during the period September 1977 to January 1978, resulting in 18 deaths. The patients consisted mainly of the paediatric age group. Sixty two per cent of the cases were males and 38 per cent were females.

The children presented symptoms of high grade fever and altered sensorium invariably progressing to coma. Convulsions, neck rigidity, altered pupils and hemiparasis were the other predominant clinical features. Polymorphonuclear leucocytosis was observed in many patients. CSF analysis showed pleocytosis with lymphocytic preponderance, moderately raised protein levels and normal sugar values.

HI, CF and N tests were performed on four paired and 29 single convalescent sera from survivors. In 29 cases, the results of the serological tests were compatible with a diagnosis of JE. JE virus neutralizing antibodies were also demonstrated in 28 of the 66 contacts.

A serological survey in humans was conducted immediately after the outbreak of encephalitis. One hundred and forty six sera were tested for neutralizing antibodies to JE virus. Twenty per cent of the sera tested had JE virus neutralizing antibodies.

1.1.2 Serological survey of mammals and birds

Fifty per cent of the 146 pig sera had JE virus neutralizing (N) antibodies. Of the 33 cattle sera tested, 6 had JE N antibodies. HI antibodies for JE/WN were detected in 8 of the 82 cattle sera. Incidence of Flavivirus HI antibodies was not significant in the sera of other species tested. However, it is noteworthy that 13 out of 67 domestic fowl sera had HI antibodies for WN and 1 had HI antibodies against JE.

1.1.3 Entomological studies

A total of 15,723 adult mosquitoes belonging to 33 species were collected in 499 man-hours during January 1978 and from June to October, 1978 from different habitats. The following six species accounted for 85.3 per cent of the total collection which in the order of preponderance were: *Anopheles subpictus* (3657), *Culex tritaeniorhynchus* (1736), *C. vishnui* (2249), *C. pseudovishnui* (1395) and *C. fuscocephala* (1170). Other species recorded in fairly good numbers were: *An.hyrcanus*, *C. gelidus*, *C. whitmorei*, *C. bitaeniorhynchus* and *Armigeres obturbans*.

A total of 717 pools comprising 14,161 mosquitoes belonging to 22 species were processed for virus isolation. Four strains of virus were recovered in suckling mice. Two strains from *C. vishnui* and one from *An subpictus* have been tentatively identified as JE virus.

1.2 INVESTIGATIONS IN DHANBAD, BIHAR AND ASANSOL, WEST BENGAL

The outbreak in the Dhanbad-Burdwan area had commenced in mid-June 1978 and it subsided in September 1978. The clinical picture, both in Dhanbad and Asansol was moderate to high fever, severe headache, vomiting, drowsiness and convulsions of 2-3 days duration. In more severe cases patients were admitted to hospital in semicomatose or comatose condition. Signs of meningeal irritation with paresis or paralysis or aphasia were common. The CSF pressure was raised in most of the cases. Increased proteins and increased lymphocyte in CSF were common features.

Attempts were made to isolate virus from the CSF and brain tissues collected from Dhanbad and Asansol cases. JE virus was isolated from 2 CSF samples.

It was noted that in Dhanbad, adults were affected and the clinical picture was atypical in many cases. Total mortality was higher at Dhanbad but the mortality among the patients in the age groups of 17 years and above, particularly in females, was higher at Asansol.

One hundred and fifty seven bird sera were tested in neutralization test against JE. One out of the 13 pond herons, one of the four crows, two of the 15 cattle egrets, nine of the 35 little egrets showed N antibodies to JE virus.

1.2.1 Entomological investigations

In Asansol, 1,218 mosquitoes belonging to 20 species were collected in 13 man hours. Of these, Anopheles vagus and Culex tritaeniorhynchus amounted to more than 50 per cent of the collection. The other eight species in order of abundance were: C. gelidus (9.2%), C. whitmorei (6.3%), An. subpictus (4.5%), C. p. fatigans (4.3%), C. pseudovishnui (4.3%), Armigeres subalbatus (4.1%), C. fuscocephala (3.9%), and Mansonia uniformis (3.2%) respectively. The remaining 10 species amounted to only 8.2 per cent.

In Dhanbad district, 5,414 mosquitoes belonging to 29 species were collected in 136 man-hours. Thirteen species amounted to more than 90 per cent of the total collection. These in order of preponderance were: C. tritaeniorhynchus (16.9%), An. subpictus (15.7%), An. vagus (14.5%), C. p. fatigans (13.6%), C. pseudovishnui (10.8%), C. gelidus (9.0%), C. fuscocephala (4.3%), An. annularis (3.1%), An. culicifacis (3.1%), M. uniformis (1.0%) and C. whitmorei (1.0%) respectively. The remaining 16 species accounted for 4.8 per cent of the total collection.

So far, 167 pools consisting of 2053 mosquitoes have been processed for virus isolation. Mice inoculated with the suspension of a pool of An. tessellatus mosquitoes resisted challenge of 1000 LD 50 of TR 1751 (dengue 2) virus. No other virus has been isolated from the mosquitoes so far.

1.3 INVESTIGATIONS IN DIBRUGARH, ASSAM

During September 1978, information was received about the admittance of a large number of clinically diagnosed cases of encephalitis at Assam Medical College, Dibrugarh. The earliest cases started in the first week of August and the peak was reached sometime in the fourth week of August. The Hospital data up to 29th October 1978 showed 213 cases out of which 124 expired. The age distribution as obtained from 269 cases is as follows:

0-5	years	42
5-10	years	56
11-20	years	79
21-40	years	70
Over 40	years	22

This shows that considerable number of cases occurred in the older age groups. There was a preponderance of cases among males. The male to female ratio was approximately 7:5.

A total of 33 paired sera were obtained from different hospitals. Ten pairs showed rise in titres against JE virus/sero-conversion in HI and CF tests. Eight pairs did not show any distinct serological response. Two pairs showed fall in titre of JE antibodies. This was attributable to the time of collection of the sera. Nine pairs showed high titred antibodies reacting against JE antigen in both acute and convalescent sera but the sera were also sensitive to mercaptoethanol treatment showing thereby recent infection with JE. One of the four brain necropsies tested yielded JE virus.

The duck, fowl and cattle sera collected from this area have been tested in HI tests and some of them have been tested by N tests as well. Two of 76 sera from ducks and 16 out of 101 sera from cattle were positive in HI with JE antigen. Six out of 108 sera from fowls had WN antibodies and one fowl serum had antibody which reacted with JE and WN antigens. In the NT carried out with JE virus 733913 strain, 10/65 fowl sera and 34/35 cattle sera neutralized JE virus.

1.4 VIRUS ISOLATION FROM ENCEPHALITIS CASES FROM MADHYA PRADESH

There had been reports of encephalitis cases from the northern districts of Madhya Pradesh. Brain specimens were received one each from Bhopal, Shahdol and Indore. One brain specimen from Bhopal BHEL Hospital yielded JE virus.

1.5 INVESTIGATIONS IN TIRUNELVELI DISTRICT AND SURROUNDING AREAS OF TAMIL NADU

The epidemic commenced in November 1977 and subsided during the month of April 1978, with the peak of February and March 1978. The clinical features were typical of Japanese encephalitis (JE). The incidence was predominantly in children in the age group of 5 to 15 years.

Eleven brain specimens, 58 CSF specimens and 50 early/late acute phase sera were inoculated into infant mice for virus isolation. JE virus was isolated from one CSF specimen obtained from an 11 year old male patient.

Twenty six of the 32 paired sera were tested for HI antibodies. In 10 cases, there was conversion from negative to positive or a four-fold or greater rise in titre to JE virus. Conversion or rise in titre of antibodies to WN or the dengue viruses was either absent or at least four-fold

lower than JE virus. In 3 cases, the titres were 1:80 or higher to JE virus in both serum samples. In the remaining cases, HI antibodies to the Flaviviruses tested were either not detectable or low. The result of tests for N antibodies to JE virus with these 26 paired sera, correlated with the results of the HI tests.

1.5.1 Serological survey of humans

A serological survey of the human population was carried out during the months of April and May when the epidemic had declined. Sera were tested from residents of: High incidence areas, Low incidence areas, and Areas from which no cases of encephalitis were reported.

A total of 1229 sera selected by stratified random sampling were tested. The geometric mean HI antibody titres to JE, WN and DEN-2 viruses were 14.5, 10.8 and 10.8 respectively. There was a progressive increase in the proportion of HI positive reactors: 39.1 per cent in the 0 to 4 years age group; 44.5 per cent in the 5 to 9 years age group; 51.4 per cent in the 10 to 14 years age group; 61.6 per cent in the 15 to 29 years age group and 81.7 per cent in the 30 years and above age group. The high proportion of positive reactors in the 0 to 4 years age group indicates a high rate of infection with one or more of the Flaviviruses. The porportion of HI positive reactors from the high incidence areas, low incidence areas and the areas which did not report any cases of encephalitis was not significantly different.

A sub-sample of 307 sera was selected for the presence of N and CF antibodies. The results indicate that about fifty per cent of those possessing HI antibodies to the Flaviviruses were infected by WN, DEN-2 or some other Flavivirus other than JE, and the JE positive HI reaction in these cases represented cross-reactions.

Eighty two of the 307 sera have so far been tested for CF antibodies to the above mentioned Flaviviruses. CF antibodies to one or more of these viruses were detected in 39 sera:

	HI positive	HI negative	Total
CF positive	39	0	39
CF negative	11	32	43
Total	50	32	82

The findings of this serological survey indicate that there was high prevalence of Flaviviruses particularly JE virus infections in Tirunelveli district.

1.5.2 Serological survey of domestic animals

One thousand five hundred and eighty three serum samples were obtained: 701 from pigs, 313 from cattle, 202 from sheep, 180 from goats, 124 from donkeys, 59 from buffaloes and 4 from horses. The results of HI tests indicate that infection of pigs and cattle with JE virus is extensive.

1.5.3 Mosquito collections

A total of 17,314 mosquitoes belonging to 24 species were collected. *C. tritaeniorhynchus*, the proven vector of JE in South India, was found to be the predominant species amounting to 46.4% of the total catch followed by *An. subpictus* (25.7%), *C. pseudovishnui* (7.4%), *C. vishnui* (5.9%) and *An. hyrcanus* (4.4%).

A total of 509 pools comprising 11,140 mosquitoes have been processed for virus isolation. So far no virus has been isolated.

1.6 INVESTIGATIONS IN UTTAR PRADESH

The outbreak of encephalitis in Uttar Pradesh had commenced in early part of October 1978, continued in November and declined during the month of December 1978. Till 18th December 1978 there were 1,088 deaths out of 3,441 reported cases.

All the sera have been tested by HI/CF tests with JE, WN, DEN-2 and CHIK viruses. In HI test presence of 2-mercaptoethanol sensitive antibodies were also tested. The results so far analysed indicate that out of 70 serum samples collected (33 paired and 37 single acute/convalescent phase), 55 cases (28 paired and 37 acute/convalescent phase) showed serological evidence of JE infection on the basis of conversion/rise in titre and sensitive antibodies to JE virus only.

Two thousand six hundred eighty three mosquitoes belonging to 19 species from human dwellings, cattlesheds, and pigsties were collected. The important species were *C. fatigans*, *C. pseudovishnui*, *An. annularis* and *An. subpictus*. Other species collected in good numbers were *An. vagus*, *C. gelidus*, *C. vishnui*, *C. tritaeniorhynchus*, *Arm. subalbatus* and *M. annulifera*. The mosquitoes are being processed for virus isolation.

1.7 INVESTIGATIONS IN MYSORE AND MANDYA DISTRICTS OF KARNATAKA STATE

Several cases of encephalitis were reported between March and June 1979 from the villages of Mysore and Mandya districts. Out of 30 cases admitted to the hospitals 16 died. The clinical picture of some of the cases was typical of JE. However, others showed symptoms similar to Rye's syndrome. The HI antibody response in two paired serum samples was suggestive of JE virus infection.

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Table 1. JE epidemics investigated during 1977-78

State/District	Period	Reported mortality	Male-female ratio	Isolation Human	Serological evidence: Single convalescent	Serological evidence: Paired acute-convalescent	Age distribution by years: 0-5	6-10	11-15	16-35	>36
Karnataka/Kolar	Sep. 77 to Mr. 78	18/72 (25%)	1.6:1	-	25/29 (86.2%)	3/4 (75%)	12/16	13/14	0/0	3/3	0/0
Tamil Nadu/ Tirunelveli and surrounding areas	Nov. 77 to Apr. 78	99/299 (33.1%)	1.8:1	1 CSF	73/93 (78.5%)	20/32 (62.5%)	33/44 Total:	39/55 93/125	11/14 (74.4%)	9/10	1/2
West Begal/ Many districts	June to Sep. 78	532/1190 (44.7%)									
Burdwan district	Hospital cases	33/123 (26.83%)	1.4:1	2 CSF	--	26/33 (78.7%)	6/8	9/14	0/0	6/6	5/5
Bihar/Many districts	June to Dec. 78	123/349 (35.2%)*									
Dhanbad district	Hospital cases	25/57 (43.9%)	0.9:1	--	--	9/16 (56.2%)	1/3	1/2	3/5	2/4	2/2
Uttar Pradesh/ Many districts	Oct. 78 to Nov. 78	703/2478 (28.4%)*	2:1	--	27/37 (72.9%)	28/33 (84.8%)**	1/1 Total:	12/15 55/70	11/12 (78.5%)	29/39	2/3
Assam/ Dibrugarh	Aug. 78 to Nov. 78	137/288 (44.09%)	1.4:1	1 Br.	--	13/33 (39.3%)***					
Madhya Pradesh/ Bhopal	--	--	--	1 Br.	--	--					

*As of 9th November 1978. **Single convalescent and paired sera. ***In addition to the paired sera showing rise in titre/seroconversion 10 pairs showed high titred HI antibodies which were sensitive to 2-ME, indicating presumbly recent JE infection.

Table 2: Important species of mosquitoes collected from various parts of India, during or subsequent to JE epidemic.

State/District months of collection.	Karnataka/ Kolar (Jan. to June to Oct.)	Tamil Nadu/ Triunel-veli (Mar. to May)	West Bengal/ Asansol (July to Sept.)	Bihar/ Dhanbad (July to Sept.)	Uttar Pradesh/ Basti (Nov.)	Uttar Pradesh/ Goraghpur (Nov.)
Total No. of Man-hours	499	364.75	13	136	18	44
Total No. of mosquitoes	15,723	17,314	1,218	5,409	794	1,889
Species	No. of mosquitoes/Man-hour					
An. annularis	N	0.2	1.6	1.3	19.8	5.9
An. barbiorostris	N	0.1	0.1	Nil	0.4	0.1
An. hyrcanus	0.6	4.4	0.8	0.1	0.1	0.1
An. subpictus	7.3	12.2	4.2	6.3	5.9	3.1
An. vagus	0.2	N	27.7	5.8	0.6	1.3
C. bitaeniorhynchus	0.3	0.3	1.1	0.3	0.1	N
C. epidesmus	--	--	1.6	0.5	--	--
C. fatigans	6.4	0.4	4.1	5.5	5.2	16.9
C. fuscocephala	2.3	0.1	3.7	1.7	0.2	N
C. gelidus	1.4	0.4	3.7	3.6	0.3	0.5
C. pseudovishnui	2.8	3.5	4.1	4.3	6.4	11.0
C. trataeniorhynchus	3.5	22.0	21.1	6.7	3.4	1.1
C. vishnui	4.5	2.8	0.4	0.3	0.2	0.4
C. whitmorei	0.3	--	5.8	0.4	--	N

N= Negligible.

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

1978 epidemic of Japanese encephalitis in West Bengal - INDIA

A preliminary report on epidemic of Japanese encephalitis in several districts of West Bengal was provided in the INFO Exchange No. 35, which was further continuing.

The epidemic started in the premonsoon (early May, 1978) and lasted till early winter (December, 1978). A total of 1256 people, mostly young and youngadults, were affected with an approximate mortality of 43.3 percent and for virological investigations 544 cases were referred to us.

Samples received were - paired blood 26; single blood - 244, C.S.F. - 125 and post mortem brain tissues - 5. Mosquitoes numbering 983 and comprising mostly of Anopheles and Culicine varieties were collected from field and sent to us for investigations.

Results : Japanese encephalitis virus was isolated and identified from one of the 5 autopsy brain tissues. No virus could however be isolated from mosquitoes during this epidemic. The recurrent isolation of JE virus from *Culex vishnui* in previous epidemics of 1973 and 1976 seem to suggest this species of mosquito to be an important vector for JE in this area. Fourfold or more rise of HI and/or CF antibody against JE was found in 69.2% of paired sera while in 55.7% of single sera high titre JE antibody was detected (HI titre 160 or more
CF " 16 or more).

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Effect of challenge by Dengue - 2 on mice after Dengue 1-4 vaccination - a preliminary report.

In a previous work, it was shown that when groups of mice were injected intraperitoneally (I.P.) with 10 LD_{50} of adult-adapted dengue type - 1 (D_1) and later challenged intracerebrally (I.C.) with 10 LD_{50} of adult-adapted dengue type - 2 (AD_2) one, two and four weeks later, microscopic haemorrhage of various grades was found in the internal organs (liver, kidney, brain, lungs and heart) of the sick mice. Subsequently it was found that as a result of I.C. challenge by 10 LD_{50} of AD_2 in mice which previously received I.P. injection of dengue - 1 antiserum, microscopic haemorrhage could be detected in the internal organs of the mice. That is, Dengue - 2 infection could produce haemorrhage in some internal organs of mice which had previous experience of Dengue - 1 virus infection or Dengue - 1 antiserum. This was not the case after dengue - 2 infection of mice, who had no such experience.

At various centres attempts are being made to develop attenuated single polyvalent dengue type 1-4 vaccine mainly to prevent dengue haemorrhagic fever (DHF). It was thought worthwhile to use the mouse model to find out the effects of injecting Ad_2 in mice which had received all the four types of dengue viruses.

The attenuated strains of dengue virus were not available and therefore laboratory maintained live dengue virus strains were used for vaccination. Three groups (Group A, B and C) of 4-6 weeks old mice were taken. Group A mice were injected I.P. with 0.2 ml of virus (infected mouse brain) suspension containing 10 LD_{50} of each of the four types of dengue virus. This group, one week later was challenged, I.C. by 10 LD_{50} of AD_2. When the mice were ill, autopsy was performed and the internal organs were examined histopathologically for haemorrhage.

For control studies, Group B mice were inoculated with 10% normal mouse brain suspension through I.P. route and later challenged by AD_2 . When the mice were ill, autopsy was performed and the internal organs of the sick mice.

Mice of the Group C were inoculated with all the four dengue types through I.P. route as above just to see the effect of the multiple virus infection.

RESULT

None of the organs of sick mice of Gr. A and Gr. B showed haemorrhage. Gr. C mice showed no sickness.

COMMENTS

From the preliminary experiment it appears that Dengue - 2 infection of mice which received all the 4 types of dengue virus as single inoculum previously, does not produce haemorrhage in internal organs, although in a previous work, dengue -2 infection was found to cause haemorrhage in mice after previous experience of dengue - 1 virus.

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

It is now becoming clear that enzootic foci of MVE exist in North-West Australia (Kimberley) and that clinically severe human cases occurred in 1969, 1974, 1978 and 1979. Rapid antibody conversion of sentinel fowl and other vertebrates suggests pulsations of activity correlating with higher virus isolation rates at the end of the wet season compared with those at the end of the dry season (see Table I).

Tables II, IIIA and IIIB show the numbers and the characterization of viruses from five species of mosquitoes. We continue to isolate MVE from *Culex annulirostris* each year.

Changes have been introduced in the technique of mosquito capture following the introduction by Dr. Telford H. Work of the EVS/CO_2 light trap. Table IV shows the total and percentage of captures of the common mosquitoes of North-West Australia with 16 field trips made between 1972 and 1979. Tables V.A and B and VI.A and B compare the results with bait and light traps.

The predominance of *Culex annulirostris* is striking. It is also the mosquito which yields viruses pathogenic for infant mice. A total of 49 mosquito species has been collected.

Further major environmental changes are being planned for North-West Australia and continued monitoring before and during these changes is required prior to increased human population densities.

(N.F. Stanley, P. Liehne, A. Wright, P. Pihu, D. Britten; joined in July, 1979 by *T.H. Work* and *M. Jozan).*

TABLE I

VIRUS ISOLATIONS[I] N.W. AUSTRALIA

SEASONAL VARIATION

(1972 - 1977)

VIRUS ISOLATES	MOSQUITO CAPTURES	
	NOV. - DEC. (? END OF DRY)	APRIL - MAY (? END OF WET)
MVE	1.3% [4[x]/317]	8.3% [52/627]
ALL VIRUSES	11.4% [56/488][xx]	28.1% [195/691]

x = 1974 UNUSUALLY HEAVY RAIN

xx = NO. ISOLATES/NO. POOLS

I = BY INFANT MOUSE INOCULATION

TABLE II

VIRUS ISOLATIONS FROM MOSQUITOES CAPTURED IN N.W. AUSTRALIA 1972 - 1977

MOSQUITO SPECIES	NO. POOLS TESTED	NO. ISOLATES	%
CULEX ANNULIROSTRIS	944	209	22.2
AEDEOMYIA CATASTICTA	112	37	33.0
AEDES NORMANENSIS	18	3	16.7
AEDES TREMULUS	9	1	11.1
CULEX FATIGANS	96	1	1.0
TOTAL	1,179	251	21.3

TABLE IIIA

VIRUS ISOLATIONS FROM W.A. MOSQUITOES

CHARACTERIZATION OF 251 ISOLATES FROM 5 VECTORS

I

VECTOR	VIRUS GROUPS										SUB-TOTAL	TOTAL
	FLAVIVIRUS B			ALPHAVIRUS A		KOONGOL			COR	ANOPH A/B		
	MVE	KUN	KOK	SIN	RR	KOO	WON	NON HA	COR	OR 540		
CULEX ANNULIROSTRIS	55	21	2	15	1	4	29	64	1	6	198	209
AEDEOMYIA CATASTICTA									32		32	37
AEDES TREMULUS		1									1	1
AEDES NORMANENSIS				2							2	3
CULEX FATIGANS	1										1	1
TOTAL	56	22	2	17	1	4	29	64	33	6	234	251

MVE = MURRAY VALLEY ENCEPHALITIS

KUN = KUNJIN

KOK = KOKOBERA

SIN = SINDBIS

RR = ROSS RIVER

WON = WONGAL

TABLE IIIB

VIRUS ISOLATIONS FROM W.A. MOSQUITOES

CHARACTERIZATION OF 251 ISOLATES FROM 5 VECTORS

II.

VECTOR	UNGROUPED RHABDOVIRUS			UNKNOWN UNGROUPED ⊥			UNCLASSIFIED		
	KNA* OR 194	PC* OR 189	KIM* OR 250	I OR 379	II OR 512	III OR 869	WGR	SUB-TOTAL	TOTAL
CULEX ANNULIROSTRIS		3	1	2	2		3	11	209
AEDEOMYIA CATASTICTA	1					4		5	37
AEDES TREMULUS								0	1
AEDES NORMANENSIS							1	1	3
CULEX FATIGANS								1	1
TOTAL	1	3	1	2	2	4	4	18	251

* NOW RECOGNIZED AS NEW TYPES

⊥ TESTED AGAINST WORLD GROUP ANTISERA AND AUSTRALIAN ANTISERA AND FOUND TO BE NON-REACTING

KUN = KUNUNURRA

PC = PARRY'S CREEK

KIM = KIMBERLEY

WGR = WONGORR

TABLE IV

TOTALS OF MOSQUITOES COLLECTED DURING THE ARBOVIRUS MONITORING PROGRAMME [1, 2].

THE 10 MOST COMMON SPECIES [3]

SPECIES	TOTAL CAUGHT	PERCENTAGE CATCH [4]
Culex (Culex) annulirostris	98,227	71.80
Anopheles (Cellia) annulipes	12,162	8.89
Culex (Culex) fatigans	8,043	5.88
Aedeomyia catasticta	6,694	4.89
Anopheles (Cellia) amictus	5,480	4.00
Aedes (Ochlerotatus) vigilax	1,369	1.00
Aedes (Ochlerotatus) normanensis	1,123	0.82
Anopheles (Anopheles) bancroftii	799	0.58
Coquillettidia xanthogaster	743	0.54
Culex (Culex) australicus	405	0.30
Remainder (39 species)	1,750	1.30

Total identified: 136,795

Total unidentified unsorted material: approximately 46,000

Total captures: approximately 182,800

[1] These collections include all sites - Kimberley and Pilbara

[2] The collections cover 1972 - June 1979 (16 field trips)

[3] A total of 49 species have been collected

[4] % of total identified catch.

TABLE V.A.

SUMMARY OF 48 PAIRED TRAP COLLECTIONS COMPARING EVS/CO_2/LIGHT TRAPS AND ANIMAL BAIT TRAPS[1]

	BAIT TRAP	EVS/CO_2/LIGHT
Total number mosquitoes	2,586	20,979
Average catch per night	53.88	437.06
Species diversity	10	23

[1] Comparisons made at Balgo and Ord Study site only (June, 1978).

TABLE V.B.

COMPARISON OF MAJOR SPECIES COLLECTED IN 48 PAIRED COLLECTIONS WITH THE EVS/CO_2/LIGHT TRAP AND ANIMAL BAITED TRAPS.[1 2 3]

	BAIT TRAP	EVS/CO_2/LIGHT
Culex annulirostris	1,355 (52.40)	7,765 (37.01)
Anopheles annulipes	8 (0.31)	6,525 (31.10)
Aedes species [4]	1,166 (45.09)	4,119 (19.63)
Anopheles amictus	3 (0.12)	1,728 (8.24)
Totals collected	2,586	20,979

[1] Comparisons made at Balgo and Ord in June, 1978

[2] In almost all cases, the EVS/CO_2/Light collected greater numbers of each species where both were collected in the same collection. Only one species, one individual of *Culex starkiae*, was collected only from bait traps.

[3] The percentage is given in parentheses

[4] A mixture of *Aedes (Ochlerotatus)* species and *Aedes (Pseudoskuses) bancroftianus* as yet unsorted.

TABLE VI.A.

COMPARISONS OF THE EVS/CO_2/LIGHT TRAP AND BAIT TRAPS AS MOSQUITO COLLECTING METHODS AND OVERALL SUMMARIES OF MOSQUITO COLLECTING[1]

	Trips 1-12 Bait traps	Trips 13 and 14[2] Bait	Trips 13 and 14[2] EVS/CO_2/Light	Trips 15 & 16[3,4]
Total number caught	85,685	4,833	48,150	App. 45,200
No. of trap nights	1,972	113	296	330
Average catch/trap night	43.45	42.77	162.67	App. 36.96
Species diversity	33	17	30	

[1] From April, 1972 to June, 1979, Field Trips 1 - 16.

[2] EVS/CO_2/light traps only used on Trip 14, Trip 13 used both bait and light traps

[3] EVS/CO_2/light traps only used on trips 15 and 16

[4] Mosquitoes unidentified and unsorted

TABLE VI.B.

OVERALL SUMMARY (TRIPS 1 to 16)

	Bait	EVS/CO_2/Light
TOTAL NUMBER CAUGHT	90,518	Approx. 93,350
NUMBER TRAP NIGHTS	2,085	626
AVERAGE CATCH	43.42	Approx. 149.12
SPECIES DIVERSITY	33	30

REPORT FROM CSIRO, DIVISION OF ANIMAL HEALTH, LONG POCKET LABORATORIES, PRIVATE BAG NO. 3, P.O., INDOOROOPILLY, QUEENSLAND, AUSTRALIA 4068.

Separation of D'Aguilar Into 3 Serotypes

The group of viruses known as D'Aguilar virus by complement fixation test at the Queensland Institute of Medical Research (Doherty, 1977), consist of at least 3 separate strains which are easily distinguished by a cross-neutralization test in Vero cells. Table 1 shows the titres obtained using 100 $TCID_{50}$ of virus per .025 ml, with antiserum prepared in rabbits.

TABLE 1

Antiserum	Virus		
	CSIRO58	CSIRO11	B8112
CSIRO58	>2048	<1	1
CSIRO11	2	256	3
B8112	1	1	>256

Since September, 1976, we have made 47 isolates of D'Aguilar virus of which 6 were strain B8112, the type strain (Doherty *et al.*, 1972), 6 were strain CSIRO11 and 35 were strain CSIRO58. These were made from the midges *Culicoides brevitarsis* (CSIRO11 and CSIRO58), *C. schultzei* (CSIRO58) and whole blood out of cattle (CSIRO11, CSIRO58 and B8112).

The following results indicate that infection in cattle with one strain of D'Aguilar does not protect against infection by another strain. Four of the cattle bled yielded 2 different strains at different times as shown in Table 2.

TABLE 2

Sentinel Animals Bled on Four Different Farms.

Animal Number	Date Bled	Strain of D'Aguilar Isolated
1	2. 1.79	CSIRO58
1	19. 2.79	CSIRO11
2	30.11.78	CSIRO58
2	21. 2.79	B8112
3	2. 1.79	CSIRO58
3	21. 2.79	B8112
4	12.12.78	CSIRO11
4	17. 1.79	CSIRO58

One cow on a farm near Brisbane, Queensland, was bled daily for 60 days and the CSIRO11 strain of D'Aguilar virus was isolated 9 times in 11 days (St.George and Dimmock, 1976). The animal had a titre of greater than 32 to strain B8112 throughout this time.

References

Doherty, R.L. 1977. The Australian Journal of Experimental Biology and Medical Science, Vol. 55, Part 2 pp.103-130.

Doherty, R.L., Carley, J.G., Standfast, H.A., Dyce, A.L. and Snowdon, W.A. 1972. Aust. vet. J. 48: 81.

St.George, T.D. and Dimmock, Corinne K., 1976. Aust. vet. J. 52: 598.

(D.H. CYBINSKI and T.D. ST.GEORGE)

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

Ross River Virus in Fiji and New Zealand

In Fiji, beginning in April, 1979, a large outbreak of an illness characterised by polyarthritis and rash occurred. Although dengue virus is active at a low level in Fiji, patients showed no antibody rise to this group and the symptoms suggested infection with either Chikungunya or Ross River viruses, neither of which had been reported in Fiji before.

The epidemic began in the western and northern coastal areas of Viti Levu later spreading to Suva and other areas and involving possibly 30,000 people. The outbreak began to decrease in July and even fewer cases are being reported in August.

Dr J. U. Mataika of the Wellcome Virus Laboratory, Suva, using suckling mouse brain inoculation methods, isolated two strains of a virus from the blood of polyarthritis patients. These viruses were subsequently shown to be Ross River virus, a finding confirmed by the Queensland Institute of Medical Research in Brisbane, Australia.

In May, numerous New Zealand travellers, on returning from Fiji or within a short time after having done so, became ill with symptoms similar to those described for the Fijian infections. Efforts were made to obtain details of travel history, mosquito exposure and symptoms, and serum samples were examined in this Unit for antibodies to dengue and Ross River viruses using HI, CF and NT methods based on one of the Fijian strains, 9073, as antigen. No isolation attempts have been made from New Zealand patients so far.

Of 123 suspected cases of Ross River virus infection in New Zealand travellers, 69 have been confirmed by one or other of the tests. Some of the antibody has been shown to be IgM by protein A absorption methods and many of the paired sera showed four-fold or greater rises in Ross River virus antibody. Of 278 Fiji patients examined for antibody in this Unit, 162 were positive by one or more tests. Symptoms in Fijian and New Zealand patients varied but they included those of influenza with pyrexia, diarrhoea with or without fever, swelling of the joints with pain but minimal fever and in many cases, rash. Most of the people from New Zealand who were positive for antibody, gave histories of having been bitten by mosquitoes while visiting Fiji. No cases of infection in persons not having visited Fiji have been discovered so far in this country.

Ross River virus is a mosquito-borne alphavirus until now only known to occur in Australia, although antibodies thought to be specific for this virus have been found in Papua-New Guinea. The probable vectors of the virus in Australia are Aedes vigilax and Culex annulirostris. Both of these species occur in Fiji, but epidemiological evidence suggests that A. vigilax is the vector there. Neither A. vigilax nor C. annulirostris is found in New Zealand, but the vector potential of the indigenous mosquitoes remains unknown and this will be investigated shortly.

Ross River virus has been isolated in Australia from mosquitoes on numerous occasions, but there has been only a single isolation reported from human serum in spite of numerous attempts from polyarthritis cases.

This outbreak is the first reported introduction of an arbovirus other than dengue affecting the populations of Fiji and New Zealand and it emphasises the ease with which these agents can be spread when viraemic individuals can move so far so rapidly. A combination of such travel with suitable climatic conditions and appropriate local vectors can rapidly establish an arbovirus in a new region and give rise to epidemic or endemic states. It is probable in the case of Ross River virus in Fiji that the virus was carried to that country either by an Australian visitor, or a Fijian resident returning from Australia. It is perhaps ironic that cases are now being reported in Australia among Australian visitors returning home from Fiji (Australia Communicable Disease Intelligence, 79/15 p.2). Whether the virus becomes endemic in Fiji or whether secondary cases occur in New Zealand remains to be seen.

(F. J. Austin, T. Maguire, J. A. R. Miles)

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUT PASTEUR DE NOUMEA - NOUVELLE - CALEDONIE

Dengue

Type 1 virus appeared in New Caledonia, in March 1975, three years after an explosive dengue 2 outbreak. From 1976, each year, the number of cases rose sharply in March, reached a maximum in April and decreased more or less abruptly after. Ultra-low volume malathion sprayings and refreshment of atmospheric temperature in "winter" (from June to August) contributed to reduce the virus transmission.

Aedes aegypti was found infected in Nouméa, Ouvéa (Loyalty Islands) and Aoba (New Hebrides) ; strains were also obtained from Aedes polynesiensis during an outbreak in Futuna (Horne Islands) and from a specimen of Aedes vigilax caught in Nouméa ; intra-thoracic inoculation of dengue 1 strain and subsequent inoculation, 7 days after, to baby mice proved that virus replication may occur in Aedes vigilax specimens reared from larvae.

Clinical manifestations of type 1 infections were rarely severe ; minor haemorrhagic forms, mainly epistaxis, were noted in 2 % of patients ; one dengue 1 strain was recovered from blood, in a fatal case of encephalitis. Sex ratio of patients with positive serology was 47 men/53 women against 52/48 in New Caledonia population. Percentage of suspected cases confirmed by laboratory examination fell from 22 % in 1976-77, to 6.4 % in 1978. Influenza and adenoviruses were responsible of 12 % of dubious cases. Annual report of positive cases/suspected cases was as follows in New Caledonia :

Year	1976	1977	1978
positive cases	290	247	61
suspected cases	1189	1086	952
% positive	24.4	22.7	6.4

No primary or secondary response was noted between september 1978 and january 1979. In February 1979, an imported case occured in La Tontouta ; the patient, a woman of 45, returned from Tahiti ; no subsequent case was detected in that locality free of Aedes aegypti. Two other cases, imported from Tahiti, were found in March : they affected a traveller of 61, returning to France, and a woman of 37, dwelling in Nouméa ; three subsquent cases and Aedes aegypti breeding places were discovered in and around her home. Control measures were taken immediately in the block and the town was sprayed on April 6, by air, using naled (70 ml/ha).

Distribution of cases, from January 1 to June 30, 1979 was a follows :

1) by month :

January	February	March	April	May	June	Total
0	1	11	10	5	4	31

2) by sex :

Men	Women
9	22

3) by age group :

0-4	5-9	10-19	20-29	30-39	40-49	50-59	60 or more
0	2	1	10	6	6	5	1

4) by ethnic group :

Europeans	Melanesians	Wallisians	Indonesians
22	4	1	2

Tahitians	Asiatics
1	1

Virus isolation was successful in 13 of these cases (42 %). Three strains were identified as dengue type 4, by complement fixation test :

	den 1	den 2	den 3	den 4	Origin	Date
H.NC - 172/3657	64/32	8/8	8/16	64/128	Papeete	11/03
H.NC - 173/3689	32/32	16/16	33/32	64/128	Nouméa	21/03
H.NC - 174/3709	64/32	32/32	64/32	64/128	Nouméa	23/03

There is no previous record of dengue 4, in New Caledonia. The two indigenous cases occured respectively 10 and 12 days after the imported case.

Other arboviruses

Survey on natural infection of mosquitoes caught in rural areas was conducted along the western and north-eastern coasts of New Caledonia : 34 strains were isolated from specimens collected between January and July 1979.

Genus and species	N° of pools	N° of specimens	Positive	Origin of strains
Aedes aegypti	4	16	0	
alternans	3	41	0	
nocturnus	5	174	2	La Tontouta (2)
notoscriptus	4	89	2	Bouloupari (2)
vigilax	93	4355	18	Pam (2) Tiari (2) La Tontouta (9) Bouloupari (4) RT 1 Km 6 (1)
Coquil. xanthogaster	12	423	1	Païta (1)
Culex annulirostris	24	982	7	La Tontouta (6) Païta (1)
bitaeniorhynchus	12	519	2	La Tontouta (2)
iyengari	1	6	0	
p. fatigans	4	60	2	Païta (1) Pam (1)
Tript. caledonicus	2	18	0	
	164	6683	34	

33/34 strains produced pathogenic effect on baby mice only after a blind passage. First identification tests indicate that two strains are Flaviviruses (group B).

P. Fauran and G. Le Gonidec

The following report had been submitted earlier by Drs. Fauran and Le Gonidec but arrived too late for inclusion in Info-Exchange No. 36 (March, 1979). It is included here for your information. The editor.

Dengue

In the research area of the laboratory, dengue cases occured only in the main town Nouméa (New Caledonia) and in Ouvéa (Loyalty Islands). On 952 suspected cases, 68 (7,14 %) were confirmed by sero-diagnosis and/or by virus isolation, from january 1, to December 31, 1978. The foci of New Hebrides, Wallis and Futuna disappeared.

Sero-diagnosis : positive results were obtained from 61 patients (6,41 %), by hemagglutination inhibition test, using dengue 1 antigen : monthly records were as follows :

	01	02	03	04	05	06	07	08	09	10	11	12	Total
Primary response	4	7	3	5	1	5	0	2	0	0	0	0	27
Secondary response	4	2	0	1	1	1	0	0	0	0	0	0	9
Presumptive	3	2	4	6	1	1	4	1	0	3	0	0	25
Total	11	11	7	12	3	7	4	3	0	3	0	0	61

Virus isolation from patients with :	01 L	01 M	02 L	02 M	03 L	03 M	04 L	04 M	05 L	05 M	06 L	06 M	07 L	07 M	08 L	08 M	09 L	09 M	10 L	10 M	11 L	11 M	12 L	12 M	Total L	Total M
Primary response	2	1	6		1		2	1	1						1										13	2
Secondary response	1	1					1																		2	1
non significant			1	1	3										1		1								6	1
	3	2	7	1	4	0	3	1	1	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	21	4
Total	5		8		4		4		1		0		0		2		1		0		0		0		25	

L = intra-cerebral inoculation of baby mice litters
M = intra-thoracic inoculation of *Aedes aegypti* mosquitoes

507 litters were inoculated with human sera, giving 57 virus isolations : 23 from patients with positive serology, 6 from patient with negative serology, 5 from sera taken in acute phase without convalescent specimen, 23 for re-isolation ; 9 of these strains originated from sera stocked in 1976-1977.

149 human sera were inoculated to *Aedes aegypti* mosquitoes, giving 21 strains (14 %) : 17 confirmed a former virus isolation by litter inoculation ; 4 were obtained only by intra-thoracic route.

49 strains isolated from man in 1977-1978 were identified as dengue type 1. Apparently, it was the lone serotype transmitted during that period.

Survey on transovarial transmission

Larvae, pupae and adults emerged from pupae, collected in premises were dengue cases occured were pooled and tested for virus infection by intra-cerebral inoculation of baby mice. On 12 pools tested, none produced pathogenic effect but in one case (TTO 201) sera of inoculated mice showed IH antiflavivirus antibodies when tested with dengue 1 antigen. Control is under way.

Vectors survey

The following species were tested for natural infection :

	N° pools	N° specimens	Strains	Origin of strains
Aedes aegypti	56	571	1	Nouméa, N.C.
Aedes nocturnus	6	44	0	
Aedes notoscriptus	8	54	0	
Aedes vigilax	32	1217	2	Bouloupari, N.C.
			1	Teramba, N.C.
Culex annulirostris	5	70	0	
Culex fatigans	18	575	0	
Culex iyengari	4	20	0	
Culex sitiens	1	1	0	
Cogu. xanthogaster	3	69	0	
Boophilus microplus	3	3	0	

Provisory identification of the strain AR 167/24 from Aedes aegypti, 1 ♀ caught in Noumea, on Febr. 2, 1977 is dengue type 1.

Strains from Aedes vigilax are under study.

Birds survey

During a study by french ornithologist, R. de Naurois, 65 specimens were checked for natural infection by inoculation to baby mice of ground organs (brain, heart, liver). Species tested were :

Corvus moneludoïdes (1) - Rhipidura fuliginosa (5) - Gerygone flavolateralis (2) - Coracina caledonica (2) - Collocalia esculenta (2) - Aerodroma spodiopygia (1) - Lichmera incana (2) - Accipiter haplochrous (1) - Rallus philippensis (1) - Sterna fuscata (2) - Anous stolidus (3) - Fregata ariel (2) - Turdus poliocephalus (6) - Trichoglossus haematodus (2) - Erythrura psittacea (1) - Pterodroma rostrata (7) - Myiagra caledonica (2) - Halcyon sancta (1) - Zosterops xanthochroa (2) - Zosterops griseonota (1) - Eunymphicus cornutus (2) - Aplonis striata (2).

One strain was isolated from Corvus moneludoïdes, paralyzing the mice 13 days after inoculation, identification pending.

Sera of mice inoculated with organs of Gerygone flavolateralis, Lichmera incana, Eunymphicus cornutus, Turdus poliocephalus, Trichoglossus haematodus, Erythrura psittacea, Zosterops xanthochroa, showed IH antiflavivirus antibodies, while those of mice inoculated with organs of Accipiter haplochrous and Coracina caledonica showed IH anti-alphavirus antibodies.

P. Fauran et G. Le Gonidec

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

Virus isolation from dengue hemorrhagic fever patients using virus-sensitive clone C6/36 of *Aedes albopictus* cells (Singh)

In 1978, 4 strains of viruses were isolated from DHF patients in Thailand under cooperation with the Virus Research Institute, Department of Medical Sciences, Ministry of Public Health, using a virus-sensitive clone C6/36 of *A. albopictus* cells which was established by Dr. Akira Igarashi of our Department.

Clone C6/36 cells were cultured in plaque bottles and inoculated with 0.2 ml of acute phase sera of serologically confirmed DHF patients. CPE of syncytium formation of the mosquito cells appeared on the 4-6th day after inoculation. The culture fluids of C6/36 cells with CPE were inoculated onto BHK-21 cells in Lab-Tek 8 chamber slides and the virus infectivities were assayed by the peroxidase-anti-peroxidase (PAP) staining technique.

Identification of the isolates was carried out by neutralization test against standard anti-dengue rabbit sera using 50% focus reduction method. All 4 strains isolated and passed with the mosquito cells were not neutralized with either type of standard anti-dengue sera. Then the isolates were transferred to suckling mice. After 4th passage with them, one isolate (SI-8) was neutralized with anti-DEN-2 serum but other two isolates (No.124 and 176) were scarcely neutralized as shown in the table (another isolate No.177 is under adaptation to sucklin mice).

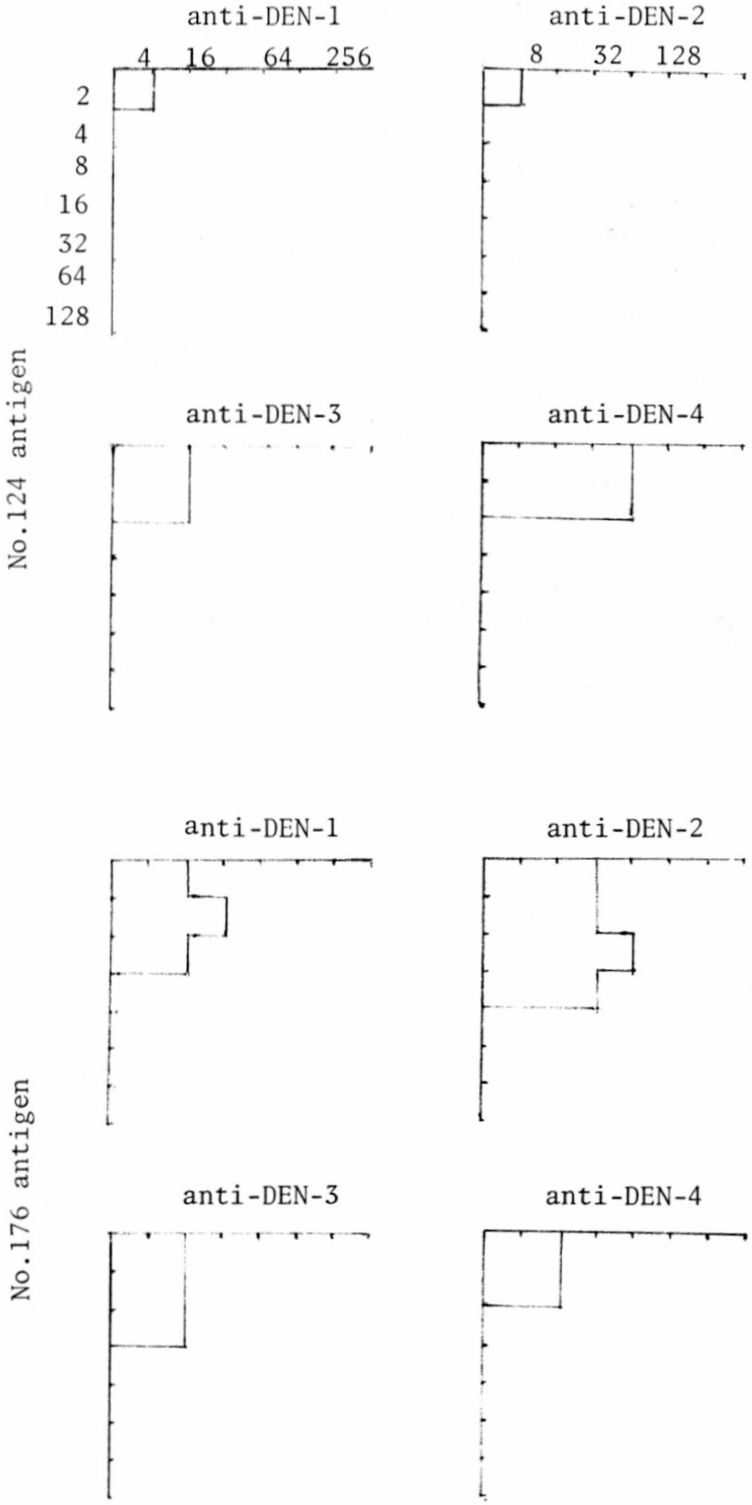
Complement-fixing (CF) antigens of No.124 and 176 isolates were prepared from infected suckling mouse brains by sucrose-acetone extraction and CF-tests were carried out with anti-dengue type 1-4 mouse sera. Results of the CF-tests were shown in the figure and thus the isolates No.124 and 176 were identified as dengue type 4 and type 2, respectively.

Table. Results of neutralization tests of the isolates

Isolates	Anti-DEN-1 (6400)	DEN-2 (1800)	DEN-3 (3200)	DEN-4 (2000) (a) (c)
SI-8:SA4	< 200	< 200	< 200	< 200
SI-8:SA4SM4	< 200	1200	< 200	< 200
	Anti-DEN-1 (780)	**DEN-2 (1600)**	**DEN-3 (1500)**	**DEN-4 (880) (b) (c)**
No.124:SA4	61	< 20	63	< 20
No.124:SA4SM4	20	68	20	166
No.176:SA4	< 20	< 20	< 20	< 20
No.176:SA4SM4	< 20	66	< 20	< 20

SA; C6/36 cell passage. SM: Suckling mouse passage.
(a): Anti-sera used in Virus Research Institute, Bangkok.
(b): Anti-sera used in Osaka University.
(c): Homologous neutralizing titer.

Figure. Results of CF-tests



(Toshihiko Fukunaga)

REPORT FROM

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

Of the first 8000 mosquitoes of 3 species examined to date, which were collected throughout the Yukon Territory, Canada between latitudes 61 and 68°N during June and July 1979, no virus has been isolated by intracerebral injection of newborn mice. Tests are continuing on the remainder of the mosquito collection.

In laboratory experiments conducted with wild-caught Culiseta inornata mosquitoes, 1978 mosquito isolates of snowshoe hare (SSH) (California group) and Northway (NOR) (Bunyamwera group) viruses have replicated after one month's incubation at 4°C and 13°C following intrathoracic injection both of unpassaged virus, and after the viruses received one intracerebral passage in newborn mice. Laboratory-reared Aedes aegypti mosquitoes supported replication of unpassaged SSH and NOR viruses after 3 week's incubation at 28°C both after viruses were fed to or injected into mosquitoes. However NOR virus did not replicate after mosquitoes were fed and incubated at lower temperatures.

(D. M. McLEAN).

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE,
DEPARTMENT OF MEDICAL MICROBIOLOGY,
UNIVERSITY OF TORONTO,
TORONTO, ONTARIO, CANADA.

A Case of Powassan Encephalitis in Ontario, 1979.

A patient was admitted to the Toronto General Hospital on June 2, 1979 with clinical signs of a severe viral encephalitis. A history of tick bite at a cottage on a lake about 80 miles northeast of Toronto on or about May 20 was obtained. Onset of the patient's illness was May 30 which indicated that the incubation period was approximately nine days.

Serological studies were undertaken on serial serum samples as well as cerebrospinal fluid against the following arbovirus antigens - Eastern equine encephalitis, Western equine encephalitis, Powassan, St. Louis encephalitis, California encephalitis (Snowshoe hare serotype) and Colorado tick fever. Hemagglutination inhibiting (HI) and complement fixing antibodies were detected to Powassan but not to any of the other arbovirus antigens. Serology showing a diagnostic rise in serum antibody titers to Powassan antigen is presented in Table 1. Powassan antibody was also detected in cerebrospinal fluid.

This was the sixth case of Powassan encephalitis contracted in Canada and the fourth case encountered in Ontario. It marked the first Canadian case of Powassan infection to be diagnosed in spring months.

Limited field studies were carried out in the area where the infection occurred. Sera was collected from five chipmunks (*Tamius striatus*) and nine dogs. Four of the dogs showed HI antibodies to Powassan antigen. However, no antibodies were found in the chipmunks tested. Three *Ixodes angustus* ticks collected from one chipmunk were tested and found negative for the presence of virus.

L. Spence, H. Artsob and C. Th'ng, National Arbovirus Reference Service, Toronto, in collaboration with;
D.R. Crapper, Staff Physician, Toronto General Hospital and G.A. Surgeoner, Department of Environmental Biology, University of Guelph.

Table 1

Powassan serology of diagnosed case

SPECIMEN	DAYS POST ONSET OF ILLNESS	HI TITERS	CF TITERS
Blood	4	< 1:10	< 1:4
	9	< 1:10	1:16
	12	1:10	1:32
	28	1:20	1:32
	34	1:40	1:64
	42	1:20	1:64
Cerebrospinal fluid	22	Not tested	1:8

REPORT FROM ARBOVIRUSES AND SPECIAL PATHOGENS LABORATORY
LABORATORY SERVICES BRANCH, ONTARIO MINISTRY OF HEALTH
TORONTO, ONTARIO, CANADA

Potential Importation of Dangerous Exotic Arbovirus Diseases. A Case Report of Rift Valley Fever with Retinopathy

Travellers can import viruses highly pathogenic to humans from endemic or affected areas. At least ten times since 1976 our laboratories have handled specimens from suspect cases of dangerous exotic diseases (DED). Some of these viruses such as Rift Valley fever (RVF) pose threats to both livestock and human public health and may have significant economic implications. The possibility that infection with this virus was introduced into South Africa by air traffic or by direct contiguity was raised by Gear et al in 1951. Animal quarantine measures largely prevent direct spread of enzootic diseases which are occasionally transmitted to humans; no such restrictions exist for man.

The case of a 41-year old Canadian worker in Jeddah, Saudi Arabia, illustrates this possibility. She left Jeddah with her son and husband on January 25 for a recreational trip to Kenya and flew directly to Nairobi. They participated in a Safari from Nairobi to Mombasa and spent one night at Amboselly Park. The patient stated that there were a lot of "bugs" in the Park and that they had no contact with animals although they had seen many "cats".

While in Mombasa she became ill on January 29. She complained of fever, sweating, aches and pains, chills, dizziness, weakness, fatigue, some sore throat and severe headache and neck rigidity which lasted two to three days. The patient did not complain of ocular involvement at that time and did not have any rash. The family stayed in Mombasa during this illness and returned to Nairobi by train on January 31 and then flew back to Jeddah. She did not feel well and "could not move" for about two weeks. Early in February she complained of blurring of vision and "difficulty in focusing" then became aware of definite impairment of her vision. When she was seen by a physician during that time he advised her that it was a "flu-like" illness. When her condition did not improve, she was referred to an opthalmologist in Jeddah on March 4. He diagnosed her case as Rift Valley fever and told her that he was "not aware" of any other cases in Jeddah but knew of cases in Cairo.

The patient had no previous history of travel to Africa including Egypt, and her husband and son were well during and following their trip. The patient returned to Canada and was referred for consultation (JAP) and was seen also at the Tropical Disease Unit of Toronto General Hospital. Opthalmological examination (JAP) on March 8th, 1979, revealed normal external findings, motility, refraction, and intraocular pressures bilaterally.

The right eye vision was 20/20, the media were clear, there was

an opaque white exudative lesion 10° temporal to the fovea, 5° in diameter. Angiography showed fluorescein leakage at the site of the lesion and retinal hemorrhage inferior to it. Adjacent vessels were normal, and there was no cellular reaction in the overlying vitreous.

The left eye vision was 20/600, there was minimal aqueous flare, no cells, and clear vitreous. A white exudative lesion involved the left fovea and extended nasally. Fluorescein angiography revealed multiple foci of leakage within the lesion, and retinal hemorrhage inferiorly. Adjacent vessels were patent.

On the basis of fluorescein angiography, Cohen and Luntz,1976, suggest that the lesions seen are ischemic infarcts of retina secondary to small vessel occlusion due to virus proliferation in the endothelium of the vessel wall. There may be leakage from larger vessels, hemorrhage, and cellular reaction in aqueous and vitreous. The lesions in this case are consistent with this explanation and with other reports of retinopathy secondary to Rift Valley fever.

Serological examination of a serum specimen collected on March 8 showed antibody titre of 320 by the haemagglutination inhibition (HAI) test to RVF antigen obtained from the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland (AMRIID).

The patient had no detectable antibodies to the following arbovirus antigens by the HAI and complement fixation tests: Eastern, Western, and California encephalitis; St. Louis encephalitis, Powassan, Dengue, and Banzi. No antibodies were detected to cytomegalovirus and toxoplasmosis by complement fixation and immunofluorescence tests. There was no decline in her HAI antibody titre to RVF antigen in specimens collected on March 18 and June 29.

These results were confirmed by plaque reduction neutralization tests through the kind co-operation of Lt. Col. Dr. C.J. Peters of U.S. AMRIID. The first two serum specimens had neutralizing antibody titres of 5,120 and 10,240 respectively; the third specimen has not been examined yet. Serum specimens from the patient's husband and son collected on June 29 had no detectable antibodies to RVF antigen by the HAI test.

In the absence of an alternative diagnosis and on the basis of the patient's history of travel and disease, these serological results are consistent with the findings by fluorescein angiography and the clinical diagnosis of RVF.

Following a suspect case of Lassa fever in 1976 we have been monitoring specimens referred to our laboratories for histories of travel to areas affected by dangerous exotic diseases. Between

1976 and 1978 specimens from 41 patients returning from 17 different countries were identified. Serological results consistent with dengue fever and travel to dengue affected areas were identified in 11 cases; a patient returning from Nigeria and suspected to have Lassa fever was found negative for LF and developed seroconversion indicative of flavivirus infection. Eight more travellers were investigated till April 1979, three of them for RVF; two cases were negative and the third is described here.

Since the description of "enzootic hepatitis", RVF by Daunbey et al in 1931, activity of this virus was reported in 14 African countries. Virus outbreaks or cases in animals with limited or extensive human involvement were reported; alternatively, virus activity was demonstrated through virus isolation and/or serological surveys. Before 1977, the disease was limited to Sub-Saharan Africa. In that year, however, an extensive outbreak occurred in Egypt with significant involvement of humans and with at least several hundred deaths associated with or attributed to it.

The epidemic in Egypt well demonstrates the potential transmission of this disease far beyond the Sub-Saharan Africa. The spread of disease within Egypt in 1978 raises the possibility of its extension to other Mediterranean countries. The extensive travel between this part of the world and North America, the presence of Canadian and U.S. Armed forces personnel in that area and our and others' experiences in suspect cases of DED are all factors which emphasize the threat of importation of this disease in particular and other exotic diseases in general.

Because of the high concentration of virus in the blood during viremia, man may become a vehicle for spreading this virus into new areas. This potential is further emphasized because of the large number of species of mosquitoes reported as possible vectors of RVF virus.

In the case reported here, the patient's travel history suggests that she was viremic when she returned to Jeddah. Had she travelled directly to her rural Ontario home instead of Saudi Arabia, she could have become a source of infection for local livestock, potentially establishing an enzootic focus in Canada.

In conclusion, it is apparent that (a) the possibility of importing cases of DED is a reality that should not be underestimated, (b) the potential importation and possible spread of a disease such as RVF from man to man and man to domestic livestock under suitable conditions, raises important human and veterinary public health and economic implications, and (c) RVF should be considered in travellers from affected areas who may have ocular or other RVF manifestations. To our knowledge, this is the first reported such case imported to North America, fortunately not during the phase of viremia.

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Arboviral Encephalitides With Special Reference to St. Louis Encephalitis (SLE) In Ontario 1975-1978, An Overview

This composite paper is an overview based on a Report (in press) by the Committee for the Prevention of Mosquito-Borne Encephalitis, Ontario Ministry of Health, edited by M.S. Mahdy*, L. Spence, and J.M. Joshua. As this paper appears in print, the complete Report will be available and contributions to it are not precluded from publications elsewhere.

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The opinions and views expressed both in this paper and in the Report are those of the authors and do not represent directives or policies of their institutions.

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Arboviral Encephalitides With Special Reference to St. Louis Encephalitis (SLE) In Ontario 1975-1978, An Overview

Prior to 1975, there were no records of St. Louis encephalitis (SLE) disease outbreaks in Canada. The occurrence of sporadic cases of undiagnosed SLE during this period might have been a remote possibility and cannot be entirely excluded. During the summer of 1975, the first recorded epidemic of SLE occurred in Canada and involved 68 laboratory documented cases, 66 of them (97%) in southwestern Ontario and one each in Manitoba and Quebec. A presumably imported case of SLE was identified in Espanola, Northern Ontario; the patient apparently acquired infection in Ohio, U.S.A. This case, therefore, is not included further in this discussion

The emergence of SLE in Ontario was epidemiologically linked to the most extensive outbreak of SLE ever recorded in the U.S. The exact number of confirmed SLE cases and deaths during the 1975 epidemic in U.S.A. is uncertain. Originally, 1815 confirmed SLE cases, including 142 deaths were reported, but another communication gave a total number of 2,131 cases which included 171 deaths. The actual number could be even higher and is still under investigation by the Center for Disease Control (CDC), Atlanta, Ga., U.S.A. The extensive spread of SLE in 1975 to areas in North America which had no earlier records of the disease and the occurrence of cases over several months, from late June to October, add to the difficulties involved in this investigation.

Given the climatological conditions of 1975 in southwestern Ontario, an extension of the U.S. epidemic to this part of Ontario perhaps was not surprising. Weather conditions at that time can be described as equivalent to advancing the summer season by one month or having southwestern Ontario moved several hundred miles southward. The prevalent temperatures and the rainfall were favourable for increased mosquito populations and for the early arrival of migratory birds from the south, where SLE virus activity was widespread.

All reported cases of locally acquired SLE in Ontario, at the onset of disease, were residents of an area south of a line extending from the northern limits of Toronto to Sarnia. This line was chosen by the Committee on Programs for the Prevention of Mosquito-Borne Encephalitis, to divide the province into a non-epidemic area and a southwestern belt which was designated a high risk or epidemic zone (Fig. 1). The outbreak consisted of 50 cases in Windsor-Essex, one in Kent-Chatham, four in Lambton-Sarnia, six in the Niagara Regional Area, two in Hamilton-Wentworth and three in Metropolitan Toronto. Five of these 66 cases resulted in death, a case fatality rate of 7.58%

The aetiological agent of this outbreak was identified as St. Louis encephalitis virus by: a) serological results compatible with SLE virus infection in clinically diagnosed cases; b) pathological findings in two fatal cases which revealed changes in the brain indicative of viral encephalitis; c) the isolation, from one of these cases, of a viral agent which was identified as SLE virus both by complement fixation (CF) and neutralization (neut) tests using arbovirus reference antisera.

Specific diagnosis of SLE was made on basis of four-fold or more increase or decrease of complement fixing antibody titre to SLE virus antigen in paired sera or a CF antibody titre of $\geqslant$ 32 in cases with single serum specimens. These criteria were based on those of CDC Atlanta, on the absence of previous records of SLE disease in Ontario and on clinical manifestations consistent with the diagnosis of SLE.

Out of the 66 cases locally acquired in Ontario, 29 (44%) showed seroconversion, 35 (53%) had CF antibody titres of $\geqslant$ 32, one fatal case (1.5%) yielded virus and in another fatality (1.5%) histological changes in brain specimens were compatible with SLE. A 74-year old female patient, who had confirmed SLE disease, was also infected with herpes simplex virus. This was demonstrated by isolating herpes virus from the throat during the acute stage of her illness, then by seroconversion employing the complement fixation and neutralization tests.

The first case of SLE occurred in Windsor in the last week of July. This was soon followed by the large outbreak in Windsor-Essex. The majority of cases in all the affected areas occurred in the four-week period between the second week in August and the third week in September.

The epidemic affected 30 males and 36 females. Most of the cases were in adults; all fatalities were among the 35 (53%) persons who were over 45 years of age. Only six patients (10%) were under 9 years of age.

In addition to the 66 cases of SLE, there were 445 cases of central nervous system (CNS) involvement in Ontario. Viral aetiology was established only in four of these cases. Thus, 441 cases (86%) were of indeterminate origin. The large proportion of undiagnosed cases of encephalitis is not an unusual experience. The picture changes in epidemic years as illustrated by Windsor-Essex in 1975 when SLE virus accounted for 50 out of 154 cases with CNS involvement. The experience in the U.S. is similar. In a non-epidemic year like 1974, almost 70% of the cases of encephalitis were of undetermined aetiology. In contrast, the cause was not established only in 40% of the cases in 1975.

The frequency of sequelae in cases of SLE in the Ontario

outbreak was reviewed six months after the onset of disease. Thirty-three patients (54%) from the 61 survivors had residual symptoms while eight only (13%) had residual organic defects.

Eight to nine months after SLE disease, sera were obtained from 51 patients and tested for residual antibodies. Their titres were below 8 by the CF test in 57% of the cases and below 20 by the haemagglutination inhibition (HAI) test in 33% (Fig. 2).

In 1976, the Committee on Programs for the Prevention of Mosquito-Borne Encephalitis was established under the auspices of the Ontario Ministries of Health and of the Environment. Membership in this Committee included representatives of these Ministries, the University of Guelph, the National Arbovirus Reference Service, Medical Officers of Health and Health and Welfare Canada. Furthermore, other specialists from these Institutions and from other agencies were invited to assist the Committee in its proceedings whenever the need arose.

The Committee made recommendations for mosquito control programs in each of these three years (1976-78) and discussed and proposed plans for extensive investigations on arboviral encephalitides most of which were carried out by various members of the Committee and are presented in this report.

The representation in this Committee and the co-ordination of efforts of those involved have been key factors in the successful implementation of the programs which included: 1) surveillance of arboviruses in southwestern Ontario to detect renewed SLE virus activity or the occurrence of other arboviral infections; 2) assessing the status of SLE and other arboviruses of existing or potential health importance in southwestern Ontario; 3) determining the extent of the spread of SLE infections during the 1975 epidemic within and outside the epidemic zone; 4) estimating the ratio of SLE disease in 1975 to inapparent infections.

The most important aspect of the surveillance program was to establish, after the 1975 outbreak, early warning systems of arbovirus activity. These warning systems included: monitoring mosquito populations for species, numbers and for infection with SLE virus; serological testing of migrating birds and resident English sparrows, caught at several points in the epidemic zone, as well as testing sera from sentinel chickens placed in strategic sites within the high risk area; and surveillance of human cases with suspect acute central nervous system disease.

The population levels of mosquito vectors are important in maintaining and transmitting arboviruses to humans. A total of 209,722 mosquitoes comprising six genera and 35 species were caught between 1976-1978 in southwestern Ontario. More than 90% of these mosquitoes were from three species: *M. perturbans* (55.7%),

A. vexans (22.2%) and Culex pipiens-restuans (12.2%). These species were abundant at the time when three of the four confirmed cases of SLE occurred in 1976. Furthermore, these species were also present in large numbers in areas close to the residences of these three patients and to the location of a sentinel chicken which showed seroconversion to SLE.

Two important species (Culex tarsalis and Aedes sollicitans) which are known to be vectors of equine encephalitides, were detected in small numbers in southwestern Ontario. It is felt, therefore, that in order to forewarn against the possible activity of Eastern equine (EEE) and Western equine encephalitis (WEE) viruses, these mosquito species should be monitored.

During the study, several arboviruses were isolated from mosquito pools. These viruses included SLE, two California group viruses - Snowshoe Hare and Trivittatus -, an agent currently untyped but belonging to the Bunyavirus genus, Hart Park - Flanders and a virus antigenically related to or identical with infectious bursal agent.

In 1976, four cases of SLE were reported in southwestern Ontario and SLE virus was isolated twice from mosquito pools, once from Culex pipiens and the second from a mixed pool of Culex pipiens and Culex restuans.

Experience throughout this period of time showed that weekly monitoring for seasonal activity revealed abundance of mosquitoes during July and August. This approach appears to be a useful tool in helping to predict the possibility of SLE or other arbovirus outbreaks. Furthermore, monitoring mosquitoes and surveillance of virus activity in birds and in humans may permit determining the relationship between population levels of mosquitoes and the incidence of SLE or other mosquito-borne viral infections.

During the period from late 1975 to 1978 about 1500 migrant birds were trapped and bled at Prince Edward Point on Lake Ontario and at Long Point on Lake Erie. More than 3000 blood specimens were also obtained from sparrows caught in Essex County. All these sera were tested for HAI antibodies to SLE, EEE and WEE antigens.

The percentage of sera positive for SLE antibodies was high in sera collected late 1975 and early 1976 but dropped sharply in samples collected between June and September 1976. No HAI antibodies to SLE antigen could be detected in specimens obtained in 1977 and 1978.

Antibodies to EEE and WEE antigens were occasionally detected in 1975 and 1976 in sera from English sparrows. Except for a single EEE positive serum in 1978, sera collected from English sparrows during the 1977-78 survey did not contain detectable HAI antibodies to either EEE or WEE antigens.

Antibodies to EEE and WEE were detected by HAI in sera obtained between 1975-1977 from wild and migratory birds and only to EEE in sera collected in 1978. It appears that there is an ongoing activity of EEE and WEE viruses in these birds, however, viral infection has not been found to extend to horses or humans; perhaps ecological conditions have not been favourable for virus spread.

Sentinel chickens were placed at 11 sites in southwestern Ontario, bled weekly from May to September and tested for HAI antibodies to SLE, WEE, EEE and California encephalitis virus antigens. About 2000 sera were collected in 1976 and 1977. One chicken acquired infection and developed SLE antibodies in August 1976; all other specimens had no detectable HAI antibodies against any of the four antigens.

The use of sentinel chickens to monitor SLE activity in Ontario may be of questionable importance. During 1976, a year of limited SLE activity in Ontario, sentinel chickens were of little value as a monitoring system for early detection of SLE virus activity. The negative results of testing sentinel chickens in 1976, except in one case, may suggest, however, that their use could help in demonstrating the absence of widespread activity of SLE virus.

The possibility of using horses as monitors of SLE activity was also explored. A study on sera collected from 340 horses showed that these animals seem to be poor monitors for SLE. However, they appear to be excellent indicators for certain California encephalitis and Bunyavirus activities in Ontario.

Infections with many viruses may result in an inflammatory process involving the central nervous system. Exclusion of non-viral agents and the identification of specific viral aetiology can only be determined by laboratory tests. In many cases, however, the causative agent of the disease may remain indeterminate. Thus, laboratory surveillance of acute CNS disease in humans is an important means of monitoring arbovirus activity. This program has been a part of the systems established in Ontario following the 1975 outbreak of SLE to forewarn against renewed or newly introduced arboviral disease.

Based on the residual levels of HAI and CF antibodies eight months after disease in 51 patients with SLE in the 1975 epidemic, criteria were set for presumptive diagnosis of suspect cases in 1976 and the following years. This approach has practical importance because it helps to expedite additional mosquito control measures when presumptive diagnosis of SLE was made particularly in the high risk area.

From 1976 to 1978, laboratory tests were performed to investigate 1664 patients with any of a wide variety of clinical manifestations suggesting either definite or suspect involvement of the CNS. The effectiveness of this surveillance program was demonstrated in 1976

when four new cases of SLE were confirmed by laboratory tests. Three of them met the criteria for presumptive diagnosis and signalled the earliest alarm of the warning systems for renewed SLE activity, thus prompting additional mosquito control measures. The new cases of SLE occurred in the focal area of the 1976 epidemic: Windsor-Essex (3) and Kent-Chatham (1). The seasonal activity of SLE was evident in 1975 and 1976 and specific infection with SLE virus was confined to the epidemic zone. Fig. 3 shows the distribution of SLE cases by Health Unit and time of disease onset in 1975 and 1976.

No evidence of SLE disease was obtained in 1977 and 1978. The small number of human cases in 1976 (4) and the absence of detectable human disease in 1977 and 1978 are consistent with data obtained through surveillance of SLE virus infection of mosquitoes, English sparrows, migrant birds and sentinel chickens over the three-year period.

Arboviral infections, other than SLE, were found to be associated with illness in 13 cases. Infection with Powassan virus was identified as the cause of meningoencephalitis in a case in Eastern Ontario in 1977, the fifth recorded in Canada. Infection with dengue virus was compatible in 11 cases with clinical diagnosis and with travel history to areas affected by the widespread epidemic of dengue fever in the Caribbean 1977-78. Seroconversion to flavivirus(es) was detected in 1978 in a patient returning from Nigeria and suspected to have Lassa fever. These last 12 cases illustrate that dangerous exotic communicable diseases may be imported from affected areas by travellers.

Infections with viruses other than arboviruses were detected in 69 patients with suspect or definitive CNS disease. Coxsackie B5, echo 9 and herpes simplex viruses accounted for 43% of these infections. Furthermore, a variety of entero and adenoviruses, mumps, herpes simplex and varicella-zoster viruses were isolated over the period from 1950-1978. Poliovirus emerged again in 1978 to cause six cases of paralysis in non-immunized individuals.

The majority of the 1664 cases investigated between 1976-78 remained, however, with unestablished cause. Analysis of encephalitis surveillance data obtained from the reports of the Center for Disease Control, Atlanta, for the period from 1960-1975, shows that 54.5% from 38,128 cases remained without known cause (Fig. 4). Investigation of the large number of cases of acute CNS disease with indeterminate aetiology deserves greater attention.

Two separate serological surveys on 8885 specimens collected late in 1975, in 1976 and 1977, were undertaken by two independent institutions. The objectives were to determine the spread of the 1975 epidemic and to estimate specific SLE infection rates.

One survey involved testing 4032 sera, 1867 from residents of Niagara region and 2165 from Windsor area. No definite evidence was obtained to indicate infection with either Eastern equine encephalitis, Powassan, Trivittatus and Flanders viruses. Flanders virus has been repeatedly isolated from mosquito pools collected in southwestern Ontario during the study period.

Neutralizing antibodies, however, were detected to SLE virus in 19 sera and to Snowshoe Hare (SSH) virus in 54 sera. In the Niagara region, 7 sera were positive for SLE and 19 for SSH; in the Windsor area, 12 specific reactors to SLE and 36 to SSH were identified. The specific infection rates were then determined as the percentage of individuals with neutralizing antibodies among those prescreened and found positive to SLE antigen by the HAI test. Specific SLE infection rate was 0.37% in the Niagara and 0.55% in Windsor areas; meanwhile the ratio of diagnosed cases to estimated SLE infection in 1975 was 1:225 and 1:29 for both areas respectively.

The second survey was focused mainly on flavivirus infections; it involved samples from both the epidemic (3808) and non-epidemic (1045) zones. Seventy-seven sera (1.6%) had HAI antibodies to flavivirus antigens. Only 18 sera were specific for SLE virus by the neutralization test; 17 of them were from the high risk area (a specific infection rate of 0.45%). The remaining reactor to SLE was from the non-epidemic zone and, in the absence of an interview, the possibility of travel to SLE affected areas cannot be ruled out. The ratio of confirmed SLE cases to the estimated number of infections for the Windsor area was 1:27. These figures are very close to those of the first survey and results of both studies agree with reported ratios.

Results of both surveys support the suggestion that residents of 1975 epidemic zone in Ontario were not significantly exposed to SLE prior to that outbreak. Furthermore, human infections in 1975 have been mainly confined to the focal areas of the epidemic and did not spread widely.

In Ontario, mosquito control prior to late 1975 had been undertaken by a limited number of municipalities for nuisance and pest prevention only. Upon confirmation of St. Louis encephalitis in the Windsor area in September 1975, a large number of municipalities in southwestern Ontario were encouraged to implement mosquito abatement programs. These incorporated an integrated effort to eliminate breeding habitat by draining or filling or by treatment with approved larvicides, only after the presence of mosquito larvae had been confirmed through surveillance. Only appropriately trained and licenced personnel were permitted to apply pesticides. Data were collected on the quantities of pesticides used and their associated environmental impact, this moderated concerns of critical environmentalists. Public concern about mosquitoes as vectors of

encephalitis has declined over the past few years, therefore, actual abatement control activities in the "high risk" area have also decreased.

In facing disease outbreaks and in attempting to control and prevent them, government efforts towards these goals have to be communicated to the public in order to marshal its support and participation. A media campaign, using radio and television broadcasts, pamphlets, posters and other means, fulfilled its role during and following the 1975 epidemic. Alerting the public and promoting its interest in and support of measures to prevent and control such epidemics are important factors that deserve serious consideration and careful planning.

The events associated with the 1975 Ontario epidemic of SLE and following it have generated a major interest in the ecology of arboviruses, in arboviral infections and in acute CNS disease of indeterminate cause. The emergence of the first recorded outbreak of SLE has been a catalyst which prompted a team approach towards the investigation and prevention of SLE or other potential mosquito-borne arboviruses in Ontario. This team approach allowed the Committee to mount the integrated efforts of several disciplines at various levels of governments as well as those of academic institutions. Furthermore, excellent liaison has been established with other provincial, state and federal bodies involved in surveillance of arboviral diseases and their control.

M.S. Mahdy, Laboratory Services Branch, Ontario Ministry of Health

L. Spence, National Arbovirus Reference Service

T.P. Subrahmanyan, Laboratory Services Branch, Ontario Ministry of Health

J.M. Joshua, Communicable Disease Control, Ontario Ministry of Health

FIG. 2 RESIDUAL ANTIBODIES IN 51 CONFIRMED CASES OF ST. LOUIS ENCEPHALITIS EIGHT MONTHS AFTER ILLNESS

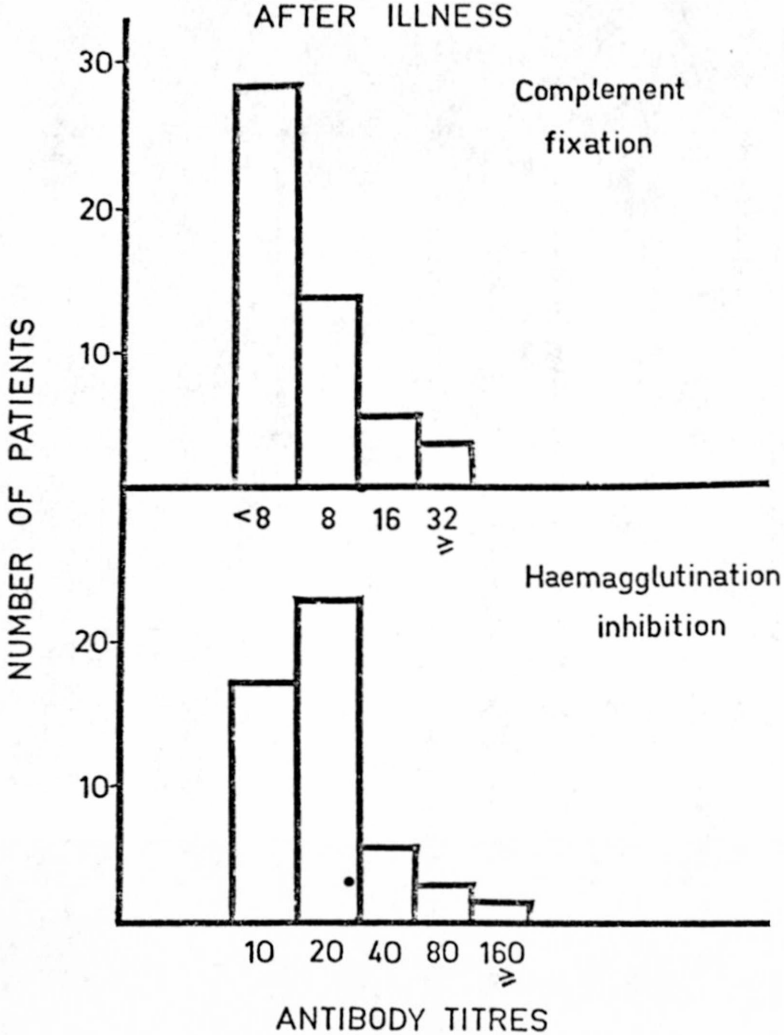
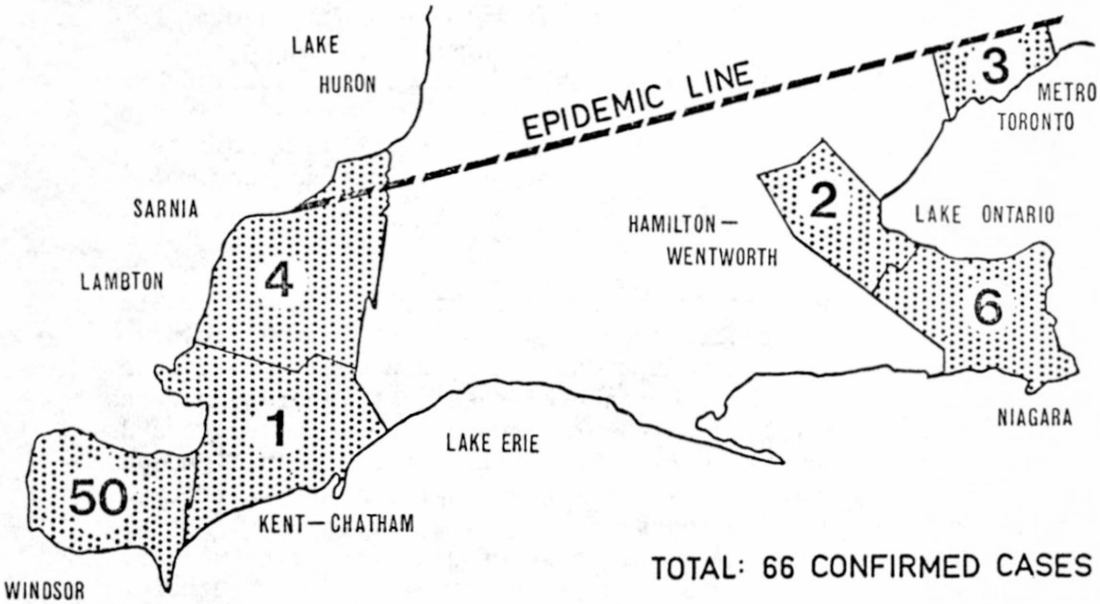


FIG. 1 ST. LOUIS ENCEPHALITIS IN SOUTHWESTERN ONTARIO IN 1975



TOTAL: 66 CONFIRMED CASES

FIG. 3 CONFIRMED CASES OF ST. LOUIS ENCEPHALITIS, ONTARIO 1975-1976 BY HEALTH UNIT AND TIME OF DISEASE ONSET

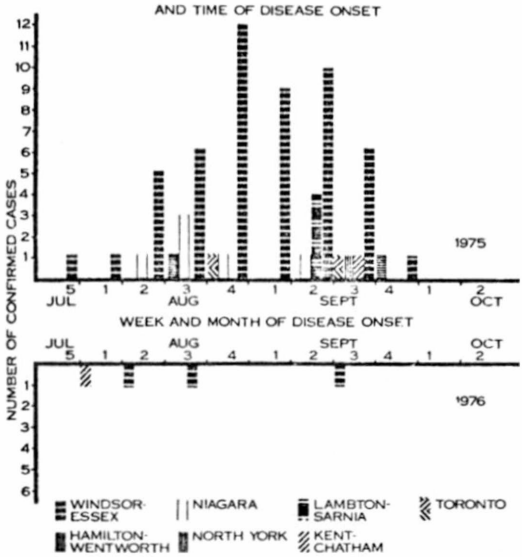
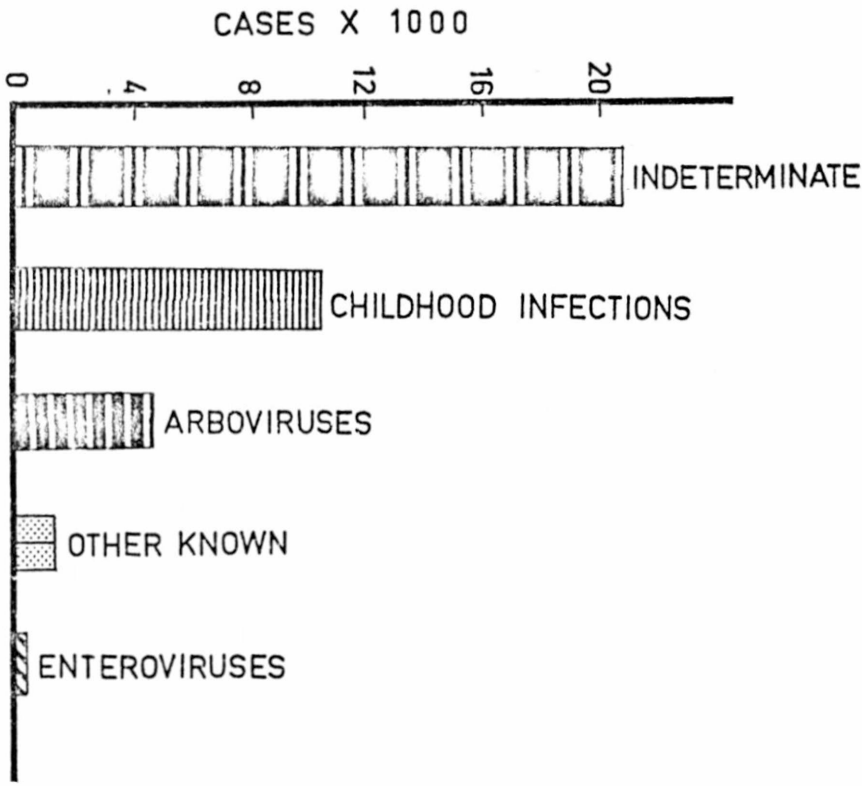


FIG. 4 CAUSATIVE AGENTS OF NONBACTERIAL ENCEPHALITIDES – 1960 – 1975, U.S.A.



Ministry of Health
Ontario

Arboviral Encephalitides in Ontario with Special Reference to St. Louis Encephalitis

The Committee on Programs for the Prevention of Mosquito-borne Encephalitis

Edited by
M.S. Mahdy
L. Spence
J.M. Joshua

1979

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M.S. Mahdy

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REPORT FROM THE VIROLOGY RESEARCH CENTRE, INSTITUT ARMAND-FRAPPIER (I.A.F
LAVAL-DES-RAPIDES, QUEBEC, CANADA

Activity in 1979 of California encephalitis group viruses in Entrelacs, Province of Québec

In 1978, human cases of California encephalitis which occurred in Entrelacs, 60 miles north of Montréal, were serologically detected by the virus diagnostic laboratory (I.A.F.).

Field work was undertaken during the spring and summer of 1979 in order to study the persistence of the virus in the area and to isolate and identify the virus strain(s) from mosquitoes and from animals.

Since May 29 1979, six indicator rabbits were placed at three different locations (at 50 m, 100 m, and 1000 m from the house where the human cases were diagnosed) and sera were collected weekly.

Acetone treated sera were tested by haemagglutination inhibition (HI) against EEE, WEE, SLE, Powassan and California encephalitis (Snowshoe Hare) antigens provided by H. Artsob (National Arbovirus Reference Service, Toronto).

Seroconversions to the California encephalitis virus group were detected in all rabbits from the three locations during June and July. Seroconversion to the other antigens tested was not observed.

Sera, collected one week prior to seroconversion and kept at -70°C were inoculated into suckling mice. Two virus isolates were obtained. Antigens, prepared from mouse brain, were identified, by CF with NIH reference ascitic fluids, as members of California encephalitis virus group.

As demonstrated for some members of the CAL group, our preliminary results suggest the persistence of the virus in the area of Entrelacs.

Larvae and adult mosquitoes collected in the same area in 1979 are under study for virus isolation and identification of the mosquitoes species acting as vector of California encephalitis virus in this region.

S. Belloncik, L. Poulin and M. Fauvel

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

A mid-season surveillance report on arbovirus activity in California is briefly summarized here; a final report for 1979 will be available for the next issue. No human cases of western equine encephalomyelitis (WEE) or St. Louis encephalitis (SLE) have been detected as yet, but at least two presumptive positive WEE cases in equines have occurred, (Imperial and Sacramento Counties) in areas where WEE virus activity in mosquitoes or sentinel chickens was also demonstrated. Mosquito pool testing, done in collaboration with the University of California, Berkeley, School of Public Health (UCBSPH), has yielded 55 strains of WEE virus, 3 strains of SLE virus, and 38 strains of other viruses (Turlock, Hart Park, Bunyamwera group) thus far. Virus isolates can be identified the same day the suckling mice become ill, and reports are telephoned to mosquito control personnel promptly. A program of statewide sentinel chicken serologic testing is being participated in by this Laboratory also in collaboration with the UCBSPH, with monthly bleedings tested on a current basis by the indirect immunofluorescent (IIF) method. Seroconversion of 87 chickens for WEE and 4 for SLE has been shown thus far. Complete comparison of these results with HAI and plaque-reduction neutralization antibody titers will be done later. Preliminary results indicate that although the IIF method is rapid and convenient, some uncertainty about interpretations at the lowest serum dilutions may occur.

Three dengue cases in overseas travelers have been detected thus far in 1979 and are being studied serologically by IIF, complement fixation (CF), and fluorescent focus inhibition methods. A suspected case of Ross River virus disease in a Californian returned from Fiji was referred to the CDC Laboratory, Fort Collins, Colorado, where stationary Ross River antibody titers were shown in paired sera, suggesting previous infection but not substantiating the virus as a cause of the current illness. Other examples of "exotic" arboviral infections will doubtless occur in the future.

Only 5 cases of Colorado tick fever have been detected in 1979, 2 from known endemic areas in California, one from tick exposure in Utah, and 2 from tick exposure in Colorado.

Rhabdovirus studies continue, including characterization of rabies virus strains isolated from apparent live virus-vaccine-induced cases of paralytic disease in dogs and cats, ecology of rabies in bat species, natural and experimental pathogenesis of rabies by immunofluorescent and histopathologic methods, and study of a human case of rabies in July, 1979 (presumed exposure in Mexico).

(R.W. Emmons)

REPORT FROM THE VECTOR-BORNE DISEASES DIVISION, CENTER FOR DISEASE CONTROL
FORT COLLINS, COLORADO

LIMITED USEFULNESS OF THE CF TEST FOR DISTINGUISHING 17D YELLOW FEVER VACCINE-INDUCED FROM NATURALLY ACQUIRED IMMUNITY

On the basis of previous studies, it has long been stated that 17D yellow fever (YF) vaccine generally does not induce complement-fixing (CF) antibodies, and that the presence of CF antibodies could be used in epidemiological studies to distinguish individuals infected with wild YF virus from vaccinated persons. In January 1979, seroepidemiological investigations were conducted during a YF epidemic in the Gambia, West Africa. Since a mass vaccination campaign was also in progress, it was important to confirm that the CF test could be used for serodiagnosis and determination of the incidence of natural YF infections. The serological responses of 58 individuals who received 17D YF vaccine were studied. The vaccinees fell into three groups: (1) those with prevaccination YF neutralizing (N) antibodies; (2) immunological virgins without prevaccination YF-N antibody or hemagglutination-inhibiting (HI) antibodies to heterologous flaviviruses (Zika, West Nile, dengue 1, Uganda S, Spondweni, or Ntaya; and (3) those without prevaccination YF N antibodies but with heterologous flaviviral HI antibodies. Vaccination of persons without prior flaviviral immunological experience resulted in monotypic YF HI and/or N antibody seroconversions, but no CF antibody response. The presence of prevaccination YF N antibodies blocked serological response to the vaccine in a high proportion of the cases; however, 24% of vaccinees in this group had a marked rise in $\log_2$ YF CF antibody titer (mean increase of 3.9). Thirteen (46%) of 28 persons without prevaccination YF N, but with heterologous flaviviral HI antibodies demonstrated YF CF antibody seroconversion or increase in titer vaccination; in this group the mean increase in $\log_2$ YF CF antibody titer was 2.1. The CF antibody response was generally broadly cross-reactive, but in a few individuals the YF CF antibody response was homotypic. Nine different patterns of HI and CF homologous and heterologous antibody responses were defined. The practical significance of these studies is that they demonstrate that in a high percentage of persons with prior flavivirus exposure, anamnestic serological responses to YF vaccine result in CF antibodies similar to those induced by natural YF virus infection. In Africa and tropical America, where the background of flaviviral immunity is high, it is imperative that seroepidemiologic investigations during or after YF outbreaks be conducted prior to vaccination.

(T.P. Monath, R. Craven, C.H. Calisher, D.J. Muth, C.J. Trautt)

Transmission of St. Louis encephalitis virus from Argentina by mosquitoes of the Culex pipiens complex.

St. Louis encephalitis (SLE) virus transmission cycles in tropical America and temperate South America are poorly defined. Elucidation of these cycles may help explain the lower incidence and relatively mild nature of disease in these areas in comparison to temperate North America where epidemics occur. We examined the virus-vector relationships of a SLE viral strain and a *Culex pipiens quinquefasciatus* strain from Argentina in experimental transmission studies. A mosquito strain and a SLE viral strain from temperate North America were included in the study as controls. Colony strains of *Cx. p. quinquefasciatus* from Esperanza, Santa Fe Province, Argentina, and *Cx. pipiens* complex mosquitoes from Memphis, Tennessee, were fed on chicks infected with either of 2 SLE viral strains from Esperanza and Dayton, Ohio, incubated for 20 days at 27 to 28° C, then given an opportunity to refeed individually on uninfected chicks. The results are summarized in Table 1.

All of the Esperanza mosquitoes were infected on the 20th day of extrinsic incubation irrespective of the viral strain used. A high proportion of Memphis mosquitoes became infected (87.8 and 89.8%); however, they were less efficient in transmitting virus than their Esperanza counterparts (58.3% vs 90.0% Ohio SLE virus, and 40.0% vs 90.5% Esperanza SLE virus). These differences may be due, at least in part, to the fact that the Memphis mosquitoes had fed on viremic chicks circulating lesser amounts of virus (Table 1). In any event, our results show that *Cx. p. quinquefasciatus* from Esperanza is a very efficient vector of SLE virus under experimental conditions, and that the Experanza SLE viral strain does not differ markedly from a temperate North American strain in its ability to infect and be transmitted by members of the *Cx. pipiens* complex. It seems unlikely, therefore, that the absence of urban-suburban outbreaks of SLE in Santa Fe Province, Argentina, can be explained on the basis of low vector potential among the *Cx. p. quinquefasciatus* population.

(Carl J. Mitchell, Thomas P. Monath, and Marta S. Sabattini)

Table 1. SLE virus infection rates in mosquitoes on the 20th day of extrinsic incubation and transmission rates to clean chicks.

Virus strain	Virus titer* of infective meal	Ratio and (%) infected	Ratio and (%) transmitting
		Memphis *Cx. pipiens* complex	
Ohio (75V-16333)	4.0-4.1	44/49 (89.8)	7/12 (58.3)
Esperanza (78V-6507)	3.8-4.0	36/41 (87.8)	6/15 (40.0)
		Esperanza *Cx. p. quinquefasciatus*	
Ohio (75V-16333)	4.6-5.0	49/49 (100)	9/10 (90.0)
Esperanza (78V-6507)	4.1-4.8	49/49 (100)	19/21 (90.5)

*Log_{10} DECC PFU/ml

Evidence for transovarial transmission of SLE virus in *Culex pipiens* complex mosquitoes

Transovarial transmission of SLE virus to progeny could ensure that infected mosquitoes entered hibernation in the fall even though they had not had previously blood-fed. Emergence from hibernation, feeding on vertebrates, and subsequent TOT to first generation progeny, would provide for virus amplification both via transmission by infected hibernating females and also by those progeny infected transovarially.

As part of a project to look for differences in SLE virus transmission efficiency among geographic strains of *Culex pipiens* complex mosquitoes, an effort was also made to document the occurrence of transovarial transmission of SLE virus. A total of 7554 and 25,951 progeny of first generation *Culex pipiens* complex females collected in McLeansboro, Illinois and Memphis, Tennessee respectively were tested for evidence of TOT. Females reared from field collected adults were fed on chicks inoculated subcutaneously with an SLE stock virus (75V15838) prepared by intrathorasic inoculation of colonized *Culex pipiens* mosquitoes with the original mosquito suspension yielding SLE virus. Egg rafts were collected at varying intervals following extrinsic incubation at 24°C. All females fed on viremic chicks were individually tested for virus individually regardless of whether they oviposited or not. Infection rates were virtually 100%. Progeny were reared, held for 7-10 days and assayed for virus in pools of 50 each in primary duck embryo and Vero cell cultures. Suckling mice were inoculated with selected specimens. A number of females were initially fed on a viremic chick, eggs collected and the females refed on a normal chick. Progeny from the second oviposition were reared for testing. Mosquitoes from this procedure represent approximately 1000 of the above noted totals.

Transovarial transmission was documented once by SLE virus isolation and reisolation from a pool of female mosquitoes. The single TOT was from first oviposition progeny reared from Tennessee females blood-fed once only. The eggs were collected 24 days following the infectious blood feeding. The frequency of TOT in this study was extremely low considering the total number of progeny processed; however, the experimental design in the study was not optimal for demonstration of TOT. The significance of the observations reported is documentation of the occurrence of TOT of SLE virus in *Cx. pipiens* complex mosquitoes. Additional studies using an experimental design to increase the probability of TOT and quantitate the frequency of occurrence of this phenomena will be necessary to assess the frequency of TOT in *Cx. pipiens* complex mosquitoes and its importance in the ecology of SLE virus.

Studies to detect TOT of SLE virus in Aedes vexans and Aedes melanimon were also conducted. In contrast to the virtual 100% infection rates obtained in Culex pipiens complex mosquitoes fed on viremic chicks, only about 60% of the Aedes species became infected following a blood-feeding on the chicks. Among a smaller group fed on pledgets saturated with blood-virus mixtures, only 10% became infected despite higher virus content than in the blood of viremic chicks. No virus was recovered from any of 2001 and 831 progeny reared from the Aedes vexans and Aedes melanimon respectively.

(D. B. Francy and W. A. Rush)

Serologic comparisons of Guama group viruses:

The six recognized Guama bunyaviruses have been isolated only in the Americas. Guama and Catu viruses have been isolated from humans with fever, headache and muscle pain but Bertioga, Bimiti, Mahogany Hammock and Moju viruses have not. These six viruses appear to be transmitted in nature principally from Culicine mosquitoes to rodents.

During field studies of Rocio virus in Brazil six serologically related isolates were found to be related to the recognized Guama group viruses. This report describes the identification of two new Guama group viruses and their relationships to other members of the group and to each other. In addition, we have shown that Guaratuba and Mirim viruses, ungrouped members of the family Bunyaviridae, are closely related to the Guama group viruses and should be classified with them. Minatitlan virus, an unassigned member of the Bunyaviridae, was used as a control.

Table 1 presents brief histories of strains used in these studies. Strains 76V-25271, 77V-14662 and 76V-25643 were isolated by personnel of the Vector Ecology Branch, CDC, Ft. Collins and strains SPAn 47817, SPAn64706 and SPAn 64962 by personnel of the Secao de Virus Transmitidos Por Artropodos, Instituto Adolfo Lutz, Sao Paulo.

Table 2 presents certain characteristics of the six field isolates as well as the registered Bertioga and Guaratuba prototype viruses.

Results of cross-comparisons of the six field isolates, six recognized Guama group viruses and Guaratuba, Mirim and Minatitlan viruses by complement-fixation (CF) tests are summarized in Table 3. Bertioga, Guaratuba, Mirim and the six field isolates belong to one complex while Guama, Bimiti, Moju, Catu and Mahogany Hammock viruses belong to another. Minatitlan virus appears by these tests to be only a distantly related bunyavirus.

In an attempt to simplify further testing the six field isolates were cross-tested by serum dilution-plaque reduction neutralization (N) tests in Vero cells (Table 4). Isolates 76V-25271 and SPAn 64706 are, by these tests, essentially indistinguishable and closely related only to each other. Isolates 77V-14662 and SPAn 64962 are identical only to each other and isolates 76V-25643 and SPAn 47817 are distinct from all other viruses tested.

Additional N tests, using strains SPAn 47817, SPAn 64706, SPAn 64962 and 76V-25643 as well as the six recognized Guama group viruses, Guaratuba, Mirim and Minatitlan viruses were performed. A summary of these results are presented in Table 5.

Strain SPAn 47817 is a newly recognized virus for which we suggest the name Itimirim after the town (24°45'S, 47°48'W) in Iguape County, Sao Paulo State, Brazil where we trapped the rat from which this virus was isolated.

Strain SPAn 64706 (and, because it is identical by N tests with SPAn 64706, strain 76V-25271) is a strain of Guaratuba virus.

Strains 77V-14662 and SPAn 64962 are identical strains of a newly recognized virus for which we suggest the name Cananeia after the locality where we collected the mosquitoes from which we isolated the prototype strain 77V-14662.

Strain 76V-25643 is a strain of Bertioga virus.

While little is known of the natural history of Guama group viruses, it is clear from the work of Jonkers, Spence and Olivier (1), Causey, Causey, Maroja and Macedo (2), and others, that these viruses are transmitted principally by Culex (Melanoconion) sp. mosquitoes to rodents in tropical habitats. Because two members of the group cause human disease, the potential public health significance of the Guama bunyaviruses should be investigated further.

In Figure 1, we present a proposed classification of Guama group viruses. We suggest that Itimirim and Cananeia viruses belong to be proposed Bertioga complex, as do Guaratuba and Mirim viruses.

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References:

(1) Jonkers, A.H., Spence, L. and Olivier, O. (1968) Am. J. Trop. Med. Hyg. 17:299-307.

(2) Causey, O.R., Causey, C.E., Maroja, O.M. and Macedo, D.G. (1961) Am. J. Trop. Med. Hyg. 10:227-249.

TABLE 1. History of strains used.

Virus	Strain	Source	Location	Date Collected	Passage Level
Guaratuba	76V-25271	Culex (Mel.) sp. 53	Sao Paulo, Brazil	Feb., 1976	SM_3
Cananeia	77V-14662	Cx. (Mel.) sp. 51	" " "	" "	V_1SM_2
Bertioga	76V-25643	" "	" " "	Mar., 1976	SM_3
Itimirim	SPAn 47817	Oryzomys sp.	" " "	Nov., 1976	SM_5
Guaratuba	SPAn 64706	Sentinel (hamster)	" " "	Apr., 1978	SM_6
Cananeia	SPAn 64962	Sentinel (mouse)	" " "	Apr., 1978	SM_4
Bertioga	SPAn 1098	" "	" " "	Mar., 1962	SM_8
Guaratuba	SPAn 12252	" "	" " "	Feb., 1969	SM_2
Mirim	BeAn 7722	Sentinel (Cebus apella)	Para, Brazil	Aug., 1957	SM_3
Guama	BeAn 277	" " "	" "	Mar., 1955	SM_7
Bimiti	Tr 8362	Cx. (Mel.) spissipes	Trinidad, W.I.	July, 1955	SM?
Moju	BeAr 12590	Cx. (Mel.) sp.	Para, Brazil	Aug., 1959	SM_5
Catu	BeH 151	human	" "	Feb., 1955	SM_{13}
Mahogany Hammock	FE4-2S	Cx. (Mel.) opisthopus	Florida, U.S.A.	Jan., 1964	SM_3
Minatitlan	MEX67-U5	Sentinel (hamster)	Veracruz, Mexico	Sept., 1967	SM_6

TABLE 2. Characteristics of eight Guama group isolates from Brazil.

Virus	Strain	SM	Titer[a]			Pathogenicity[b]		HA[c]
			Vero	DE	Plaque size (mm)	ic	ip	
Itimirim	SPAn 47817	7.0(4)	7(4)	<3.0	2-3	+	-	-
Cananeia	SPAn 64962	7.0(6)	7(5)	<3.0	<1	-	-	-
Cananeia	77V-14662	6.6(5)	6.6(4)	<3.0	<1	-	-	-
Guaratuba	76V-25271	6.5(4)	6.0(4)	<3.0	<1	-	-	-
Guaratuba	SPAn 64706	7.2(4)	7.2(4)	<3.0	<1	+	-	-
Bertioga	76V-25643	8.0(6)	7.8(4)	<3.0	1	-	-	-
Bertioga	SPAn 1098	>5.0(6)	7.0(4)	<3.0	1	-	-	+
Guaratuba	SPAn 12252	8.0(7)	8.0(4)	<3.0	2	-	-	-

a) Titer given as $\log_{10}$ suckling mouse (SM) intracranial lethal dose or plaque forming units per ml. Numbers in parentheses indicate average survival time of mice or time to appearance of plaques.

b) Pathogenicity for 3-week-old mice inoculated intracranially (ic) or intraperitoneally (ip); - indicates survival of mice with antibody production, + indicates death of mice.

c) Sucrose acetone extracted SM brain antigen tested for hemagglutinin (goose cells) at pH 5.75-7.0.

TABLE 3. Results of cross complement-fixation tests with six Guama group viruses, Guaratuba, Mirim, Minatitlan Viruses and five isolates from Brazil.

		Titer[a] of Antibody to:															
Antigen	Strain	76V-25271	77V-14662	76V-25643	SPAn 47817	SPAn 64706	SPAn 64962	BER	GTB	MIR	GMA	BIM	MOJU	CATU	MH	MTN	Normal
Cananeia	77V-14662	16	≥1024	≥1024	64	≥1024	≥1024	≥1024	128	16	16	-[b]	8	-	-	-	-
Bertioga	76V-25643	16	≥1024	≥1024	128	256	≥1024	512	64	8	16	-	16	-	-	-	-
Itimirim	SPAn 47817	128	512	≥1024	256	128	≥1024	256	512	8	32	-	32	-	-	8	-
Guaratuba	SPAn 64706	128	256	≥1024	256	512	≥1024	256	512	8	32	-	32	-	-	8	-
Cananeia	SPAn 64962	16	≥1024	≥1024	128	128	≥1024	≥1024	256	8	16	-	8	-	-	-	-
Bertioga	SPAn 1098	16	≥1024	≥1024	32	64	≥1024	≥1024	64	-	16	-	8	-	-	-	-
Guaratuba	SPAn 12252	128	512	≥1024	≥1024	512	≥1024	256	512	32	64	-	64	-	-	8	-
Mirim	BeAn 7722	8	64	256	64	32	8	512	64	32	8	-	-	-	-	-	-
Guama	BeAn 277	8	-	8	-	16	8	-	-	-	≥1024	128	≥1024	128	32	-	-
Bimiti	Tr 8362	-	-	-	-	8	-	-	-	-	≥1024	512	≥1024	256	32	-	-
Moju	BeAr 12590	-	-	8	-	8	-	-	8	-	≥1024	256	≥1024	128	16	-	-
Catu	BeH 151	-	-	-	-	8	-	-	8	-	≥1024	256	≥1024	128	16	-	-
Mahog. Ham.	FE4-2S	-	-	16	-	16	8	-	8	-	≥1024	64	512	64	128	-	-
Minatitlan	MEX67-U5	-	-	16	-	-	32	8	8	8	8	-	8	-	-	512	-
Normal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a) Results given as reciprocal of highest antibody titer at optimal antigen dilution.

b) - = <8.

TABLE 4. Comparison of six Guama group isolates from Brazil by serum dilution plaque reduction neutralization tests in Vero cells.

Strain	76V-25271	SPAn 64706	77V-14662	SPAn 64962	76V-25643	SPAn 47817
76V-25271	320	640	-	-	-	-
SPAn 64706	640	≥1280	-	-	-	160
77V-14662	-	-	320	640	-	-
SPAn 64962	-	-	640	640	-	80
76V-25643	-	-	80	-	320	-
SPAn 47817	-	-	80	-	-	≥1280

- = <40

TABLE 5. Results of serum dilution plaque reduction neutralization tests, in Vero cells, with Guama group viruses, Guaratuba, Mirim, Minatitlan viruses and four isolates from Brazil.

		Titer[a)] of Antibody to:												
Virus	Strain	SPAn 47817	SPAn 64706	SPAn 64962	76V-25643	BER	GTB	MIR	GMA	BIM	MOJU	CATU	MH	MNT
Itimirim	SPAn 47817	≥1280	-	-	-	-	-	40	-	-	-	40	-	-
Guaratuba	SPAn 64706	-	640	-	-	-	320	-	-	-	-	-	-	-
Cananeia	SPAn 64962	-	-	640	-	-	-	-	-	-	-	-	-	-
Bertioga	76V-25643	-	-	-	640	320	-	-	-	-	-	40	-	-
Bertioga	SPAn 1098	-	-	-	640	320	-	-	-	-	-	-	-	-
Guaratuba	SPAn 12252	-	≥1280	-	-	-	320	-	-	-	-	-	-	-
Mirim	BeAn 7722	-	-	-	-	-	-	320	-	-	-	-	-	-
Guama	BeAn 277	-	-	-	-	-	-	-	≥1280	-	-	-	-	-
Bimiti	Tr 8362	-	-	-	40	-	-	-	-	≥1280	-	-	-	-
Moju	BeAr 12590	-	-	-	-	-	-	-	-	-	320	-	-	-
Catu	BeH 151	-	-	-	-	-	-	-	-	-	40	640	-	-
Mahog. Ham.	FE4-2S	-	-	-	-	-	40	-	160	-	40	-	≥1280	-
Minatitlan	MEX67-U5	-	-	-	-	-	-	-	-	-	-	-	-	≥1280

a) Titers given as reciprocal (90% plaque reduction)

b) - = <40

Figure 1. Proposed Classification of Guama Group Viruses

Group	Complex	Virus	Subtype
Guama	Guama	Guama	
		Bimiti	
		Moju	
		Catu	
		Mahogany Hammock	
	Bertioga	Bertioga	
		Guaratuba	
		Cananeia	
		Itimirim	
		Mirim	

REPORT FROM THE DEPARTMENT OF VETERINARY SCIENCE
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LAC x SSH reassortant virus infections in wildlife hosts. We have begun inoculation of chipmunks (*Tamias striatus*) and showshoe hares (*Lepus americanus*) with La Crosse (LAC), snowhsoe hare (SSH), and three genetically defined LAC x SSH reassortant viruses derived by Dr. David Bishop. We will compare parental LAC and SSH virus infections in their natural vertebrate hosts with those of their progeny. Parameters measured are: a) median infectious dose, b) frequency and course of disease, if any, and c) viremia duration and magnitude.

Table 1 summarizes results to date for chipmunks, natural hosts of LAC virus. Neither parent nor reassortant viruses have caused detectable disease in adult chipmunks. Parental SSH virus seems to be more virulent than the other strains because of shorter incubation period in suckling mice and higher frequency of chipmunk infection. One reassortant (LAC-LAC-SSH) may be less virulent by the same parameters. Parental LAC virus and the other two reassortants appear to be intermediate in mouse pathogenicity and chipmunk infectivity. Viremia studies are in progress. A high frequency of naturally immune wild-caught experimental animals has delayed inoculation of snowshoe hares, natural hosts of SSH virus.

Experimental inoculation of Peromyscus species with epizootic hemorrhagic disease virus. Preliminary serologic evidence has indicated that wild caught *Peromyscus* species may have naturally acquired antibody to epizootic hemorrhagic disease virus (EHDV). Our research goal was to determine if experimentally inoculated *Peromyscus* sp. could become infected, and once infected, if virus could be recovered from blood, brain, feces, urine and/or oral swabs. Low passage, tissue culture adapted EHDV-NJ, antigenic type 1, was inoculated intracerebrally or intranasally or by subcutaneous/hematoma.[1] Virus was recovered only from animals inoculated intracerebrally. Few mice responded with detectable levels of neutralizing antibody.

To determine the reliability of demonstrable antibody as an indicator of infection, mice were challenged with homologous virus intracerebrally (ic) 60 days after the initial inoculation to elicit a possible anamnestic response. More individuals had detectable antibody five days after challenge than during the primary response phase. Mice with no previous exposure to EHDV, inoculated ic on day 60, did not have detectable antibody five and eight days after inoculation. The presumed secondary antibody response appears to be commensurate with an anamenstic response. Antibody responses of all mice appear in tables 2 and 3, and figure 1. We believe that mortality following challenge was not due to EHDV infection.

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[1] subcutaneous/hematoma - an individual's heparinized whole blood was mixed 1:1 with virus and inoculated subcutaneously.

Table 1. Infection of chipmunks with SSH x LAC reassortant viruses and their prototype parent strains.

Virus	SM AST*	Chipmunk SC Dose ($SM_{ic}LD_{50}$)	Number of Chipmunks		
			Inoculated	Infected**	Dead
Parent LAC	3.2	1600	5	3	0
Parent SSH	2.5	400	5	5	0
SSH-LAC-SSH***	3.8	250	5	2	0
SSH-LAC-LAC	3.5	16	2	0	0
		3200	5	3	0
LAC-LAC-SSH	4.7	800	5	0	0
		80	4	0	0
		8	4	1	0
		.8	4	0	0

* Average survival time (days) of suckling mice inoculated ic with 100-1000 $SM_{ic}LD_{50}$.

** Criterion of infection: Neutralization in Vero cell micro test against either LAC or SSH or both viruses by plasma taken 3-4 weeks after inoculation.

*** Reassortant virus: Notation from left to right indicates parental origin of large, medium, and small genome segments respectively.

Table 2. Neutralizing antibody titers of *Peromyscus* initially inoculated subcutaneously with tissue culture adapted epizootic hemorrhagic disease virus, type 1 and challenged intracerebrally with same on PID 60.

	Day After Inoculation					
	21	26	29	60[c]	65	68
Infected						
H1	[a]--	--	--	--	--	--
H2	--	--	--	--	1:80	1:20
H6	--	--	--	--	1:10	--
H4	[b]1:40	1:40	1:20	--	1:40	Dead
H5	1:20	1:10	1:40	1:10	1:20	1:40
H3	1:40	1:40	1:40	1:10	1:40	1:80
Controls						
HD1	--	--	--	--	Dead	
ND2	--	--	--	--	--	--
CD3	--	--	--	--	--	--

[a]No detectable antibody at a 1:10 or 1:20 serum dilution.

[b]Serum dilution inhibiting more than 50% viral cytopathic effect induced by 50 $TCLD_{50}$ homologous virus

[c]Serum drawn prior to intracerebral inoculation with homologous virus. All mice including the controls were inoculated on PID 60.

Table 3. Neutralizing antibody titers of *Peromyscus* initially inoculated intranasally with tissue culture adapted epizootic hemorrhagic disease virus, type 1 and challenged intracerebrally with same on PID 60.

	Day After Inoculation					
	21	26	29	[d]60	65	68
Infected						
N6	[a]--	--	[b]1:10	--	1:20	--
N5	--	--	[c]NT	--	1:20	--
N3	--	--	1:10	--	--	--
N2	--	--	--	--	1:10	--
N4	--	--	--	--	1:40	Dead
N1	--	--	--	--	--	--
Controls						
HD1	--	--	--	--	Dead	
ND2	--	--	--	--	--	--
CD3	--	--	--	--	--	--

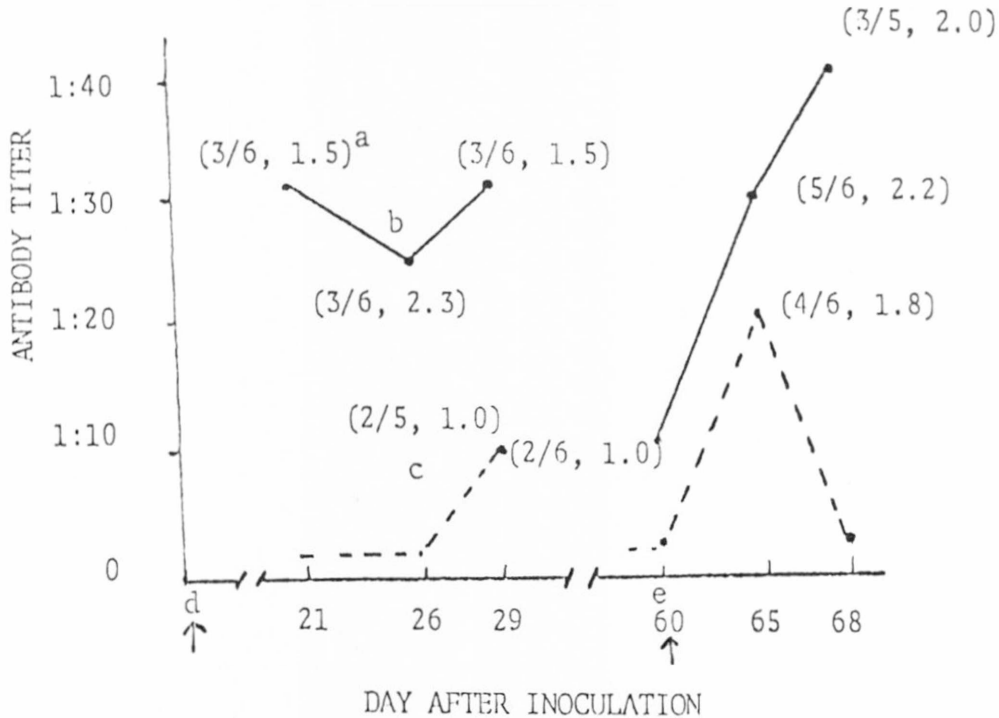
[a]No detectable antibody at a 1:10 or 1:20 serum dilution

[b]Serum dilution inhibiting more than 50% viral cytopathic effect induced by 50 $TCLD_{50}$ homologous virus.

[c]Not tested

[d]Serum drawn prior to intracerebral inoculation with homologous virus. All mice (including the controls) were inoculated on PID 60.

Figure 1. Geometric antibody responses by Peromyscus infected with epizootic hemorrhagic disease virus (type 1).



a (# with antibody/# tested, standard deviation).
b solid line represents mice inoculated subcutaneously.
c dashed line represents mice inoculated intranasally.
d PID 0 - primary inoculation date.
e PID 60 - intracerebral challenge inoculation.

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

The first known isolation of La Crosse (LAC) virus from the eastern half of Wisconsin has just been obtained from Aedes triseriatus larvae collected from a basal tree-hole in the rapidly developing suburban development area 10 miles northwest of Milwaukee.

This confirms that an area of endemic activity of LAC virus exists outside of the previously known endemic region in the southwestern third of our state, as previously defined by the geographic distribution of human cases, antibodies in animals, and virus isolation studies in mosquitoes. Occasional cases diagnosed in residents of this highly populated portion of the state have usually had histories of exposure to mosquitoes in western endemic areas, however on August 31, 1977 a 6-yr-old girl living in the Nob Hill development was hospitalized in a Milwaukee area hospital with encephalitis without exposure elsewhere. Paired serums collected on September 5th and 20th developed neutralizing antibody titers to LAC virus of 1:320 and $\geq$1:1280. She had been admitted on 8/31 with fever, repeated vomiting and severe headaches, lethargic and dehydrated. She remained febrile for five days, with continuing pain in her back and neck, and was later discharged on September 7th.

Antibodies neutralizing LAC virus were found in 2 of 3 chipmunks sampled in a woodlot back of her home during the spring of 1978. During the present 1979 season we are finding similar antibodies in chipmunks and squirrels, and acquistions of antibody to LAC have been found in a dozen chipmunks live-trapped, sampled, marked and recaptured in the woodlot behind her home, but not in surrounding woodlots in samples collected and tested by mid-August.

Oviposition sites for Aedes triseriatus found in this 80 acre wooded housing area include 15 basal tree-holes and about 35 old automobile tires mostly around two of the older homes in the area. The isolates of LAC virus were obtained from two suckling mice which had been fed upon by six female Aedes triseriatus reared from larvae collected in one-half gallon of rain water held in this tree-hole. Many other larvae collected from this and other sources are still being processed for virus isolation and establishment of fresh field colonies from this area, surrounding negative woodlots, and from endemic areas in western Wisconsin for continuing studies of transovarial, venereal and salivary transmission ability. Field strains of LAC virus are being obtained by holding females collected as larvae and later transmitting virus to mice, in the cartons in the insectory with subsequent feedings on chipmunks and mice, and collections of transovarially infected eggs. All of the old tires and other likely oviposition sites we have found have been removed and the tree-holes closed. Ovitrap collections on black cloths and paddles, established in these areas during June by Dr. Mori Zaim, are planned to be continued for monitoring vector presence, along with other studies next season.

Search for other cases of California encephalitis in this and other eastern Wisconsin areas is underway, by testing portions of serums from possible febrile CNS disease submitted to State Lab of Hygiene supplemented by visits of two medical students (James Schmidt and Barbara Stowe) to physicians in a dozen clinics. Thru mid-August 6 cases have been found in western Wis. with antibodies to LAC in their serums; no children have been found with evidence of infection with La Crosse in eastern Wisconsin, but studies are continuing.

(Wayne H. Thompson)

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

This laboratory again participated in an encephalitis surveillance program in Indiana in cooperation with the Indiana State Board of Health (ISBH). Four 2-man teams have been continuously mist-netting primarily house (English) sparrows since late March and will continue to do so through early October. All field collections are made by ISBH personnel while all laboratory testing is conducted by UNDLARS.

By mid-August over 4,300 birds had been captured, banded, bled (0.6 ml) and released. We have used the BD MICROTAINER brand of blood serum separator tubes (maximum capacity is 0.6 ml) this entire season and found them to be very convenient for field use. Teams carry small high-speed centrifuges into the field or keep them in their motel rooms and are thus able to centrifuge samples within a short time after drawing blood. The interfacing of the silicone plug allows for complete serum-clot separation and recovery of virtually all the serum. Samples can then be frozen or kept on wet ice until delivered to the laboratory; we keep all samples on wet ice.

While the initial cost of these tubes is more than other types of blood holding tubes, we have found that technician time is considerably reduced from our previous system and space utilization in our REVCO inventory systems is greatly reduced owing to the small size of the tube and the fact both blood clot (for possible future virus isolations) and the serum are held in the same tube.

All samples have been screened for antibodies to EEE, SLE, and WEE by means of HAI tests in microtiter. All HI positive samples were further tested and confirmed using serum dilution NT in microtiter. We have additionally neut tested all samples that had HI titers of < 1:20 for we find the majority of these have significant (≥ 1:8) SDNT titers.

To date (8-15) 30 birds have been detected with antibodies to SLE by both HAI and SDNT's; these include 1 adult male dove, 13 adult female house sparrows, 15 adult male house sparrows, and 1 juvenile house sparrow. One adult male house sparrow had low level (<1:20 HAI titer) antibodies to EEE. The majority of the birds positive for SLE were collected in the southern half of Indiana.

Six of the 30 birds were recaptures, having been banded and bled in 1977 (1 bird) and 1978 (5 birds). Five of the 6 were seropositive on initial capture (1 from 1977, 4 from 1978). One sparrow was captured three times in 20 days during June 1979; on initial capture (6-05) the HI titer was 1:80 and the NT titer was 1:32. The respective titers on second capture (6-12) were 1:40 and 1:32; the respective titers on third capture (6-25) were also 1:40 and 1:32.

Testing of the 4 birds seropositive in 1978 indicated antibody decay with lower HAI and NT titers. However, the male bird initially captured in 1977 (8-02-77) apparently had experienced a secondary infection with SLE since the HAI titer and the SDNT titer were 1:640 and 1:1024 respectively. This is the highest NT titer we have ever detected in the 3 years this survey has been conducted and over 180 seropositive birds tested in NT's.

Seronegative birds were frequently recaptured as well. The rate of recaptures in some areas were as high as 40-45%. Hardly any collection did not have at least one or several recaptured birds. Many of these birds were recaptured after previously having been banded in 1977 or 1978 indicating the minimal dispersion of house sparrow adults. In the past we have made recaptures throughout the winter season in a study of house sparrow dispersion. Juvenile house sparrows will disperse some distance from the nest site and apparently not return; adults on the other hand apparently remain in one general area for several years.

FURTHER EVIDENCE FOR SLE OVERWINTERING?

Our avian survey began late March again this year as last and resulted in the early detection of numerous sparrows with low-level(by HAI and SDNT) antibodies to SLE. However, several birds were captured in April and May that on testing had HAI titers to SLE ≥1:160 with corresponding NT titers of ≥1:64. We reported this phenomenon last year and suggested it may be evidence for overwintering of SLE virus locally since avian infection apparently corresponds with the emergence of adult *Culex* females from hibernacula in early April in Indiana. We have found this situation occurring for the second year in a row and in the same areas as last year, and have continued to see, for a third year in a row, endemic SLE activity in avians in several select isolated foci in the state. This supports our contention that SLE virus overwinters in Indiana by means of infected mosquitoes and/or avian hosts that experience viral recrudescence in the early spring coincident with the emergence of *Culex* species from overwintering hibernacula.

VIRUS ISOLATIONS.

Additionally we have isolated 2 strains of Flanders virus (one from *Culex pipiens*, one from *Culex* sp.) and one each of La Crosse virus and trivittatus virus from *Aedes triseriatus* and *Aedes trivittatus* females collected in late summer 1978. We would like to acknowledge the assistance of Dr. Charles Calisher in final typing of these 3 viruses, the latter two of which constitute new state records for arbovirus distribution.

HUMAN SEROSURVEY.

A serosurvey of 10,000+ Hoosiers for antibodies to EEE, WEE, SLE and CE group is essentially complete. We have received samples from 97 separate hospitals, clinics, medical foundations, blood banks and survey "donor" programs, all with informed consent. All samples were tested in microtiter by SDNT's (initial screening at 1:2 only with positives titered to their endpoints). A suprising number of samples were positive for SLE and an equally large number for CE group. Results of this study will be reported in the next issue of this Exchange.

An additional study was conducted this summer in an area of northwestern Indiana which has seen periodic EEE in horses. In conjunction with the ISBH program and the St. Joseph County Mosquito Control project, we collected all mosquitoes attracted to horses over the past summer in several selected pastures. *Coquillettidia perturbans* was the predominant species followed by *Aedes vexans*. No other species of mosquito was taken on these horses. Collections were made near the farms where horses died from EEE in 1975. All mosquitoes were pooled for virus isolation studies. Extensive avian bait and CO_2 trapping of other species was also done in the same area near a large marshland. Again the different species were pooled for isolation work.

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REPORT FROM THE ARBOVIRUS SURVEILLANCE PROGRAM

DIVISION OF LABORATORIES
ILLINOIS DEPARTMENT OF PUBLIC HEALTH
CHICAGO, ILLINOIS

Since the inception of the Arbovirus Surveillance Program by the Illinois Department of Public Health in 1976, an annual decline in apparent enzootic transmission to avians and subsequent human involvement has been observed. Thus far, results from this season have conformed to that pattern. As noted in Table 1, only 2 (0.1%) of 2,282 juvenile birds, primarily house sparrows, had HAI antibodies to SLE virus. Overall, only 12 (0.4%) of 3,104 birds, 11 house sparrows and one pigeon, had antibodies. In St. Clair County, which is adjacent to St. Louis, Missouri, approximately 1-2 percent of the adult birds had antibodies. This county has yielded the majority of the positive birds this year. Virus isolation attempts on blood of 680 nestling birds were negative. No human SLE cases have been confirmed.

This spring, studies of California encephalitis (CE) group viruses were expanded in Peoria County, where 10 human cases were confirmed in 1978. Recent information on two Peoria County CE group mosquito isolates from Dr. C. H. Calisher, Vector Borne Diseases Division of CDC, confirmed that both were LaCrosse (LAC) virus, as we had suspected. One was from adult Aedes triseriatus collected in 1976 and the other was from laboratory-reared mosquitoes collected from a treehole in 1978. Objectives this year were to further delineate the temporal and spatial distribution of LAC transmission. Ten indicator rabbits were bled weekly and 50 ovitraps (described by Grimsted and Sinsko, Vol. 35, p. 125) were strategically placed around the county and were supplemental to ongoing human surveillance.

Preliminary laboratory data through early-August indicates that four of the ten rabbits developed CE group antibodies and there have been five human CE cases, including three from Peoria County (one who resided within one mile of where the LAC isolations were made and the first 1979 rabbit seroconverted). The girl and four boys ranged in age from 4 to 13 years and had onset of illness between June 1 and July 22. Several other cases are under investigation.

This year 14 equines with suspected arboviral encephalitis were tested and had no significant antibodies to WEE, EEE, and VEE viruses. A single juvenile house sparrow from Christian County had antibodies to WEE virus. This is the same locality from which we isolated WEE virus from a nestling house sparrow in 1977, the first from a vertebrate other than an equine in Illinois. Following detailed testing, Dr. C. H. Calisher at CDC advises that this strain is WEE, as opposed to the closely-related Ponchatula, that has been seen frequently in eastern United States.

(Gary G. Clark and Harvey L. Pretula)

TABLE 1.

AVIAN SEROLOGY -ILLINOIS- 1979

REGION	COUNTY	Collection Interval									
		April		May		June		July		Mid-August	
		HY*	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages
NORTH	COOK		0/15**		0/3	0/41	0/50	0/296	0/356	0/77	0/91
	WILL					0/42	0/50	0/7	0/9		
	Winnebago					0/19	0/19	0/5	1/9		
CENTRAL	CHRISTIAN			0/39	0/39	0/46	0/51	*** 0/81	0/94	0/14	0/16
	CUMBERLAND			0/29	0/29	0/84	1/128	0/31	0/58	0/4	0/11
SOUTH	MADISON			0/53	0/53	0/68	0/68	0/4	0/4		
	ST. CLAIR			0/28	0/56	0/97	2/223	0/199	3/337	0/73	2/112
	RICHLAND			0/28	1/61	0/127	0/171	0/219	0/252	0/60	0/75
	WHITE			0/44	0/74	2/215	2/249	0/102	0/130	0/84	0/91
	HAMILTON			0/9	0/9	0/12	0/12	0/15	0/28		
	SALINE			0/3	0/23	0/18	0/36		0/1		
	Pope									0/9	0/11
	TOTAL (PERCENT POSITIVE)		0/15	0/233	1/347 (0.3)	2/769 (0.3)	5/ 1057 (0.5)	0/959	4/ 1278 (0.3)	0/321	2/407 (0.5)

*Hatching year; includes older nestlings, fledglings, and juvenile birds.

**Number positive (HAI antibody titer to St. Louis Encephalitis (SLE) virus of 1:20 or greater)/ Number tested.

***One bird with HAI antibody titer of 1:20 to Western Equine Encephalitis (WEE) virus.

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

Arbovirus Surveillance 1979

From the beginning of June to mid-August, 269 patients with signs of meningitis, encephalitis and/or fever of unknown etiology were tested for evidence of infection with Eastern encephalitis, Western encephalitis, St. Louis encephalitis, Powassan and California encephalitis (CAL) viruses. No confirmed cases of arbovirus infections were detected, but seven presumptive cases of California encephalitis were observed.

From 861 pools of approximately 42,000 wild-caught mosquitoes so far tested, nine isolates of CAL complex were obtained. Most of these isolates were from specimens collected in the northeastern region of New York State.

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

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT (YARU)
DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH
NEW HAVEN, CONNECTICUT

Antigenic Relationships Of The Crimean-Congo Hemorrhagic Fever (CCHF) Virus

A distant relationship has recently been reported between Nairobi sheep disease (NSD) and CCHF and Hazara viruses, detected by means of immunofluorescence (IF) and indirect hemagglutination tests, but not by the neutralization test (Davies et al., J. Comp. Path., 88: 519-523, 1978). An extension of these studies to include a large number of other arboviruses has shown heretofore unreported antigenic relationships among viruses currently included in the family Bunyaviridae, subdivision "Bunyavirus-like", of the classification scheme established by the International Committee on Taxonomy of Viruses - ICTV (Porterfield et al., Intervirology 6: 13-24, 1975-76).

Until now, the complement-fixation (CF) test has been the technique generally used for the antigenic classification of tick-borne viruses excluding the flaviviruses (Casals, Proceedings of a Symposium, Bratislava, 1971). Usually, a CF screening is done by testing an antiserum for the problem virus against antigens from all available tick-borne viruses; cross-reactions thus detected were subsequently investigated by neutralization test and, if antigens could be produced, by hemagglutination-inhibition (HI) test.

Initially planned as an investigation of the antigenic relationships of CCHF virus the studies summarized here have been extended to other tick-borne viruses in various antigenic groups as well as ungrouped ones; in addition, and mainly as controls for specificity in the tests, insect-associated viruses from several taxons and antigenic groups have been included. As of now, 54 different serotypes have been represented; in addition to the viruses named in the table, the following were included: Batai, Bhanja, Bwamba, California encephalitis, Caraparu, Chenuda, Chikungunya, Colorado tick fever, dengue 3, Dhori, epizootic hemorrhagic disease of deer, Grand Arbaud, Huacho, Inkoo, Japanese encephalitis, Johnston Atoll, Joscow, Kaisodi, Karimabad, Kemerovo, Lanjan, Lonestar, Manawa , Mono Lake, Oropouche, phlebotomus fever Naples, Sakhalin, Silverwater, Thogoto, Tyuleniy, Upolu, Uukuniemi, VEE, Wad Medani and West Nile.

The method used are: HI, when agglutinating antigens were available; indirect IF employing spot-slides prepared with cell cultures infected with CCHF or Dugbe viruses; and the neutralization test. The latter was: either a mouse intracerebral test inoculating a mixture of undiluted serum and a constant amount of virus, equivalent to 100 LD_{50}, to groups of 16 mice between 22 and 28 days old; or a kinetic reduction of foci of infection, detected by IF (Tignor et al., 1979, in publication). Sera in all tests were extracted with acetone and ethyl ether; for the HI test the sera were, in addition, adsorbed with goose erythrocytes. The sera were used in serial two-fold dilutions beginning at 1:5 in the HI test, and beginning undiluted in the reduction of foci of infection and IF tests; in the mouse neutralization test, the sera were used undiluted only.

The accompanying table summarizes the current status of the study. HI tests with CCHF, Hazara and Abu Minnah antigens have uncovered a relationship that includes viruses in the groups CCHF, NSD, DGK (Dera Ghazi Khan), Qalyub and possibly also Hughes; no virus outside these antigenic groups has shown any cross-reaction with the above antigens -- only ones available - even at dilution 1:5. In so far as the observations have gone, the neutralization test with CCHF virus either in mice or by reduction of foci of infection has shown cross-reactions among viruses of the same groups only, particularly marked with NSD and Qalyub groups, less so with the Hughes groups. No cross-reactions, even with undiluted sera, have been observed by IF other than between CCHF and NSD (Dugbe virus) groups.

No CF tests were performed at this time; extensive studies done previously had shown no cross-reactions between viruses belonging to these 5 antigenic groups, except for a small crossing between NSD group sera and a strain of CCHF virus, U-C 3010 (Casals, Proceedings of a Symposium, Bratislava, 1971; Davies et al., J. Comp. Path., 88: 519-523, 1978; Casals, In "Ebola Haemorrhagic Fever", S.P. Pattyn, ed, 1978).

The present observations show the existence of relationships between antigenic groups of viruses from the Bunyavirus-like set or from the unclassified category (Berge, 1975); only tick-borne viruses are thus far involved. In our estimate the described relationships do not at this time justify combining the viruses in the 5 related groups in a single group; rather, as was our conclusion from the relationships observed with other antigenic groups of Bunyaviridae, and for similar reasons (Casals, Proceedingsof the 7th International Congress of Tropical Medicine and Malaria, 1964), it would seem advisable to consider these new relationships as indicating the existence of a second antigenic supergroup in the Bunyaviridae, which could be designated NSD supergroup.

It remains for biochemical and molecular studies to determine in the future whether these reported antigenic relationships correspond to similarities in basic properties of the involved viruses, which would justify the establishment of a defined second genus in the family Bunyaviridae, to be named by the ICTV.

(J. Casals and G. H. Tignor, YARU)

Antigenic Relationships Between CCHF Group Of Viruses And Other Bunyavirus-like Viruses

Serum Antigenic group	Virus	Antigen and Test							
		CCHF				HAZ	A. Mina	Dugbe	Other #
		HI	N	RF1	1F	H1	H1	1F	H1
CCHF	CCHF	320		+	256	80	40+	8	0
	Hazara	40			32	160	10	4	0
NSD	NSD	80+	+	+		80+			0
	Ganjam	80		±	4	20		16+	0
	Dugbe	160		+	16+	80	20	16+	0
DGK	A. Hammad	40+			0	20	40+	0	0
	A. Mina	160			0	40	40+	0	0
	DGK	20			0	0			0
	Kao Shuan	40			0	5	10	0	0
	Pretoria	40			0	10	20	0	0
Qalyub	Bandia	40	+	±	0	5	5	0	0
	Qalyub	320	+		0	40	40+	0	0
Hughes	Farallon	10	±		0	5	0	0	0
	Hughes	20			0	5	0	0	0
	Soldado	5	+	±	0	0	0	0	0
Others and ungrouped	34	0	0	0	0	0	0	0	

N = neutralization test in mice. Negative sera: Bhanja, CTF, Huacho, Uukuniemi and Pathum Thani.

RF1 = reduction of foci of infection. Negative sera: Chikungunya, Huacho, Mono Lake, Pathum Thani, Tyuleniy and West Nile.

Other antigens: Bhanja, Germiston, Ketapang, Lanjan, Manawa, Naples SFF, Oropouche, Quaranfil and Uukuniemi.

Reciprocal of serum titers.

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT, 60 COLLEGE STREET, NEW HAVEN, CT 06510 AND US ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES, FORT DETRICK, FREDERICK, MD 21701

Serologic relationship between Rift Valley fever virus and viruses of the phlebotomus fever serogroup

A serologic relationship of Rift Valley fever virus has been found with viruses of the phlebotomus fever group in the *Bunyaviridae*. Rift Valley fever virus previously was believed on the basis of complement-fixation (CF) tests to be unrelated serologically to other bunyaviruses.

A Rift Valley fever (RVF) hemagglutinating antigen and antigens of phlebotomus fever group viruses were reacted with hyperimmune mouse ascitic fluids of phlebotomus fever group viruses (some of these reagents were supplied by the Research Resources Branch of the US NIH) and sheep anti-RVF serum. Inhibition of RVF antigen and Punta Toro, Chagres, and Saint-Floris and other antigens was observed with RVF sheep serum as shown in Table 1. Reciprocal reactions were observed when antibody of Punta Toro, Candiru, and Gordil (closely related to Saint-Floris virus) were used. A phlebotomus fever grouping antibody inhibited the RVF antigen in the 1:160 dilution.

Complement-fixation tests in grid titrations with Punta Toro and Gordil antigens and antibodies failed to detect cross-reactions with RVF reagents. Homologous antibody titers were RVF sheep serum 1:32, Gordil and Punta Toro antibody 1:512.

Plaque reduction neutralization tests in Vero cells (Table 2) resulted in neutralization of RVF virus by both Punta Toro and Gordil ascitic fluids as well as other antibody to phlebotomus fever group viruses. It has not yet been determined if any of the 26 distinct viruses in the phlebotomus fever group are identical to RVF, nor has the full range of relations within the group been explored.

Possible implications of these findings are 1) that heterologous reactions of phlebotomus fever group viruses may confound interpretation of RVF diagnostic reactions and serosurvey results, especially when HI and neutralization tests are involved, 2) that one or more viruses of the phlebotomus fever group might serve as a heterologous vaccine or might play a role in regulating the distribution of RVF infections because of cross-immunity of vertebrate hosts, 3) that RVF virus might have a natural reservoir in a phlebotomine fly, reasoning by analogy with the natural history of some phlebotomus fever group viruses which are maintained transovarially in phlebotomines.

(R.E. Shope and C.J. Peters)

Table 1

HI Test Reactions of RVF Virus and Phlebotomus Fever Group Viruses

	Antibody								
Antigens	RVF	SFS	SFN	CHG	ICO	PT	CDU	GOR	GP PHL
RVF	>640	0	0	0	0	640	40	160	160
SFS	0	40	10	0	10	0	0	0	20
CHG	40	0	10	10	10	40	0	20	40
PT	40	0	20	0	0	640	20	0	80
SAF	320	0	40	0	0	40	40	160	80
SAL	20	0	0	0	0	40	10	0	80
KAR	0	0	0	0	0	40	0	0	80
I47	20	0	20	0	0	20	20	20	20

[a]Reciprocal of antibody titer; 0=<10; 2 - 4 antigen units. RVF antigen was produced at USAMRIID. Abbreviations: RVF, Rift Valley fever; SFS, Sicilian; SFN, Naples; CHG, Chagres; ICO, Icoaraci; PT, Punta Toro; CDU, Candiru; GOR, Gordil; GP PHL, group phlebotomus fever; SAF, Saint-Floris; SAL, Salehabad; KAR, Karimabad; I47, Naples-like isolate.

Table 2

Neutralization of RVF Virus by Antisera to Phlebotomus Fever Group Viruses[a]

	Antibody									
Virus	RVF	PT	GOR	CDU	ICO	SFN	SFS	SAL	GP PHL	NORMAL MOUSE
RVF	10,240[b]	320	80	40	10	10	10	<10	640	<10

[a]69 to 104 pfu of RVF were incubated 1 hr at 37C with the antibody before assaying residual infectivity in VERO cells to determine the highest dilution neutralizing 80% of the inoculum.

[b]Reciprocal of titer; abbreviations as in Table 1.

REPORT FROM THE STATE OF NEW JERSEY DEPARTMENT OF HEALTH
JOHN FITCH PLAZA, TRENTON, NJ 08625

Isolations from Arthropods in New Jersey

July, 1979

Group	Virus and No. of Strains EE	WE	Isolated from	Collected in
A		1	C. melanura	Atsion
A		3	C. melanura	Bass River
A	1		C. melanura	Bass River
A		6	C. melanura	Dennisville
A		1	Coq. perturbans	Dennisville
A	1		C. melanura	Dennisville
A		4	C. melanura	New Gretna
A		2	C. melanura	Woodbine
Totals	2	17		

August, 1979

Group	Virus and No. of Strains EE	WE	Isolated From	Collected At
A	6	10	C. melanura	Bass River
A	5	8	C. melanura	Dennisville
A	9	5	C. melanura	New Gretna
A	2	13	C. melanura	Woodbine
A		1	Culex salinaris	Woodbine
Totals	22	37		

(Wayne Pizzuti)

REPORT FROM CORNELL UNIVERSITY, DEPARTMENT OF MICROBIOLOGY (MEDICAL COLLEGE, NEW YORK) AND DEPARTMENT OF ENTOMOLOGY (COLLEGE OF AGRICULTURE, ITHACA)

Mosquito vector studies in Guatemala during August 1977 showed Culex (Melanoconion) opisthopus to be the predominant mosquito where sentinel VE-susceptible, Nepuyo/Patois-immune hamsters were dying. Collections of mosquitoes by people from clothing with subsequent exposure to hamsters resulted in deaths of two hamsters and recovery of VE virus from heart tissues. VE virus was also recovered from single mosquitoes in the groups of mosquitoes that engorged on these hamsters. The prevalence of Cu. (Mel.) opisthopus transmitting VE virus during July-August 1977 at the La Avellana enzootic focus in Guatemala was 1/487. Agar-gel precipitin tests of 376 naturally engorged Cu. (Mel.) opisthopus showed the bloods to be predominantly mammalian and not avian, reptilian or amphibian. Sentinel hamsters and sentinel guinea pigs detected VE virus at this location during August 1977. Nine of ten guinea pigs became infected as evidenced by presence of VE antibodies in post-exposure plasmas, but no guinea pigs died, suggesting that the strains of virus which infected them were equine-benign.

Surveillance of sentinel horses for VE virus infections in Nicaragua was carried out during July and August 1977 by obtaining sera from 88 young "unvaccinated" horses on ranches at the western and eastern extremes of the Pacific Coast. Plaque-reduction, neutralizing antibodies were found in sera in about equal prevalences to an HI subtype I, epizootic strain and to the TC83 vaccine strain. Serum-dilution, plaque-reduction N tests revealed that antibodies in these horses could be classified as a) residual from infections during 1972 or before, plus or minus TC83 vaccination (despite the negative history of vaccination), b) engendered by attenuated live VE virus vaccines (again despite a negative history of vaccination), c) uninterpretable as to the stimulating antigen, but not likely to originate from epizootic virus infection or d) conceivably engendered by epizootic virus infection since 1972 (two horses).

Mosquito vector studies in Guatamala during 1978 have included initial attempts to colonize Cu. (Mel.) opisthopus and Aedes taeniorhynchus from La Avellana, Guatemala and semi-monthly collections of mosquitoes by permanent personnel at La Avellana to mail to New York for identification. In this manner, fluctuations are monitored in species compositions of mosquitoes collected from clothing one hour at dusk, one evening every two weeks by two persons.

A group C bunyavirus, probably Nepuyo, was isolated twice from Cu. (Mel.) opisthopus collected during July-August 1977 in Guatemala. Provided neutralization tests confirm the identities as Nepuyo within group C, these strains represent the first isolations of Nepuyo virus from this species of mosquitoes. Group C, probably NEP, virus was also isolated twice from sentinel hamsters at the same location during 1977 in a field investigator. This illness was of a duration and severity similar to that of classical dengue. Viremia was present and antibodies developed during convalescence.

Collaborative studies with the U.S. Army Medical Research Institute of Infectious Diseases (Drs. P. Jahrling and G. Eddy) concerned VE vaccinee antibody responses detected by staphylococcal protein A tests. There was no correlation between development of positive protein A tests in pre- versus post-vaccinee sera and the occurrence of early symptomatic reactions. However positive tests did correlate with development of VE viral antibodies.

A virus isolated from mosquitoes in Peru during 1975 has been tentatively identified as encephalomyocarditis virus through the kind efforts of Dr. F. Murphy at CDC.

Hydroxylapatite column chromatography was evaluated as a technique for detection and isolation of epizootic Venezuelan encephalitis (VE) virus subpopulations from mixtures of enzootic and epizootic VE viruses. Initially this involved the testing of various production lots of hydroxylapatite, disposable glass columns, gradient generators, and the adaptation of the phosphorous assay of Fiske and SubbaRow for measuring phosphate concentrations of column eluates.

Two well-characterized VE virus strains were selected to learn the conditions for obtaining optimal separation of Central American epizootic and enzootic viruses. These two virus strains were found to elute from hydroxylapatite differently. Enzootic virus, not binding to hydroxylapatite at 0.05 M phosphate, eluted immediately following the void volume. A small proportion of enzootic virus, however, did bind to hydroxylapatite and eluted over a broad range of phosphate concentrations from 0.15 to 0.40 M. Epizootic virus bound to the hydroxylapatite and eluted in a sharp band between 0.20 M and 0.26 M phosphate. Basically the same elution profiles were obtained whether the virus was derived from hamster heart, brain or kidney tissues or from chick embryonic cells.

The best procedure to date for separating epizootic from enzootic virus is to first wash the virus-loaded hydroxylapatite column with 0.15 M phosphate, pH7.0, to remove enzootic virus, and then to elute epizootic virus with a 0.15 M to 0.40 M phosphate gradient, pH7.0. This spreads the late eluting enzootic virus over a broad phosphate range while the epizootic virus elutes in a narrow zone.

(W.F. Scherer, M.E. Wiebe, R.W. Dickerman (NY) and E.W. Cupp (Ithaca))

REPORT FROM THE DIVISION OF VIROLOGY AND IMMUNOLOGY
BUREAU OF LABORATORIES
PENNSYLVANIA DEPARTMENT OF HEALTH
LIONVILLE, PENNSYLVANIA

Arbovirus Surveillance in Pennsylvania, 1979

The Pennsylvania Department of Health and Environmental Resources have conducted continual surveillance of arboviral activity in the state, similar to that conducted in 1978.

Sentinel flocks of four (4) chickens were placed at 50 locations throughout the state at the locations shown in Figure 1. There was at least one (1) sentinel flock in 28 of the 67 counties of the state. The chickens were bled weekly and the sera tested, after acetone extraction, for hemagglutination-inhibition (HI) antibodies against St. Louis Encephalitis (SLE), Western Equine Encephalitis (WEE), Eastern Equine Encephalitis (EEE), and California Encephalitis (CE) viruses. A single chicken in Crawford County demonstrated a transitory, two-week, low level (1:40-1:80) titer to SLE. There were no other seroconversions in 5163 HI tests performed through August 30, 1979.

Through August 30, 1979, a total of 26 patients were tested serologically for evidence of infection with SLE, WEE, EEE, and CEV. There were no confirmed cases.

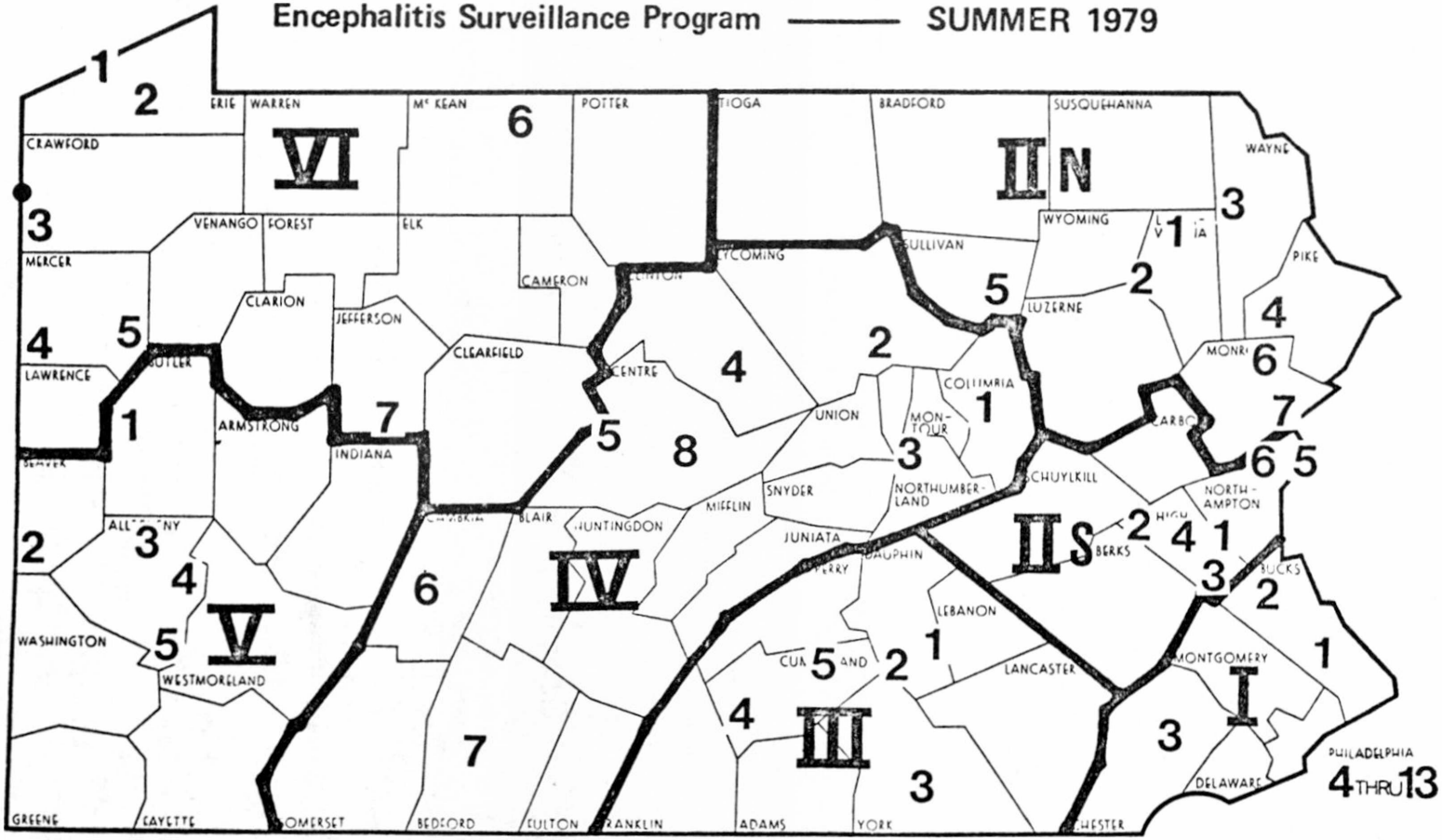
The surveillance program will be continued through September 1979.

(Bruce Kleger and Vern Pidcoe)

FIGURE 1

SENTINEL POULTRY FLOCK LOCATIONS BY REGION*

Encephalitis Surveillance Program ——— SUMMER 1979



COOPERATIVE WORKING AGREEMENT

PA DER — Vector Control

PA DH — Div. Comm. Disease Control; Bureau of Laboratories

● SLE

***COOPERATING COUNTY HEALTH DEPTS.**

Allegheny
Philadelphia
Bucks
Chester
Erie

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

CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

DATA THROUGH AUGUST

Surveillance for Human Arbovirus Infection, United States, 1979

The 1979 arboviral season has been devoid of epidemic activity to date. A total of 35 California Encephalitis (CE) cases has been identified from 6 states. Only 1 case of St. Louis Encephalitis (SLE) and none of Eastern Equine Encephalitis (EEE) or Western Equine Encephalitis (WEE) have been confirmed.

California Encephalitis

With one exception, cases have been reported from mid-western states with recognized endemic CE: Minnesota (17), Wisconsin (6), Illinois (5), Ohio (3), and Iowa (3). The age and clinical severity of cases have been unremarkable. The relatively high number of reports from Minnesota may reflect accelerated laboratory diagnoses through the counter immune electrophoresis technique.

The geographic exception and first reported case of the season involved a 13-year-old boy from Stateline, Mississippi who had onset of febrile headache on May 21. His illness worsened and he was hospitalized May 25 in Mobile, Alabama with clinical encephalitis. He improved rapidly and was discharged May 30. Sera collected on May 27 and June 19 had CF titers of < 1:8 and 1:32 respectively at the Alabama State Laboratories.

St. Louis Encephalitis

The only case of confirmed SLE reported through August occurred in a

51-year-old man who was a resident of Matagorda County, Texas. On June 15, he developed headache and fever and was hospitalized on June 20 with progressive symptoms including disturbance of gait, difficulty in focusing, tongue tremors, and somnolence. He improved and was discharged on July 28. Sera collected on July 23 and August 7 had CF titers of < 1:8 and 1:64 at the Texas State Laboratories.

Eastern Equine Encephalomyelitis*

No human cases have been reported although more than 40 horses with suspect EEE from North Central Florida have demonstrated elevated HI titers in unpaired sera.

Western Equine Encephalomyelitis

No human cases reported. Scattered equine cases with varying laboratory documentation reported from California, Colorado, Minnesota, New Mexico, Texas, and Washington.

(Karl Kappus, Melinda Moore and Lawrence Schonberger)

*Addendum: A single case of laboratory confirmed EEE in a 58-year-old resident of New Castle County, Delaware has been reported by the Delaware Health Department. The patient was hospitalized July 11 with clinical encephalitis and discharged July 18 with residual neurologic symptoms. Sera collected on July 12 and August 8 had HI titers of <1:10 and 1:160, respectively. Investigation revealed no history of travel during the period of probably infection and no other EEE activity has been detected in the area.

REPORT FROM THE OFFICE OF LABORATORY SERVICES AND ENTOMOLOGY
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES
JACKSONVILLE, FLORIDA

Again, as in 1978, extensive surveillance in Florida, January through July, 1979, failed to elicit any evidence of SLE activity in the State. EEE activity continues to circulate in the sentinel chickens, small mammals and horses.

A total of 1,111 human sera from patients with CNS symptoms were tested by HI against EEE, SLE, VEE, Dengue 2 and CAL antigens. Two patients had confirmed Dengue 2 infections presumably acquired in the Caribbean area. Fourteen patients, from endemic areas, had constant titers to Group B antigen.

A total of 4,033 sera from sentinel chickens, wild caught avians and mammals (excluding horses) were tested for SLE and EEE HI antibodies. Fifteen sera (12 chickens, 3 mammals) yielded EEE HI antibody titers. No SLE antibody was detected in any of the specimens. A total of 632 *Culex nigripalpus* mosquito pools were tested and yielded no virus.

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

REPORT FROM THE TEXAS DEPARTMENT OF HEALTH
BUREAU OF LABORATORIES
AUSTIN, TEXAS 78756

JANUARY 1, 1979 THROUGH JUNE 30, 1979

During the period indicated above, a total of 14,351 mosquitoes were tested. Listed below are the positives:

Locality	Collection Date	Species	# Isolations	Virus
Beaumont	4-25-79	C. quinquifasciatus	1	Hart Park
Beaumont	5-23-79	A. quadrimaculatus C. quinquifasciatus C. quinquifasciatus	1	Hart Park
Dallas	5-22-79	C. restuans C. salinarius C. restuans	1	Hart Park
Colorado Co.	5-25-79	A. quadrimaculatus C. quinquifasciatus C. quinquifasciatus	1	Hart Park
Hidalgo Co.	5-30-79	C. quinquifasciatus A. aegypti C. quinquifasciatus	1	Hart Park

In addition to isolation attempts, serology was used to supplement. Thirty-two canine sera were submitted by Lubbock for VEE surveillance. All were negative by HI.

Dallas and Hidalgo Co. submitted a total of 397 sentinel and wild bird sera. Only one specimen showed evidence of Arbovirus infection. A wild sparrow caught in Dallas had an HI titer of 1:20 for SLE.

Thirty-four nestling wild birds received for isolation attempts yielded no positives.

(Charles E. Sweet)

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

HUMAN SERA CONTAINING HI ANTIBODIES* TO VEE, EEE AND ILHEUS VIRUSES IN MEXICO**

AGE GROUP (years)	NUMBER TESTED	V E E		E E E		I L H	
		Nº	%	Nº	%	Nº	%
- 1	0	0	0	0	0	0	0
1 - 14	4	2	50.0	1	25.0	2	50.0
15 - 44	89	40	44.9	22	24.7	67	75.2
45 - 64	5	2	40.0	1	20.0	4	80.0
65 - +	0	0	0	0	0	0	0
TOTAL	98	44	44.9	24	24.2	73	74.5

HI = Hemagglutination-inhibition
VEE = Venezuelan equine encephalitis
EEE = Eastern equine encephalitis
* Titers ranged from 1:10 to $\geq$ 1:160 against 4 HA units of each antige
** Sera collected in southeast Mexico.

Dr. César Wong-Chía
Apartado 70-434, C.U.
México 20, D. F.

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

Dengue in Puerto Rico, 1979

Through week 35, ending August 29, 1979, 684 cases clinically compatible with dengue had been reported to the San Juan Laboratories through the surveillance system. Four-hundred thirty-five of these were reported by the end of the first quarter of 1979, and represent the tail of the 1978 dengue-1 epidemic. Laboratory confirmation rates have dropped progressively from a peak of 80% in May-July, 1978, through the peak of the epicemic, to 45% in December and January, 23% in February through April, and only 6% in June 1979. Part of the clinical "dengue" in 1979 has been shown by laboratory tests to have been due to measles and influenza B.

Nine virus isolations have been made from patients with onset of illness in 1979, all type 1, the most recent being from a case with onset in May. The most recent serologically confirmed dengue case had onset in July.

Comparison of Symptoms, 1977 and 1978 Epidemics, Puerto Rico

Computer analysis of hemorrhagic symptoms in laboratory confirmed cases from these epidemic has been partially completed, and the results are shown in the table. The ratio of primary to secondary infections reversed between 1977 and 1978, as expected since many people infected in 1977 were exposed to reinfection with the new type 1 in 1978. The sex ratio remained the same from one year to the next, and hemorrhagic symptoms were distributed by sex in the same proportion. The rate for each symptom was similar from one year to the other except for ecchymoses and blood in the vomit (possibly due to swallowing after nosebleed), which appear to have increased in 1978. A new report from with space for recording blood in the urine and vaginal bleeding was introduced part way through the 1978 epidemic.

Aedes aegypti Population Indices in Puerto Rico, 1979

Monthly larval inspections at 100 houses in each of the cities of Guayama, Ponce and Mayaguez revealed average Breteau indices of 16.6 34.3 and 11.6, respectively, for the first 8 months of 1979. The indices for Guayama and Ponce were 13% and 130%, respectively, above the long-term (1973-1978) averages for those cities, while for Mayaguez it was 44% below the long-term average for the same period.

A new trap for adult *A. aegypti*, developed by D.A. Eliason, BTD, CDC, Atlanta, is being tested in several cities. Weekly collections from these traps have ranged from 0 to 43 female *Aedes aegypti* per day during the first 8 months of 1979.

Laboratory confirmed cases:

	1977		1978$	
	No.	%	No.	%
Primary	771	57	847	37
Secondary	587	43	1347	58
Undetermined$$	-	-	126	5
	1358	100	2320	100
Males	550	41	903	39
Females	808	59	1417	61
Reporting hemorrhagic symptoms:				
Males			127	37
Females			213	63
Petechiae	130	8.7	153	6.6
Epistaxis	46	3.4	83	3.6
Bleeding gums	33	2.4	64	2.8
Ecchymoses	16	1.2	57	2.5
Blood in vomit	1	0.1	36	1.6
Blood in stool	15	1.1	32	1.4
Blood in urine	NA	-	20*	2.7*
Vaginal bleeding	NA	-	11**	2.5**
	132	9.7	340	14.7
Primary cases with hemorrhage			128	38
Secondary cases with hemorrhage			199	58
Undetermined cases with hemorrhage			13	4

* Only 752 people were asked this question in 1978.
** Only 435 females were asked this question in 1978.
$ Cases with onset from 4.1.78 through 12.31.78
$$ Virus was isolated, but no convalescent serum specimen obtained.

Dengue in Mexico, 1978-79

In January 1979, the Center for Disease Control received through the good offices of Dr. José Laguna García, Deputy Minister for Planning, Secretaria de Salubridad y Asistencia (SSA), Mexico, and Dr. Gerardo Amaro Santana, Chief of Coordinated Public Health Services of Quintana Roo State, SSA, permission for Dr. Irene Fabrikant, Visiting Fellow at the SJL, to make a collection of serum samples from people from southeast Mexico to test for evidence of recent infection with dengue virus. This was in view of the presence of epidemic dengue in Belize and Guatemala.

Out of 69 people sampled, 55 of whom had a history of febrile illness compatible with dengue in the period since November 1, 1978, nine had antibodies by the hemagglutination-inhibition (HI) test compatible with infection by dengue virus. One of these had antibody titers by the complement fixation test indicating a current infection. This was a 20-year-old female resident of Chetumal, a town on the Belize border, with onset of illness in the second half of December 1978. The other eight giving positive HI reactions were five males and three females, with ages ranging from 8 to 70 years, residing in Chetumal, Isla Cozumel, El Palmar, José María Morelos, La Unión, and Subteniente López, all in Quintana Roo State. The positive HI results in the six people more than 35 years old could have been due to infection during the 1941 epidemic, but the antibodies in an 8-year old boy from La Unión and a 21-year old man from Isla Cozumel very probably refer to recent illness.

In February, Alexander Moya-Cabrera of the Virus Laboratory of the Institute of Health and Tropical Diseases, Mexico City, came to the SJL with a collection of sera from 96 cases clinically compatible with dengue, with onset in January 1979, from Tapachula in Chiapas State, on the Guatemalan border at the Pacific Coast of Mexico. The Institute of Health and Tropical Diseases had already reported finding dengue antibodies in sera from cases in that town. Attempts at virus isolation at the SJL were unsuccessful, but one pair of sera showed a diagnostic rise in dengue antibody and 32 had dengue antibodies, including 15 with CF and HI titers indicative of secondary infection. Many of these secondary cases were less than 30 years old, including a baby of 15 months, suggesting that dengue (or another flavivirus) has been active in the southern border area of Mexico since the last recorded epidemic in 1941.

Reports to PAHO indicate that the epidemic had spread to Oaxaca state by July. The Gulf Coast of Mexico is known to be infested with *Aedes aegypti* up to U.S. border, so the probability of northward spread is high.

Dengue in Hispaniola, 1979

The contract with PAHO for dengue surveillance in this Caribbean island produced specimens from 68 patients from Haiti through July 1979, of which 4 could be laboratory confirmed as dengue cases, with onset in March (2), May and June. Two were cases of influenza B, with onset in June. Three Haitian medical epidemiologists received training at the SJL in dengue surveillance. House-to-house surveys of 100 houses for *Aedes aegypti* in Port-au-Prince, carried out in May, June and July, produced house indices of infestation ranging from 41-58%, Breteau indices of 70-156%, and container indices of 11-22%, indicating a high level of risk of transmission of *Aedes aegypti*-borne disease.

From the Dominican Republic, specimens were received from 30 patients through July 1979. None was laboratory confirmed as current dengue. The number of dengue cases reported countrywide for the first quarter of 1979 was 142. A house-to-house mosquito larvae survey of 200 houses in Santo Dmingo in May showed house indices from 5-70%, and in July from 15-65% (including 45% at the Tourism Pier, 15% of the International Airport, and 45-64% at eight hospitals inspected).

(J.P. Woodall, R.H. López-Correa, C.G. Moore, G.E. Sather, and G. Kuno)
San Juan Laboratories, Center for Disease Control, GPO Box 4532, San Juan, Puerto Rico 00936)

REPORT FROM THE GORGAS MEMORIAL LABORATORY,

PANAMA, R. P.

Recent Activity of Yellow Fever Virus in Monkeys in Darien Province of Panama

From 1970 to the end of 1974, a wave of jungle yellow fever (YF) moved east to west from the Colombian border, along the Isthmus of Panama, until it came to a halt about 40-50 km. NE of the capital city. Since the late 1940's, such waves have been spaced at intervals of about 7-9 years.

In late April 1979 we began this year's YF surveillance by sampling monkeys in the Darien forest at Alturas de Nique (#1 on map) close to the Colombian border. Seventy-four percent of 35 spider monkeys and all of the 10 howler monkeys tested (Table 1) had type-specific YF plaque-neutralizing (N) antibodies. In spider monkeys titer varied from 1:8 to 1:256, 10 of the 26 positives had titers ≥ 1:128, and the mean was 1:64. All of the howlers had titers ≥ 1:128. Animals ranged in age from infants to adults; all or almost all were no more than 7 years old. Only 2 spider monkeys had YF complement-fixing (CF) titers, at 1:8 and 1:16, respectively. (Howler sera were anti-complementary.) In view of the high percentage of immune individuals and the relatively high titers in the majority, we estimated that YF virus passed through the area sometime within the last 18 months.

In June-July, a more extensive expedition was made to the slopes of Cerro Tacarcuna (#2 on map) on the main cordillera of the Darien. Here, 19 (63%) of 31 spider monkeys and 1 (17%) of 6 white-faced monkeys had type-specific YF N antibodies (Table 1). Titers in these spider monkeys, lower in general than at Cerro Nique, varied from 1:8 to 1:128 with a mean of 1:32. Only 3 of the 19 positives had titers ≥ 1:128. The single positive *Cebus* had a low titer, 1:16. None of the spider monkeys had CF antibodies. (*Cebus* sera were anticomplementary.) Howler sera were not obtained in this area. The antibody data from Cerro Tacarcuna confirmed the findings at Alturas de Nique, that YF had been active in this part of the Darien within the last 18 months.

To attempt to determine the minimum time elapsed since the virus was present in either Nique or Tacarcuna, we tested 16 positive monkey sera with 2-mercaptoethanol. Titers of N antibodies after treatment were the same or at most only 2-fold lower than pre-treatment. The high proportion of γ-G immunoglobulin present in these sera therefore indicated that the infection had occurred more than 2 or 3 months earlier.

Virus isolation attempts on all the monkey sera are being carried out in Vero tube cultures. One *non*-yellow fever isolate has been recovered; identification is in progress.

In June and July, mosquitoes were captured at the Tacarcuna site from human bait in the canopy during the day time. One hundred twenty-two pools containing 2,533 individual mosquitoes of 8 genera were tested for virus in Vero tube cultures. No virus was isolated. Since in 1974 using the same system we were able to isolate YF virus from similar mosquito collections made at sites of current YF activity, we conclude that YF virus was no longer active in the Cerro Tacarcuna area in June-July 1979, in agreement with the conclusion drawn from the monkey antibody data.

Surveillance is continuing with an expedition in late August to a site about 100 km. farther west along the cordillera. A major aim is to set up a study site in advance of the YF wave, where we can intercept it and carry out studies on virus transmission mechanisms on the spot.

(Drs. A. Benenson, A. Adames, P. Galindo, G. Justines, P. Peralta and W. Reeves)

Table 1. Yellow fever neutralizing antibodies in wild monkeys of the Darien, Panama, 1979.

Monkey species	Nique (April-May)	Tacarcuna (June-July)
Ateles fusciceps (black spider)	26/35* (74%)	19/31 (63%)
Alouatta villosa (howler)	10/10 (100%)	--
Cebus capucinus (white-faced)	--	1/6 (17%)

* No. positive/No. tested

Panama
1
2

REPORT FROM THE INSTITUTO DE INVESTIGACIONES VETERINARIAS, SECCION DE ARBOVIRUS, APARTADO 70, MARACAY, VENEZUELA

The first isolation of EEE virus in Venezuela was made by means of sentinel hamsters exposed in 1975 in the Catatumbo area, Estado Zulia(1). As previously reported the agent was recovered in this Institute in 1976 from autopsy materials of horses from Zulia(2) and Yaracuy. In 1978 the virus was isolated once more from clinical equine cases occurring in Mérida and Zulia, and also from sentinel hamsters exposed in the swampy zone of the southern tip of the Lago de Maracaibo. During 1979 the virus was recovered again from horses dying in Yaracuy. These results and those of the study of equidae sera collected between 1972 and 1979 in several Estados compose a fair picture of the known distribution of EEE in Venezuela.

The sera were tested in HI with VEE and EEE antigens. This report is based on the findings with EEE; in each case careful scrutiny was made to decide whether the results could be attributed to EEE or VEE activity alone, to double infections, or to serological overlaps. Sera from Zulia, Yaracuy, Apure and Guárico were tested also against WEE antigen; no antibodies to this virus were found. The analysis of the combined results of HI, CF and N assays with all three viruses will be the subject of future publications. A previous report dealt with the distribution of VEE in Venezuela(3).

The adjoining map of Venezuela presents the political division of the country and indicates the areas where the sera were collected. The Lago de Maracaibo is the portion of the Caribbean within Zulia.

The following summary describes the EEE situation in the Estados that were visited either with the purpose of a serological survey or with the aim of establishing the etiological diagnosis of equine encephalitis cases:

Bolivar.Serological survey,1972. No indication of viral activity was found in 42 sera tested.

Delta Amacuro. Serological survey,1973. Intense activity was detected in 71 sera, suggesting a highly endemic situation.

Monagas. Serological surveys,1972 and 1977. The testing of 150 sera revealed moderate endemic conditions.

Sucre. Serological survey,1972. No proof of virus activity in 76 sera.

Guárico. Etiological diagnosis of clinical cases,1976. No virus was isolated. The examination of 79 sera showed present and past activity. No recent antibodies to VEE were found.

Apure. Serological surveys,1975 and 1978. The testing of 220 sera pointed to a low endemic picture.

Trujillo. Serological survey,1979. No antibodies detected in 43 sera.

Portuguesa. Etiological diagnosis of clinical cases,1975. No virus isolation. The tests of 42 sera gave no evidence of EEE activity. VEE virus

was recovered.

Cojedes. Etiological diagnosis of clinical cases,1978. No isolation of the virus was made. The study of 208 sera demonstrated current activity.

Yaracuy. Etiological diagnosis of clinical cases,1976 and 1979. On both occasions the agent was isolated. The serological results with 29 sera of 1976 and 52 of 1979, for a total of 81 specimens, were similar and indicated an endemic situation.

Mérida. Etiological diagnosis of clinical cases,1978. The virus was recovered. The examination of 17 sera confirmed recent activity.

Táchira. Etiological diagnosis of clinical cases,1978. No virus was isolated. The serology of 107 specimens indicated intense recent activity.

Zulia. Serological surveys,1973 and 1974. At that time 566 sera were collected. It became apparent that in the northern semi-desert areas facing the Caribbean, the viral activity was practically none. Advancing to the south along the eastern side of the Lago de Maracaibo, the picture was compatible with moderate activity of the virus. On the western side of the Lago the situation was that of a highly endemic condition.

Etiological diagnosis of clinical cases,1976 and 1978. In the area to the south west of the Lago the virus was isolated in 1976 but not in 1978; the study of 190 sera confirmed the existence of a highly endemic picture. In the eastern side of the Lago the virus was isolated from cases occurring in 1978; the serological results of 163 specimens signaled intense viral activity.

Falcón. Serological survey,1972. The testing of 59 samples collected in the semi-desert peninsula showed no antibodies.

Etiological diagnosis of clinical cases,1979. The episode occurred in the south east of Estado. Virus was not isolated but the assay of 10 sera substantiated present activity.

According to the preceding information the activity of EEE in equidae is, as expected, straightly related to the presence of permanent surface water such as that of swamps, marshes, bogs, pressumably associated with birds populations and mosquitoes; this situation is patent in Delta Amacuro and in the area to the south west of the Lago de Maracaibo, Estado Zulia. The reverse conditionis present in the semi-desert zones, where stagnant ground water, if any, is found only for short periods during the brief rainy season; such is the case of the Caribbean coast of Zulia and Falcón, where EEE does not exist. The different epidemiology of EEE and VEE is clearly illustrated in these dry areas where recurrent explosive epidemics of the latter do occur. Between the two extreme conditions described , there is a gradient of ecological combinations manifesting themselves by varied degrees of EEE activity, including localized small outbreaks of the encephalitis. Up to now there is no evidence of EEE affecting man in Venezuela.

(J.de Siger, D.Parra, M.Pérez, E.Pulgar, C.Sanmartín)

References:

(1) Walder,R. and O.M.Suárez.1976. Primera evidencia en Venezuela de la encefalitis equina del Este (EEE) en circunstancias silentes. Boletín Dirección Malariología y Saneamiento Ambiental, Vol.XVI,N°2: 119-125.

(2) de Siger,J.,Mettler,N. and Castañeda,J.1976. First isolation of eastern encephalomyelitis virus from a horse in Venezuela. 19th.Annual Proceedings Amer Assoc Veterinary Lab Diagnosticians: 229-236.

(3) Parra,D.,de Siger,J. and Mackenzie,R.1976. Prevalencia del virus de encefalitis equina venezolana en animales domésticos de algunas regiones de Venezuela,1972-1975. Veterinaria Tropical, 1 (1):93-115.

MAP OF VENEZUELA

HI ANTIBODIES FOR EEE IN EQUIDAE 1972 - 1979

AREAS WHERE SERA WERE COLLECTED

REPORT FROM THE ANIMAL VIRUS LABORATORY

INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS

CARACAS, VENEZUELA

Studies on the virulence of Yellow Fever Viruses:

This is a preliminary report on the differentiation of two Yellow Fever Virus (YFV) strain populations on the basis of the *in vivo* virulence markers. A preliminary quantitation of expression of virulence of strains PHO 42 H SMB-1 (isolated from liver of a human fatal case) and 17 D has been made by a titration of each virus in NMRI/IVIC mice of different ages.

Results summarised in Fig. 1 show responses obtained with the two YFV strains at defined animal ages in terms of efficiency of infection and outcome of infection (as survival or death). In each experiment the test strain of virus was titrated in defined ages of mice by the intracerebral (*i.c.*) and intraperitoneal (*i.p.*) route. The strain PHO 42 H SMB-1 by the *i.c.* route invariably kills efficiently animals up to 13-15 days old, whereas, by the *i.p.* route will efficiently kill only up to 7-9 days old and afterwards changing to a prolonged intermediate-mixed response with considerable diminished efficiency (~ 80 ID50/PLD50). On the other hand, the 17D strain is only lethal for the early ages (2-3 days old) by the *i.c.* route with an efficiency of 1 ID50/LD50, but a change occurs at 4-6 days where it becomes less efficient for mixed responses (~ 30 ID50/PLD50). However, by the *i.p.* route the 17D strain results less virulent from the very early ages of mice as shown by the intermediate responses up to 7-9 days old animals (~ 40-90 ID50/PLD50) with a change to efficient protection (~ 1-2 ID50/PD50).

(Thais Fajardo, Raúl Walder).

FIG. 1

Y F V STRAIN PHC 42 E SME - 1

Age (days)	Inoculation Route	Efficiency of Infection	Outcome of Infection		
			Mortality (M)	Mortality-Protection	Protection (P)
		SMicLD50/ID50	SMicLD50/LD50	SMicLD50/PLD50	SMicLD50/PD50
2 - 3	ic	1	1	-	-
4 - 6	ic	1	1	-	-
7 - 9	ic	1	1	-	-
10 -12	ic	1	1	-	-
13 -15	ic	1	3	-	-
2 - 3	ip	1	1	-	-
4 - 6	ip	1	2	-	-
7 - 9	ip	1	1	-	-
10 -12	ip	1	-	81 P > M	-
13 -15	ip	1	-	79 P > M	-

Y F V STRAIN 17 D

Age (days)	Inoculation Route	Efficiency of Infection	Outcome of Infection		
			Mortality (M)	Mortality-Protection	Protection (P)
		SMicLD50/ID50	SMicLD50/LD50	SMicLD50/PLD50	SMicLD50/PD50
2 - 3	ic	1	1	-	-
4 - 6	ic	1	-	36 M > P	-
7 - 9	ic	1	-	28 M > P	-
10 -12	ic	1	-	66 M > P	-
13 -15	ic	1	-	33 M > P	-
2 - 3	ip	3	-	88 M > P	-
4 - 6	ip	1	-	51 P > M	-
7 - 9	ip	1	-	44 P > M	-
10 -12	ip	1	-	-	2
13 -15	ip	1	-	-	1

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

Oropouche virus outbreak in Pará State, Brazil, 1979 .

An extensive outbreak of Oropouche (ORO) virus disease broke out in the east section of Pará State, during the first semester of 1979. The epidemic affected Belém, capital of Pará, and nearby towns and small villages (Fig) .

The outbreak was first detected in the town of Santa Izabel (40 Km east of Belém), at the beginning of March . The two initial cases were first examined on March 2nd . One of them was a 46 y.o. nun residing in Santa Izabel, who reported an illness compatible with Oropouche fever . She was on the 7 day post onset and a blood specimen collected from her on this day was virus negative . A serological conversion to ORO virus, however, was documented by the HI test in paired sera of this patient . The second case was one of our laboratory technicians (Bel 1447, OVS) a 37 y.o. male who had spent two week ends in Santa Izabel (Feb 18 and 25) prior to the beginning of his illness . He was on the 2nd day of illness and a blood specimen collected from him on this day yielded ORO virus . During a short visit to Santa Izabel in the afternoon of March 2, 3 of 6 cases with acute febrile illness which were examined and bled yielded ORO virus .

From 9th to 16th of March a serological survey for ORO virus was carried out in Sta. Izabel . A total of 537 person from 96 families were randomly selected and bled . Every person was questioned for evidence of recent fever . This sample corresponds to approximately 6% of the 8618 residents in the urban area of Sta. Izabel . In addition, every family was visited twice weekly after the initial bleeding until the end of June and every febrile person was bled for attempted virus isolation . A convalescent serum was also collected from these cases . In july 202 out of the 537 persons that were free of ORO antibody and that reported no febrile illness throughout the surveillance period were rebled for antibody studies .

The March survey revealed (Table 1) an HI antibody prevalence rate of 29.6% (159/537) . When tested by CF test (indicative of recent infection) 90 of the 159 HI positive sera reacted with ORO antigen . The 90 CF positive cases plus 11 persons with no CF antibody but that had HI titers equal or greater to 1:160 were considered as recently infected by ORO virus . Thus, the serological incidence of ORO virus at mid March was 18.8% (101/537) (Table 1) . The incidence among females was 21.5% (61/283) and among males it reached 15.7% (40/254) . All age

groups were infected by the virus, although variations in the antibody rates were noted . Males aged 0 to 5 and 16 to 20 y.o. had the highest rates —— 19.4% (7/31) and 23.6% (9/38) respectively —— whereas those over 50 y.o. exhibited the lowest rate of recent infection —— 6.2% (2/32) . Among females the incidence was higher for those less than 5 y.o. and 21 to 30 y.o. —— 26% (12/46) and 34% (11/32) respectively —— whereas those older than 50 y.o. showed the lowest rate —— 7.8% (3/38) . The rate of old infection was 10.8% (58/537), and no sex differences were noted (Table 1) .

During the surveillance period (March 9 to June 30) 95 of the 537 persons reported an ORO like illness of which 47 were laboratory proven as due to ORO infection . Table 2 shows the monthly distribution of the infections in Sta. Izabel according to the laboratory method of confirmation . Proven infections among unselected persons are also included in Table 2 . It is clear that the virus was very active during March-April, when 45 cases were documented . A marked decline of the outbreak was observed in May, when only 2 cases were confirmed, and no more cases were observed in June .

During March-April the virus was detected in the localities of Benevides, Caraparú and Jundiaí, that were near Santa Izabel (Table 3) . Short term investigation were negative in Castanhal and Ananindeua, but no definite conclusions can be drawn at moment .

The Belém outbreak was detected in early May . The virus was quite active in the Nova Marambaia district, where most investigations were concentrated . As seen in Table 3, 20 infections were confirmed, 14 of which by virus isolation . By the end of June no more cases were documented . Studies are under way to see if other sections of the city were affected by the outbreak .

Vector studies were carried out in Santa Izabel and in Belém . Insects collected are observed on Table 4 . No virus could be recovered from the 16791 insects from Sta. Izabel inoculated into infant mice as 289 pools . It should be noted that the great majority of the collections comprised Culicoides paraensis . Most of these midges were taken during March and April, when the epidemic was still active . C. paraensis were also predominant in the Belém catches, although a substantial number of Culex quinquefasciatus was obtained . Most of the Cx. quinquefasciatus was caught from resting sites, inside houses . One strain of ORO virus was recovered from one pool of 30 C. paraensis collected on May 29 .

This is the 3rd outbreak of Oropouche virus disease recognized in Belém . Previous records of the virus in Belém occurred in 1961 and 1968 . Other records of outbreaks due to the virus were in Bragança (1967) Baião (1972), Itupiranga (1975), Santarém area (1975) and Tomé-Açu (1978),

all in Pará State . It is possible that the presently described outbreaks originated from Tomé-Açu . This assumption is made in view of the frequent movement of people from Tomé-Açu to Santa Izabel and Belém and vice versa . Viremic patients are believed to be important carriers in the spread of ORO virus in urban environments, acting as a source of the agent to its main urban vector, the midge C. paraensis . Tomé-Açu and the other 2 places are only 3 to 4 hours away from each other by car. The Tomé-Açu outbreak occurred in the second half of 1978 and ORO cases were found up to the end of September when the surveillance ceased . The epidemic in Santa Izabel was detected in March, but it probably started in January 1979 or even in December 1978 . The Belém outbreak was detected in May but it could have started in March-April . This suggests that the introduction of the virus in Belém originated from Sta. Izabel or nearby localities, rather than from Tomé-Açu .

The isolation of ORO virus from one pool of C. paraensis collected in Belém reinforces the hypoteses that these midges are the main vectors for the agent in urban settings . It is intriguing, however, that the virus could not be recovered from the 15.836 midges caught in Sta. Izabel, a number 3 times greater than the Belém collections .

Francisco P. Pinheiro, Amélia P.A.Travassos da Rosa, Jorge P.A.Travassos da Rosa, R.B. Freitas, M.A.V. Santos . Evandro Chagas Institute, FSESP, Brazilian Ministry of Health .

Fig Oropouche virus epidemic sites in Pará, Brazil, 19797 .

Table 1 . Old and recent infections due to ORO virus among inhabitants of Santa Izabel, Pará, examined from March 9 to 16, 1979 .

Sex	Total sampled	Persons with HI antibody	Old infections*	Recent infections **
Male	254	67(26%)	27(10.6%)	40(15.7%)
Female	283	92(32.5%)	31(10.9%)	61(21.5%)
TOTAL	537	159(29.6%)	58(10.8%)	101(18.8%)

* HI antibody titer 1:80 or lower and no CF antibody
** CF antibody positive or HI titer 1:160 or greater but no CF antibody

Table 2. Monthly distribution of Oropouche cases in Santa Izabel,Pará,1979

Month	Selected group			Unselected group			Total		
	I	C	Pos/examined	I	C	Total	I	C	Total
March	12	14	26/43	6	4	10	18	18	36
April	10	9	19/41	1	0	1	11	9	20
May	2	0	2/9	0	0	0	2	0	2
June	0	0	0/2	0	0	0	0	0	0
TOTAL	24	23	47/95	7	4	11	31	27	58

I = virus isolated C = serological conversion (virus negative)

Table 3 . Oropouche virus infections diagnosed in the first half of 1979, according to the locality .

Locality	Febrile cases examined	Cases confirmed			Period
		I	C	Total	
Santa Izabel	122	31	27	58	March-May
Benevides	3	3	0	3	March
Caraparú	2	2	0	2	March-April
Jundiaí	2	2	0	2	April
Belém	192	14	6	20	May-June

I = virus isolation C = serological conversion

Table 4 . Arthropods collected in Santa Izabel and Belém during Oropouche virus outbreaks in 1979 .

Arthropods	Santa Izabel (March-May)		Belém (May-June)
	Human bait	CDC light trap	Human bait
Aed. (H) fulvithorax	1(0)		
" (O) scapularis		3(0)	
Culex spp		5(1)	
" Belém nº 8		1(0)	
" (C) corniger	1(0)	64(3)	
" (C) cornator	1(0)	62(3)	
" (C) declarator	4(1)	103(4)	
" (C) quinquefasciatus	695(43)	5(1)	2685(203)
" (Melanconion) sp		11(1)	
Mans. (M) titillans		1(0)	
Coquillettidia (R) venezuelensis		1(0)	
Aedeomya squamipennis		2(0)	
Psorophora (G) cingulata		6(1)	
Uranoteania sp		2(0)	
Anopheles (N) nunez-tovari		3(0)	
Culicoides paraensis	15836(231)		5045(302)
TOTAL	16538(275)	269(14)	7730(505)

( ) number of pools inoculated into mice

REPORT FROM THE LABORATORY OF ARBOVIRUS - DEPARTAMENT OF VIROLOGY
INSTITUTO DE MICROBIOLOGIA - UFRJ ILHA DO FUNDÃO - RIO DE JANEIRO
BRAZIL - CEP 20.000

I - Isolation of the vírus WEE from Haemagogus janthinomys mosquitoes

From a single pool of Haemagogus janthinomys captured in the forest region of the municipality of Linhares in the state of Espirito Santo, Brazil, a virus was isolated and identified by serological methods as WEE virus.

The region situated on the left margin of Rio Doce, localised between the 19° and 20° parallels south and the 40° and 41° meridians west and to the south of Juparanã lake (map 1).

The mosquitoes were captured by Dr. L.S. Otero and were identified by Drs. L.S. Otero and J.N. Belkin.

The virus was isolated by passage in suckling mouse brain and its antigen, prepared by the method of sucrose - acetone, demonstrated hemagglutinating properties with goose erythrocytes at pH 6,2 and at temperatures of 4°C and 26°C.

When submitted to sensibility tests such as ether and sodium desoxicholate, it showed sensibility with a reduction of more than 5 logs in comparison with the controls.

The study of susceptibility to various cell cultures such as chick embryo, mouse embryo, LLC/MK_2, BHK - 21, Hela, Hep-2 IBRS, Aedes albopictus and Aedes aegypti noted that only the chick embryo and BHK-21 cells demonstrated CPE.

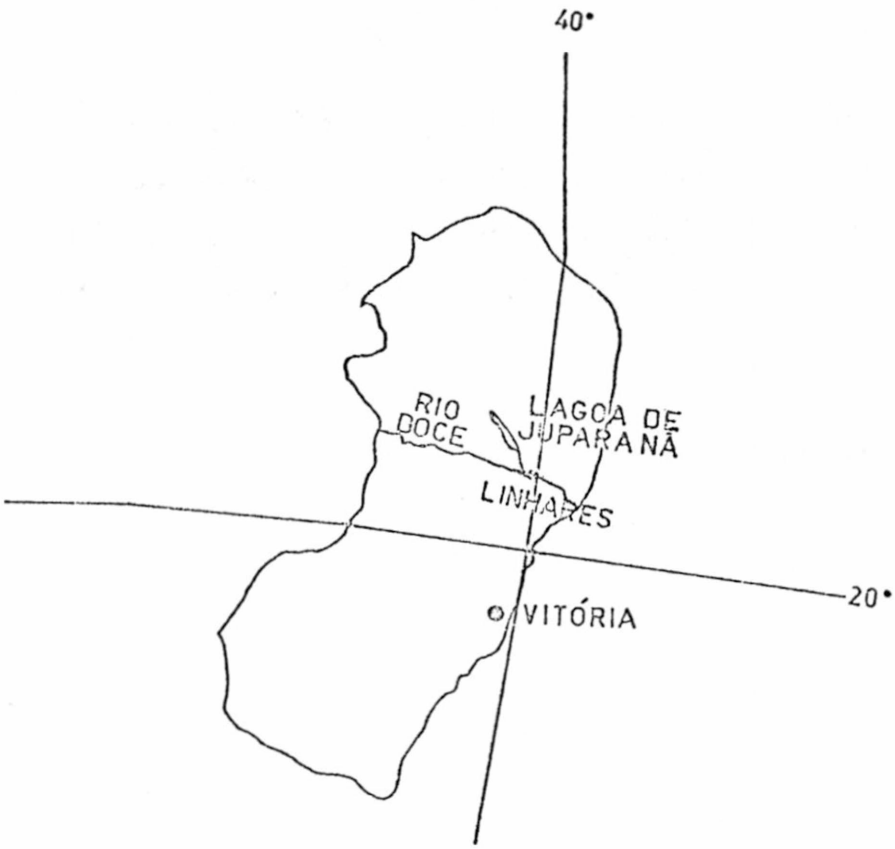
Hemagglutination - inhibition tests were employed with 2,975 human serum sample origination from 20 states representing 5 geographical regions of the country, and resulted in 6.4% positivity.

HI tests with equine serum samples resulted in possitivity of 6.2%.

Neutralization tests performes with immune ascit fluids, provided by the National Institute of Health, Bethesda, Maryland, USA, revealed the sample 3744 - IMDV to be WEE virus.

(Marcia Dutra Wigg, Claudio Andrade & Luiz Soledade Otero)

MAPA 1. LOCALIZAÇÃO DO MUNICÍPIO DE LINHARES
ESTADO DO ESPIRITO SANTO



2 - Serological relationship between different viruses of VSV group

In this research testing the serological relationships between the viruses of the VSV group, we observed little cross reaction between Indiana (VSI), Cocal (COC) and Alagoas (VSA) by complement fixing (CF) tests with dilutions of hyperimmune sera (table 1).

On the other hand when the tests were made using dilutions of antigen we did not obtain cross reactions (table 2).

Those results sugest the existence of antigenic sub-group in the VSV group constituted by the three samples formerly described.

By the neutralizing tests (NT) (table 3) we verified that there was individuality of those samples studied as follows: Indiana (VSI), Cocal (COC), Alagoas (VSA), New Jersey (VSNJ), Piry (PIRY) and Chandipura (CHP), moreover these results show the Alagoas strain is a new viral entity.

(Carlos Mitihiko Nozawa e Claudio Moraes Andrade)

Table I - CF titers of the hyperimmune sera against the different VSV antigens (with two antigenic units).

Serum / Antigen	Indiana	Cocal	Alagoas	N. Jersey	Piry	Chandipura
Indiana	320*	20	20	0	0	0
Cocal	20	160	40	0	0	0
Alagoas	20	40	320	0	0	0
N. Jersey	0	0	0	160	0	0
Piry	0	0	0	0	80	0
Chandipura	0	0	0	0	0	80

* Reciprocal titer of CF antibody

0 Negative in the dilution of the 1:5.

Table 2 - CF titers of the VSV antigens against the hyperimmune sera (with two units of the antibodies).

Serum / Antigen	Indiana	Cocal	Alagoas	N. Jersey	Piry	Chandipura
Indiana	640*	0	0	0	0	0
Cocal	0	320	0	0	0	0
Alagoas	0	0	640	0	0	0
N. Jersey	0	0	0	320	0	0
Piry	0	0	0	0	160	0
Chandipura	0	0	0	0	0	160

* Reciprocal titer of CF antigen.

0 Negative in the diluition of the 1:5.

Table 3 - NT titers of the VSV hyperimmune sera, against the homologous and heterologous antigens (Tests carried out in baby-suckling mice).

Antigen / Serum	Indiana	Cocal	Alagoas	N. Jersey	Piry	Chandipura
Indiana	1300*	0	0	0	0	0
Cocal	0	730	0	0	0	0
Alagoas	0	0	1100	0	0	0
N. Jersey	0	0	0	1250	0	0
Piry	0	0	0	0	350	0
Chandipura	0	0	0	0	0	420

* Reciprocal of NT titer which protected 50% of the animals.

0 Negative in the diluition of the 1:5.